DOCTOR'S FORMULAS

ΦΟΡΜΟΥΛΕΣ ΥΓΕΙΑΣ

βιβλιογραφία

CARDIOTONIC

περιεχόμενα

CARDIOTONIC

ΦΟΡΜΟΥΛΑ
καρδιαγγειακού

για την υγιή λειτουργία του καρδιαγγειακού

Καρνιτίνη | Συνένζυμο Q10 | Ταυρίνη

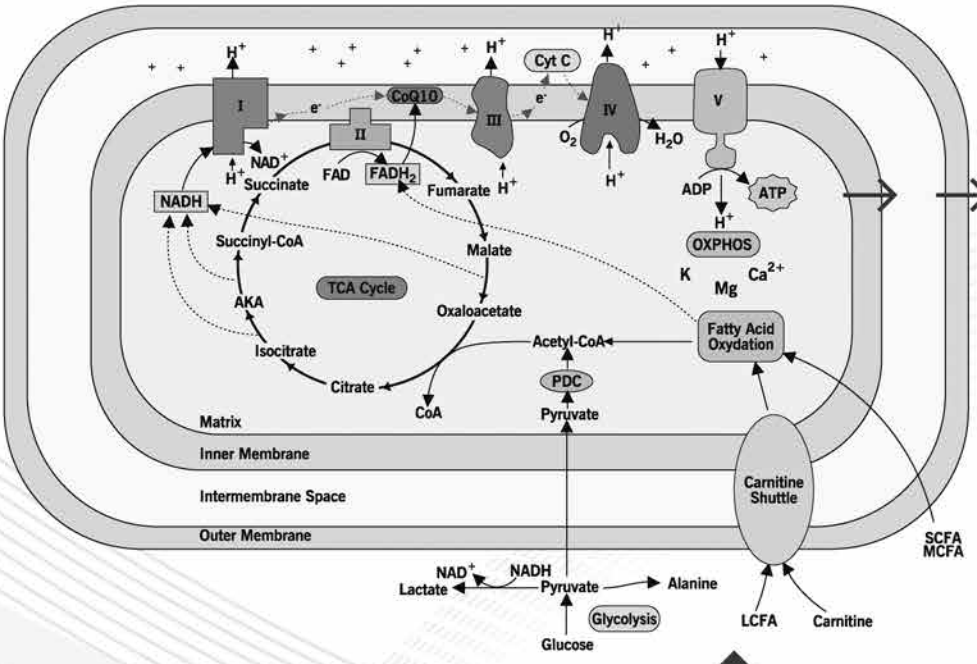
60 δισκία

Η συνεργιστική δράση των:

2 Συνένζυμο Q10

Δρα σαν **μεταφορέας ηλεκτρονίων** στη διαδικασία της οξειδωτικής φωσφορυλίωσης.

*Το **Q10** έχει την ικανότητα να διακινεί ηλεκτρόνια σύμφωνα με τις κυτταρικές ανάγκες. Είναι πανίσχυρο αντιοξειδωτικό και ο καρδιακός μυς περιέχει το περισσότερο **Q10** από οποιοδήποτε άλλο όργανο στο σώμα μας.*



αποτέλεσμα: παραγωγή κυτταρικής ενέργειας

1 L - Καρνιτίνη

Επιτρέπει τη **μεταφορά μακράς αλύσου λιπαρών οξέων** από το κυτταρόπλασμα στα μιτοχόνδρια ενεργοποιώντας:

- την β' οξείδωση
- τον κύκλο του Krebs
- την οξειδωτική φωσφορυλίωση

*Η **Καρνιτίνη** βρίσκεται σε μεγάλες συγκεντρώσεις στον καρδιακό μυ. Πολλές κλινικές μελέτες έχουν αξιολογήσει τη χρήση της **Καρνιτίνης** για την αντιμετώπιση πολλών καρδιακών παθήσεων.*

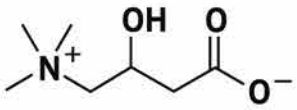
3 L - Ταυρίνη

Η καρδιά χρειάζεται την ταυρίνη για την ρύθμιση της ομοιόστασης των Ασβεστίου, Μαγνησίου και Καλίου. Ρύθμιση της Ca2+ ATPase του σαρκοπλασματικού δικτύου.

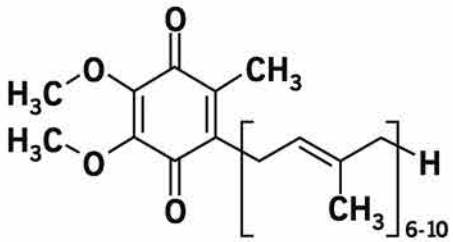
στην αντιμετώπιση της καρδιαγγειακής νόσου

CARDIOTONIC: η συνέργεια των 3 προϊόντων σε 1

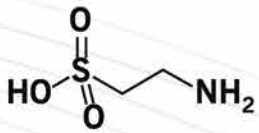
Καρνιτίνη *340mg*



CoQ10 *50mg*



Ταυρίνη *100mg*



► τριπλή άμυνα

ΜΙΑ ΜΟΝΑΔΙΚΗ ΣΥΝΘΕΣΗ ΠΟΥ ΕΝΔΕΙΚΝΥΤΑΙ ΣΤΙΣ ΕΞΗΣ ΠΕΡΙΠΤΩΣΕΙΣ

- Μεταβολικής βλάβης του Μυοκαρδίου σε περιπτώσεις ισχαιμικής καρδιοπάθειας.
- Αντιμετώπισης της περιφερικής αγγειοπάθειας.
- Έλλειψης σε συνένζυμο Q10 ή αλλαγές στον μεταβολισμό του Μυοκαρδίου στην οξεία ή χρόνια καρδιοπάθεια.

ΚΑΤΑΛΛΗΛΟ ΓΙΑ

- **Καρδιαγγειακή προστασία**
- **Καρδιαγγειακή ανεπάρκεια**
- **Στηθάγχη**

ΔΙΑΤΡΟΦΙΚΕΣ ΠΛΗΡΟΦΟΡΙΕΣ	ανά δισκίο
L-καρνιτίνη (from 500mg of L-Carnitine Tartrate)	340mg
L-ταυρίνη	100mg
Συνένζυμο Q10	50mg

ΔΟΣΟΛΟΓΙΑ

Ένα με δύο δισκία ημερησίως.

Βιβλιογραφία

1. DiNicolantonio JJ, Lavie CJ, Fares H, Menezes AR, O'Keefe JH. L-carnitine in the secondary prevention of cardiovascular disease: systematic review and meta-analysis. Mayo Clin Proc. 2013 Jun;88(6):544-51. doi: 10.1016/j.mayocp.2013.02.007. Epub 2013 Apr 15.
2. Sharma S, Black SM. Carnitine homeostasis, mitochondrial function, and cardiovascular DISEASE. Drug Discov Today Dis Mech. 2009;6(1-4):e31-e39.
3. Ferrari R, Merli E, Cicchitelli G, Mele D, Fucili A, Ceconi C. Therapeutic effects of L-carnitine and propionyl-L-carnitne on cardiovascular diseases: a review. Ann N Y Acad Sci. 2004 Nov;1033:79-91.
4. Chapman, R.A., Suleinan, M.S. & Earm, Y.E. (1993) *Taurine and the heart*, Cardiovascular Research, Volume 27, issue 3, (pp. 358-363).
5. L-Carnitine and Its Role in Medicine: From Function to Therapy. Ed R. Ferrari, S. Dimauro, G. Sherwood.
6. Nutraceutici. Brian Lockwood, Lisa Rapport. Ed. Italiana di Bruno Berra.
7. Medicina Integrativa AMINOACIDI. Integratori mirati per salute e piena efficienza. Prof. Ivo Bianchi. Ed. MOS MAIORUM.

doi: 10.1111/j.1751-7133.2011.00239.x

REVIEW PAPER

Nutritional Assessment in Heart Failure Patients

Heart failure (HF) is a growing epidemic worldwide with a particularly large presence in the United States. Nutritional assessment and supplementation is an area that can be studied to potentially improve the outcomes of these chronically ill patients. There have been many studies reporting the effect of various nutrients on HF patients, often with mixed results. Amino acids such as taurine, which is involved in calcium exchange, has been reported to improve heart function. Coenzyme Q10, a key component in the electron transport chain, is vital for energy production. L-carnitine, an amino acid derivative, is responsible for transport of fatty acids into the mitochondria along with modulating glucose metabolism. Thiamine and the other B vitamins, which serve as vital cofactors, can often be deficient in HF patients. Omega-3 fatty acid supplementation has been demonstrated to benefit HF patients potentially through anti-arrhythmic and anti-inflammatory mechanisms. Vitamin D supplementation can potentially benefit HF patients by way of modulating the renin-angiotensin system, smooth muscle proliferation, inflammation, and calcium homeostasis. Although supplementation of all of the above nutrients has the potential to benefit patients with HF, more studies are needed to solidify these recommendations. Congest Heart Fail. *2011;17:199–203.* ©*2011 Wiley Periodicals, Inc.*

John H. Lee, MD;[1] Tara Jarreau, MD;[1] Amit Prasad, MD;[1] Carl Lavie, MD;[1] James O'Keefe, MD;[2] Hector Ventura, MD[1]
From the John Ochsner Heart and Vascular Institute, Ochsner Clinical School, The University of Queensland School of Medicine, New Orleans, LA;[1] and Mid America Heart and Institute and University of Missouri, Kansas City, MO[2]

Address for correspondence:
John H. Lee, MD, John Ochsner Heart and Vascular Institute, Ochsner Clinical School-The University of Queensland School of Medicine, 1514 Jefferson Highway, New Orleans, LA 70121
E-mail: johlee@ochsner.org

Heart failure (HF) is a growing epidemic worldwide with a particularly large presence in the United States. There are approximately 5 million persons in the United States who have HF, with more than 550,000 new patients diagnosed each year.[1,2] In 2010, $39.2 billion was spent in the United States for the management of HF.[1] Because of the high prevalence and incidence of HF, much research has been devoted to the care of these patients. This research has led to the current guidelines for HF therapy, which include β-blockers, angiotensin receptor blockers, aldosterone antagonists, diuretics, cardiac resynchronization, and implantable cardiac-defibrillators.[2] Although these therapies have been shown to decrease morbidity and mortality and even improve quality of life, additional management strategies need to be studied to further improve the outcomes of these chronically ill individuals. One area of HF management that has limited study and application is nutritional assessment and supplementation. This review will describe the different nutrients that are potentially important for the HF patient and provide some of the supporting evidence (Table).

Micronutrients

There are extreme metabolic demands on the adult human heart, which is responsible for pumping approximately 5 L of blood per minute at rest and up to 24 L/min during vigorous exercise. The heart will circulate more >7200 L/d and >2.6 million L/y. During the course of 80 years, the average heart will pump more than 3 billion times. The predominant energy source is fatty acids, but the heart can also easily utilize carbohydrates or both carbohydrates and fatty acids simultaneously. Both of these energy sources are converted into adenosine triphosphate (ATP), which is hydrolyzed by the heart to continue its pump function. Micronutrients and macronutrients are essential for maintaining this highly efficient machine's parts for the life of the human it occupies by way of renewing enzymes, membranes, and structural elements with amino acids, lipids, and carbohydrates that are either synthesized or consumed in the diet. Micronutrients of importance include coenzyme Q10 (Co Q10), L-carnitine, thiamine, amino acids such as taurine, omega-3 fatty acids, and vitamins. Many of these micronutrients have been noted to be deficient in patients with HF.[3,4]

Amino acids are a vital nutrient for cardiac metabolism in that they are the foundation from which proteins are constructed as well as serve as an intermediary metabolite. Of particular importance is taurine, which is not involved in protein synthesis; however, taurine is reported to make up one fourth of the amino acid pool in the heart and functions as an antioxidant and regulates calcium homeostasis.[4] Taurine modulates a variety of calcium exchange mechanisms to ensure optimal levels and prevent cellular overloads or deficiencies.[5] In a recently published randomized controlled trial of taurine supplementation in HF patients, those who received taurine supplements had

Table. Nutrients Important in Heart Failure Patients

Amino acids: taurine
Coenzyme Q10
L-carnitine
Thiamine (vitamin B1)
Riboflavin (vitamin B2)
Pyridoxine (vitamin B6)
Omega-3 fatty acids
Vitamin D
Magnesium, potassium, zinc, selenium

better exercise capacity than those who received placebo.[6] Taurine supplementation has also been shown to lower left ventricular (LV) end-diastolic pressures[6] as well as improve systolic function.[8,9] In a study of taurine transporter knock out mice, it was observed that they reverted to a fetal cardiac phenotype and had cardiomyocyte atrophy, mitochondrial and myofiber damage, and ultimately cardiac dysfunction.[10]

Co Q10, also known as ubiquinone, is an important component of the electron transport chain in the mitochondria and is essential for the production of the heart's major energy source, ATP. Co Q10 has also been shown to act as an antioxidant that decreases low-density lipoprotein oxidation.[11] Unfortunately, in patients with HF, serum and tissue levels of Co Q10 have been shown to be lower.[12] In addition to this fact, statins, commonly used by HF patients, are HMG-CoA reductase inhibitors that can further deplete the body of this vital nutrient.[13] It has been suggested that the degree of Co Q10 deficiency can correlate to worse LV function and mortality.[14,15] For this reason, several studies looked at the supplementation of Co Q10 in HF patients. Significant improvement in ejection fraction,[16] stroke volume,[16,17] cardiac output,[17] pulmonary artery pressure,[17] functional capacity,[18,19] and quality of life[20] has been shown in trials of Co Q10. Although there is a good deal of evidence supporting Co Q10 supplementation, some studies showed no benefit,[21,22] leaving us with mixed results. Recently, however, meta-analyses have shown significant benefit of Co Q10 supplementation in HF patients.[23] Co Q10 also has been shown in a meta-analysis to lower systolic blood pressure by up to 17 mm Hg and diastolic blood pressure by up to 10 mm Hg, without major adverse side effects in patients with essential hypertension.[24]

L-carnitine is derived from amino acids and is responsible for the transport of fatty acids into the mitochondria from the cytosol.[25] It also has a role in modulating glycolysis, the Krebs cycle, and glucose metabolism. Propionyl-L-carnitine, a derivative of L-carnitine, has also been shown to be involved in the Kreb's cycle as well as increased glucose oxidation and improved contractile function.[26] Although L-carnitine can be produced endogenously as well as provided by diet, low levels have been observed in HF patients.[27] In a study by Serati and colleagues,[28] treatment with L-carnitine resulted in improved diastolic parameters by echocardiography when compared with those who received placebo. In a randomized, double-blind, placebo-controlled multicenter trial conducted by Iliceto and colleagues,[28] supplementation of L-carnitine post–myocardial infarction was shown to attenuate LV dilatation. Rizos demonstrated a mortality benefit in New York Heart Association class II or IV patients treated with L-carnitine.[30] Despite the positive results in studies investigating the effect of L-carnitine on HF, there have been others that have shown no benefit.[31] In summary, L-carnitine supplementation has been shown to improve exercise capacity, maximum exercise time, peak heart rate, and peak oxygen consumption as well as hemodynamic and echocardiographic parameters in HF patients in a variety of studies.[4] However, more studies are needed to truly demonstrate the benefits of L-carnitine supplementation in HF patients.

Thiamine, otherwise known as vitamin B1, serves as a key cofactor in carbohydrate metabolism. It is not synthesized in humans, and little thiamine is stored endogenously. As such, continual ingestion is required to prevent thiamine deficiency.[3] The effects of thiamine deficiency and the benefit of thiamine supplementation are disease- and medication-dependent.[3] Severe thiamine deficiency can result in severe vasodilatation and high-output HF, known as wet beriberi. This form of thiamine deficiency clearly warrants thiamine supplementation; however, wet beriberi is increasingly uncommon.[3] The benefit of thiamine supplementation in less severe forms of thiamine deficiency is still unclear. Thiamine deficiency in HF has typically been attributed to the use of loop diuretics, which promote the excretion of thiamine and other water-soluble B vitamins.[32,33] However, poor dietary intake is likely a contributing factor in many patients.[34] A series of studies have shown thiamine deficiency to be fairly common in the HF population, with prevalence ranging from 13% to 33%.[35,36] The prevalence may be even higher in hospitalized and elderly patients with HF.[36,37] A number of small studies have shown improved markers of LV function after thiamine supplementation. Shimon and colleagues[38] enrolled 30 patients with thiamine deficiency taking loop diuretics and provided 7 weeks of thiamine supplementation.[38] Twenty-seven of the 30 patients showed a mean improvement in LV ejection fraction of 22%. Another study by Seligmann and colleagues[32] treated 23 HF patients with a 7-day course of intravenous thiamine and demonstrated a mean improvement in ejection fraction of 13% and a mean increase in systolic blood pressure of 10 mm Hg. Studies have shown that the use of spironolactone helps to abate the excretion of thiamine and improve serum thiamine levels.[39,40] Moreover, even small doses of thiamine (1.5 mg/d) appear to prevent thiamine deficiency in HF patients.[35] Other studies have shown mixed results from thiamine supplementation in HF.[37,41] Larger studies are necessary to examine the benefit of thiamine supplementation in HF, but it appears reasonable to supplement thiamine in chronic HF patients, especially those taking high-dose loop diuretics.

Riboflavin and pyridoxine are water-soluble B vitamins that play a key role in the beta-oxidation of lipids, carbohydrate metabolism, and red blood cell production. Like thiamine, these

vitamins show increased excretion with loop diuretics.[42] Riboflavin and pyridoxine deficiencies have been demonstrated in chronic HF patients. One study of 100 patients reported riboflavin deficiency in 27% and pyridoxine deficiency in 38%[42] ; however, data regarding supplementation and its effect on cardiac function are lacking.

Hyperhomocysteinemia has been established as an independent risk factor for the development of HF even in the absence of myocardial infarction.[43,44] Vitamin B12 and folate deficiencies are the most common cause of elevated plasma homocysteine levels in adults.[45] Supplementation of these vitamins can effectively reverse elevated plasma homocysteine levels.[46] Studies show that levels of B12 and folate correlate poorly with the development of HF, and supplementation with vitamin B12 or folate has not been investigated.[47,48]

Other potential important micronutrients in the HF patient include magnesium, potassium, zinc, selenium, and creatine. Magnesium and potassium deficiencies have been associated with arrhythmias. Zinc and selenium are antioxidants that have also been reported to be low in HF patients.[4] Creatine is a key component of energy metabolism in the heart muscle and has also been reported to be deficient in patients with severe HF.[4]

Omega-3 Fatty Acids

Although reasons for the potential benefits of omega-3 polyunsaturated fatty acids (PUFAs) are not completely understood, omega-3 fatty acids appear to confer cardiovascular benefits largely through docosahexaenoic acid– and eicosapentaenoic acid–enrichment of membrane phospholipids.[49] The incorporation of omega-3 PUFA into the membranes of target cells and tissues is likely to produce a reduction in electrical excitability, thus decreasing the potential for arrhythmic events.[50] Other beneficial physiologic effects include inhibition of thromboxane production, increased production of prostacyclin, increased fibrinolytic activity of plasma, modification of leukotriene and cytokine production to reduce inflammation, reduction in vasospastic response to catecholamines, reduction in blood viscosity, decreased platelet-activating factor and platelet-derived growth factor, and oxygen free-radical generation.[51] These cumulative effects ultimately lead to increased arrhythmic thresholds, reduction in arterial blood pressure, improvement in arterial and endothelial function, reduced platelet aggregation, and favorable affects on autonomic tone.[52] Fish oils also decrease tumor necrosis factor (TNF) production in HF and improve body weight.

Omega-3 PUFA supplementation may represent a novel therapeutic approach in late-stage HF characterized by cardiac cachexia.[53,54] Supplementation with omega-3 PUFAs has also been of potential interest as a therapy for HF. Trials in primary and secondary prevention of coronary heart disease showed that omega-3 fatty acid supplementation results in a relative risk reduction of 10% to 20% in fatal and nonfatal cardiovascular events.[55] The Cardiovascular Health Study showed an inverse association in the intake of baked or broiled fish and incidence of congestive HF.[56,57] This result was supported by recent data from the Atherosclerosis Risk in Community (ARIC) study, showing an inverse relationship between omega-3 PUFA intake and incident HF in women.[58]

Further evidence on the benefit of omega-3 PUFA in HF was shown by the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Heart Failure (GISSI-HF) investigators. Almost 7000 patients with New York Heart Association class II through IV chronic HF were randomized to receive 1 g/d of omega-3 PUFAs or matching placebo.[59] Death from any cause was reduced from 29% with placebo to 27% in those treated with omega-3 fatty acids (adjusted hazard ratio, 0.91; 95.5% confidence interval, 0.833–0.998; P=.041). The co-primary outcome of death or admission to hospital for a cardiovascular event was also reduced. Although the improvements in clinical outcomes were modest, the therapy was safe, well tolerated, and additive to those of other therapies that are standard of care in HF.

Animal studies in cardiac remodeling suggest that higher doses of omega-3 PUFA may be useful.[60] In a small 18-week study of 14 patients with class II through IV HF, there was marked improvement in inflammatory cytokines, TNF-α and interleukin 1, with 5.1 g/d of eicosapentaenoic acid and docosahexaenoic acid.[53] These findings were echoed in the GISSI-HF study. Effect of n-3 PUFAs in patients with chronic HF in the GISSI-HF trial, a randomized, double-blind, placebo-controlled trial,[59] and a Japanese epidemiological study[61] both suggest that higher pharmacologic doses of omega-3 PUFA are needed to obtain maximal clinical benefits in patients with HF.

Further studies are needed to determine not only the optimal dose of omega-3 PUFA protection in different stages of HF, but also the underlying mechanism of action responsible for these benefits. It is clear that supplementation with omega-3 PUFA provides significant overall benefit with minimal risk. In a recent editorial published in *The Lancet*, Fonarow[62] concludes that "supplementation with omega-3 PUFAs should join the short list of evidence-based life-prolonging therapies for HF."[62]

Vitamin D

Vitamin D deficiency has been reported to be associated with many of the traditional CV risk factors such as diabetes mellitus, hypertension, and dyslipidemia either directly or indirectly.[63] Of particular interest for this review is the association of vitamin D deficiency with increased parathyroid hormone levels and subsequent effect on the modulation of the renin-angiotensin system, cardiac contractility, and smooth muscle proliferation leading to LV hypertrophy.[64] The greatest source of vitamin D is sunlight exposure and endogenous production in the skin. Vitamin D can also be obtained from dietary sources but to a much lesser degree.[63] Vitamin D deficiency has been reported to be a problem in several different populations ranging from the young and healthy to the elderly and is prevalent in HF patients as well. A study published by

Schierbeck and colleagues[65] indicated that both low vitamin D and elevated PTH were independently associated with mortality in HF patients.

Future of Nutrition in HF

Nutrition is an extremely important part of managing HF patients, particularly because many of them may be malnourished. With the large energy and nutrient demands of the human heart, it is essential to keep up with consumption to prevent the further fall in heart function in these already disadvantaged patients. There are physiologic reasons to supplement all of the nutrients we have discussed in this review, yet data still do not exist to be able to make solid recommendations. In addition, have many of the studies that have investigated nutrient supplementation simply fulfilled the need of one nutrient to potentially unmask the need for another? As discussed above, some basic treatment modalities of HF patients such as diuretics can, in and of themselves, contribute to some of the nutrient deficiencies. It is time to investigate the benefits of nutrient supplementation by designing a large randomized controlled trial comparing patients with repleted nutrients vs those who receive placebo. The formulation of nutrients in this study should include many if not all of the ones discussed in this review to answer the question of whether there is benefit.

References

1 Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics – 2010 update: a report from the American Heart Association. *Circulation*. 2010;121:e46–e215.

2 Hunt SA, Abraham WT, Chin MH, et al. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association task force on practice guidelines (writing Committee to update the 2001 guidelines for the evaluation and management of heart failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. *Circulation*. 2005;112: e154–e235.

3 Sole MJ, Jeejeebhoy KN. Conditioned nutritional requirements and the pathogenesis and treatment of myocardial failure. *Curr Opin Clin Nutr Metab Care*. 2000;3:417–424.

4 Soukoulis V, Dihu JB, Sole M, et al. Micronutrient deficiencies: an unmet need in heart failure. *J Am Coll Cardiol*. 2009;54:1660–1673.

5 Schaffer SW, Kramer J, Chovan JP. Regulation of calcium homeostasis in the heart by taurine. *Fed Proc*. 1980;39:2691–2694.

6 Beyranvand MR, Kadkhodai Khalafi M, Roshan VD, et al. Effect of taurine supplementation on exercise capacity of patients with heart failure. *J Cardiol*. 2011;57:333–337.

7 Jeejeebhoy F, Keith M, Freeman M, et al. Nutritional supplementation with MyoVive repletes essential cardiac myocyte nutrients and reduces left ventricular size in patients with left ventricular dysfunction. *Am Heart J*. 2002;143:1092–1100.

8 Azuma J, Hasegawa H, Sawamura A, et al. Taurine for treatment of congestive heart failure. *Int J Cardiol*. 1982;2:303–304.

9 Azuma J, Sawamura A, Awata N. Usefulness of taurine in chronic congestive heart failure and its prospective application. *Jpn Circ J*. 1992;56:95–99.

10 Ito T, Kimura Y, Uozumi Y, et al. Taurine depletion caused by knocking out the taurine transporter gene leads to cardiomyopathy with cardiac atrophy. *J Mol Cell Cardiol*. 2008;44:927–937.

11 Littarru GP, Tiano L. Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. *Mol Biotechnol*. 2007;37:31–37.

12 Folkers K, Langsjoen P, Langsjoen PH. Therapy with coenzyme Q10 of patients in heart failure who are eligible or ineligible for a transplant. *Biochem Biophys Res Commun*. 1992;182:247–253.

13 Strey CH, Young JM, Molyneux SL, et al. Endothelium-ameliorating effects of statin therapy and coenzyme Q10 reductions in chronic heart failure. *Atherosclerosis*. 2005;179: 201–206.

14 Folkers K, Vadhanavikit S, Mortensen SA. Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q10. *Proc Natl Acad Sci U S A*. 1985;82:901–904.

15 Molyneux SL, Florkowski CM, George PM, et al. Coenzyme Q10: an independent predictor of mortality in chronic heart failure. *J Am Coll Cardiol*. 2008;52:1435–1441.

16 Langsjoen PH, Vadhanavikit S, Folkers K. Response of patients in classes III and IV of cardiomyopathy to therapy in a blind and crossover trial with coenzyme Q10. *Proc Natl Acad Sci U S A*. 1985;82:4240–4244.

17 Munkholm H, Hansen HH, Rasmussen K. Coenzyme Q10 treatment in serious heart failure. *Biofactors*. 1999;9:285–289.

18 Keogh A, Fenton S, Leslie C, et al. Randomised double-blind, placebo-controlled trial of coenzyme Q, therapy in class II and III systolic heart failure. *Heart Lung Circ*. 2003;12:135–141.

19 Belardinelli R, Mucaj A, Lacalaprice F, et al. Coenzyme Q10 and exercise training in chronic heart failure. *Eur Heart J*. 2006;27:2675–2681.

20 Morisco C, Trimarco B, Condorelli M. Effect of coenzyme Q10 therapy in patients with congestive heart failure: a long-term multicenter randomized study. *Clin Investig*. 1993;71: S134–S136.

21 Watson PS, Scalia GM, Galbraith A, et al. Lack of effect of coenzyme Q on left ventricular function in patients with congestive heart failure. *J Am Coll Cardiol*. 1999;33:1549–1552.

22 Khatta M, Alexander BS, Krichten CM, et al. The effect of coenzyme Q10 in patients with congestive heart failure. *Ann Intern Med*. 2000;132:636–640.

23 Sander S, Coleman CI, Patel AA, et al. The impact of coenzyme Q10 on systolic function in patients with chronic heart failure. *J Card Fail*. 2006;12:464–472.

24 Rosenfeldt FL, Haas SJ, Krum H, et al. Coenzyme Q10 in the treatment of hypertension: a meta-analysis of the clinical trials. *J Hum Hypertens*. 2007;21:297–306.

25 Arsenian MA. Carnitine and its derivatives in cardiovascular disease. *Prog Cardiovasc Dis*. 1997;40:265–286.

26 Schonekess BO, Allard MF, Lopaschuk GD. Propionyl L-carnitine improvement of hypertrophied heart function is accompanied by an increase in carbohydrate oxidation. *Circ Res*. 1995;77:726–734.

27 Masumura Y, Kobayashi A, Yamazaki N. Myocardial free carnitine and fatty acylcarnitine levels in patients with chronic heart failure. *Jpn Circ J*. 1990;54:1471–1476.

28 Serati AR, Motamedi MR, Emami S, et al. L-carnitine treatment in patients with mild diastolic heart failure is associated with improvement in diastolic function and symptoms. *Cardiology*. 2010;116:178–182.

29 Iliceto S, Scrutinio D, Bruzzi P, et al. Effects of L-carnitine administration on left ventricular remodeling after acute anterior myocardial infarction: the L-Carnitine Ecocardiografia Digitalizzata Infarto Miocardico (CEDIM) Trial. *J Am Coll Cardiol*. 1995; 26:380–387.

30 Rizos I. Three-year survival of patients with heart failure caused by dilated cardiomyopathy and L-carnitine administration. *Am Heart J*. 2000;139:S120–S123.

31 Study on propionyl-L-carnitine in chronic heart failure. *Eur Heart J*. 1999;20:70–76.

32 Seligmann H, Halkin H, Rauchfleisch S, et al. Thiamine deficiency in patients with congestive heart failure receiving long-term furosemide therapy: a pilot study. *Am J Med*. 1991;91:151–155.

33 Zenuk C, Healey J, Donnelly J, et al. Thiamine deficiency in congestive heart failure patients receiving long term furosemide therapy. *Can J Clin Pharmacol*. 2003;10:184–188.

34 Brady JA, Rock CL, Horneffer MR. Thiamin status, diuretic medications, and the management of congestive heart failure. *J Am Diet Assoc*. 1995;95:541–544.

35 Hanninen SA, Darling PB, Sole MJ, et al. The prevalence of thiamin deficiency in hospitalized patients with congestive heart failure. *J Am Coll Cardiol*. 2006;47:354–361.

36 Kwok T, Falconer-Smith JF, Potter JF, Ives DR. Thiamine status of elderly patients with cardiac failure. *Age Ageing*. 1992;21:67–71.

37 Pfitzenmeyer P, Guilland JC, d'Athis P, et al. Thiamine status of elderly patients with cardiac failure including the effects of supplementation. *Int J Vitam Nutr Res*. 1994;64: 113–118.

38 Shimon I, Almog S, Vered Z, et al. Improved left ventricular function after thiamine supplementation in patients with congestive heart failure receiving long-term furosemide therapy. *Am J Med*. 1995;98:485–490.

39 Da Cunha S, Albanesi Filho FM, Da Cunha Bastos VL, et al. Thiamin, selenium, and copper levels in patients with idiopathic dilated cardiomyopathy taking diuretics. *Arq Bras Cardiol*. 2002;79:454–465.

40 Rocha RM, Silva GV, De Albuquerque DC, et al. Influence of spironolactone therapy on thiamine blood levels in patients with heart failure. *Arq Bras Cardiol*. 2008;90:324–328.

41 Smithline HA. Thiamine for the treatment of acute decompensated heart failure. *Am J Emerg Med.* 2007;25:124–126.

42 Keith ME, Walsh NA, Darling PB, et al. B-vitamin deficiency in hospitalized patients with heart failure. *J Am Diet Assoc.* 2009; 109:1406–1410.

43 Herrmann M, Kindermann I, Muller S, et al. Relationship of plasma homocysteine with the severity of chronic heart failure. *Clin Chem.* 2005;51:1512–1515.

44 Vasan RS, Beiser A, D'Agostino RB, et al. Plasma homocysteine and risk for congestive heart failure in adults without prior myocardial infarction. *JAMA.* 2003;289:1251–1257.

45 Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem.* 2004;50:3–32.

46 Stanger O, Weger M. Interactions of homocysteine, nitric oxide, folate and radicals in the progressively damaged endothelium. *Clin Chem Lab Med.* 2003;41:1444–1454.

47 Herrmann W, Herrmann M, Obeid R. [Hyperhomocysteinemia and B-vitamin deficiency Current clinical aspects]. *Med Monatsschr Pharm.* 2006;29:291–302.

48 Herrmann M, Stanger O, Paulweber B, et al. Effect of folate supplementation on N-terminal pro-brain natriuretic peptide. *Int J Cardiol.* 2007;118:267–269.

49 Lavie CJ, Milani RV, Mehra MR, Ventura HO. Omega-3 polyunsaturated fatty acids and cardiovascular diseases. *J Am Coll Cardiol.* 2009;54:585–594.

50 London B, Albert C, Anderson ME, et al. Omega-3 fatty acids and cardiac arrhythmias: prior studies and recommendations for future research: a report from the National Heart, Lung, and Blood Institute and Office Of Dietary Supplements Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis Workshop. *Circulation.* 2007;116:e320–e335.

51 Leaf A, Weber PC. Cardiovascular effects of n-3 fatty acids. *N Engl J Med.* 1988;318: 549–557.

52 Lee JH, O'Keefe JH, Lavie CJ, et al. Omega-3 fatty acids for cardioprotection. *Mayo Clin Proc.* 2008;83:324–332.

53 Mehra MR, Lavie CJ, Ventura HO, Milani RV. Fish oils produce anti-inflammatory effects and improve body weight in severe heart failure. *J Heart Lung Transplant.* 2006; 25:834–838.

54 Wang C, Harris WS, Chung M, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr.* 2006; 84:5–17.

55 Tavazzi L, Tognoni G, Franzosi MG, et al. Rationale and design of the GISSI heart failure trial: a large trial to assess the effects of n-3 polyunsaturated fatty acids and rosuvastatin in symptomatic congestive heart failure. *Eur J Heart Fail.* 2004;6:635–641.

56 Mozaffarian D, Bryson CL, Lemaitre RN, et al. Fish intake and risk of incident heart failure. *J Am Coll Cardiol.* 2005;45:2015–2021.

57 Lavie CJ, O'Keefe JH, Milani RV, et al. New data on the clinical impact of exercise training, fish oils, and statins in heart failure. *Phys Sportsmed.* 2009;37:22–28.

58 Yamagishi K, Nettleton JA, Folsom AR. Plasma fatty acid composition and incident heart failure in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J.* 2008;156:965–974.

59 Tavazzi L, Maggioni AP, Marchioli R, et al. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet.* 2008;372: 1223–1230.

60 Freeman LM, Rush JE, Kehayias JJ, et al. Nutritional alterations and the effect of fish oil supplementation in dogs with heart failure. *J Vet Intern Med.* 1998;12:440–448.

61 Yamagishi K, Iso H, Date C, et al. Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. *J Am Coll Cardiol.* 2008; 52:988–996.

62 Fonarow GC. Statins and n-3 fatty acid supplementation in heart failure. *Lancet.* 2008;372:1195–1196.

63 Lee JH, O'Keefe JH, Bell D, et al. Vitamin D deficiency: an important, common, and easily treatable cardiovascular risk factor? *J Am Coll Cardiol.* 2008;52:1949–1956.

64 Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol.* 2006;92: 39–48.

65 Schierbeck LL, Jensen TS, Bang U, et al. Parathyroid hormone and vitamin D – markers for cardiovascular and all cause mortality in heart failure. *Eur J Heart Fail.* 2011;13: 626–632.

Nutritional supplementation with MyoVive repletes essential cardiac myocyte nutrients and reduces left ventricular size in patients with left ventricular dysfunction

Farida Jeejeebhoy, MD,[b] Mary Keith, PhD,[a] Michael Freeman, MD,[b] Aiala Barr, PhD,[d] Michele McCall, MSc, RD,[d] Regina Kurian, BSc,[c] David Mazer, MD,[c] and Lee Errett, MD[a] ***Toronto, Ontario, Canada***

Background Congestive heart failure depletes the myocardium of carnitine, coenzyme Q10 (CoQ10), and taurine—substances known to influence mitochondrial function and cell calcium. We hypothesized that feeding patients a nutritional supplement that contained carnitine, CoQ10, and taurine would result in higher myocardial levels of these nutrients and improve left ventricular function.

Methods Forty-one patients who underwent aortocoronary artery bypass with an ejection fraction ≤40% at referral were randomly assigned to a double-blind trial of supplement or placebo. Radionuclide ventriculography was performed at randomization and before surgery. Surgical myocardial biopsies, adjusted for protein content, were analyzed for carnitine, CoQ10, and taurine levels.

Results The groups were well matched. Minor exceptions were supplement group versus placebo group for digoxin use (7 vs 0, respectively; $P = .009$) and age (62 ± 11 years vs 69 ± 5 years, respectively; $P = .04$). There were significantly higher levels in the treated group compared with the placebo group for myocardial levels of CoQ10 (138.17 ± 39.87 nmol/g wet weight and 56.67 ± 23.08 nmol/g wet weight; $P = .0006$), taurine (13.12 ± 4.00 μmol/g wet weight and 7.91 ± 2.81 μmol/g wet weight; $P = .003$), and carnitine (1735.4 ± 798.5 nmol/g wet weight and 1237.6 ± 343.1 nmol/g wet weight; $P = .06$). The left ventricular end-diastolic volume fell by −7.5 ± 21.7 mL in the supplement group and increased by 10.0 ± 19.8 mL in the placebo group ($P = .037$).

Conclusions Supplementation results in higher myocardial CoQ10, taurine, and carnitine levels and is associated with a reduction in left ventricular end-diastolic volume in patients with left ventricular dysfunction before revascularization. Because the risk of death for surgical revascularization is related to preoperative left ventricular end-diastolic volume, supplementation could improve outcomes. (Am Heart J 2002;143:1092-100.)

Left ventricular dysfunction leading to congestive heart failure (CHF) affects approximately 1.5% of the population and is most frequently caused by ischemic heart disease. Currently, with best medical practice, the death rate ranges from 50% in 5 years to as high as 40% to 50% in 2 years, depending on the severity of the heart failure and the underlying cause. There is an urgent need for therapies that improve left ventricular function and outcomes in patients with CHF.

It is known that CHF leads to malnutrition in 50% to 68% of patients with CHF.[1] Severe malnutrition in patients with CHF is termed cardiac cachexia,[2] which is an independent risk factor for mortality.[2] Traditionally, it is believed that a deficit of protein and energy intake is the most important cause of malnutrition in patients with CHF. However, supplementation of protein-calories in patients with CHF does not improve cardiac function, despite a gain in lean body mass.[4] Conversely, patients with severe CHF have lower levels of adenosine triphosphate in skeletal muscle, which also does not improve with protein-calorie supplementation,[5] suggesting an abnormality of muscle energetics rather than macronutrient deficiency in CHF. To support this concept, studies have shown that patients with ventricular dysfunction have an abnormality of

From the Divisions of [a]Cardiovascular Surgery, and [b]Cardiology, and the [c]Department of Anesthesia, St Michael's Hospital, [d]University of Toronto, and [e]University Health Network, Toronto, Ontario, Canada.
Supported by a grant from Medifoods, Inc.
Submitted May 31, 2001; accepted November 29, 2001.
Reprint requests: Lee Errett, MD, Chief, Division of Cardiovascular and Thoracic Surgery, St Michael's Hospital, 30 Bond St, 8 Bond Wing, Suite 002, Toronto, Ontario, Canada M5B 1W8.
E-mail: errettl@smh.toronto.on.ca

0002-8703/2002/$35.00 + 0 ***4/1/121927***
doi:10.1067/mhj.2002.121927

the mitochondrial respiratory chain[6] and that their myocytes are depleted of carnitine,[7,8] coenzyme Q10,[9,10] and taurine.[11] In a small series, the severity of depletion has been shown to be related to the severity of the heart failure.[12] In patients with CHF in some controlled trials, repletion of L-carnitine[13] and coenzyme Q10[14] improves survival and reduces episodes of pulmonary edema, respectively.

In addition, there is some evidence that L-carnitine supplementation will reduce the amount of left ventricular dilatation after myocardial infarction.[15] A randomized, double-blind, placebo-controlled, multicenter trial was conducted to address this question and found that supplementation with L-carnitine resulted in attenuation in the left ventricular dilation during the first year after an acute myocardial infarction.[15]

Supplementation with coenzyme Q10 in patients with CHF may have a slight effect on maximal exercise capacity and quality of life[16]; however, the data are conflicting, with another randomized trial showing no effect on ejection fraction (EF), peak oxygen consumption, or exercise duration in patients receiving standard medical therapy.[17]

Myocardial calcium accumulation occurs in the failing heart and in hamster cardiomyopathy; taurine supplementation reduces calcium accumulation and myocardial injury.[18] Thus, there is potential for taurine, carnitine, and coenzyme Q10 to improve ventricular function in patients with heart failure. A recent review article emphasized the potential relation between micronutrient deficiency and CHF and stressed the need for a large-scale trial of dietary micronutrient supplementation in patients with CHF.[19]

We hypothesize that feeding a mixture of carnitine, coenzyme Q10, and taurine to patients with ventricular dysfunction who undergo elective coronary artery bypass surgery will increase myocardial levels of these nutrients and also improve left ventricular function as assessed by radionuclide ventriculography.

Methods

Study design

This was a single-center, randomized, double-blind, placebo-controlled study. Informed consent was obtained. The research protocol was approved by the institutional review board.

Patient selection

Stable patients taking medical therapy who were scheduled for elective aortocoronary bypass surgery were approached for consent if referral EF was ≤40% on the basis of contrast ventriculography or 2-dimensional echocardiography, and if ischemic heart disease only was present; the presence of symptomatic CHF was not required for enrollment in the study.

Table I. Composition of MyoVive

Component	Amount per 250 mL
Energy (kcal)	200
Protein (g)	15
Carbohydrates (g)	17.7
Fat (g)	7.8
Carnitine (g)	3.0
Coenzyme Q10 (mg)	150
Taurine (g)	3.0
Creatine (g)	2.25
Sodium (mg)	108
Potassium (mg)	750
Chloride (mg)	203
Calcium (mg)	315
Phosphorus (mg)	183
Magnesium (mg)	20
Iron (mg)	1.0
Zinc (mg)	15
Copper (mg)	1.5
Manganese (mg)	3.0
Fluoride (mg)	1.0
Molybdenum (μg)	50
Selenium (μg)	50
Chromium (μg)	33
Iodine (μg)	100
Retinol ester (μg)	688
Cholecalciferol (μg)	5
α-Tocopherol acetate (mg)	538
Thiamin (mg)	25
Riboflavin (mg)	3.0
Niacin (mg)	20
Pantothenate (mg)	4.0
Pyridoxine (mg)	6.0
Folate (μg)	600
Cynocobalamin (μg)	3.0
Biotin (μg)	100
Ascorbate (mg)	250

Patients with significant valve disease and/or planned valve surgery, unstable blood pressure and/or heart rhythm, major comorbid disease, and patients taking supplements containing carnitine, taurine, and coenzyme Q10 were excluded from the study.

Consecutive outpatients fulfilling the criteria were enrolled from the St Michael's Hospital population of candidates for cardiovascular surgery from September 1999 to August 2000.

Supplement

A palatable drink, MyoVive (Numico Research, Zoetermeer, The Netherlands), which contains a mixture of carnitine, coenzyme Q10, and taurine, was given to patients receiving the supplement. The composition of the supplement is given in Table I. Although the supplement contains other components, these components did not influence the primary aim of the study, which was to determine if supplementation increased myocardial concentrations of taurine, carnitine, and coenzyme Q10. A placebo drink, containing carbohydrate, coloring, and flavoring in identical cartons, was provided for this trial, and an independent pharmacist dispensed the cartons through the hospital investigational drug service.

Protocol

At the time of enrollment, the patients had a baseline assessment of Canadian Cardiovascular Society (CCS) class angina, New York Heart Association (NYHA) class CHF, complete blood count, liver and renal function biochemistry, and radionuclide ventriculography. Patients were then randomly assigned to receive supplement or placebo in a 1:1 ratio at a dose of 250 mL per day for the duration of the study (until their 30- to 45-day visit after the procedure). Investigators and patients were unaware of the treatment. At the patients' routine preoperative visit, the assessment was repeated. During the operation, a single left ventricular cardiac muscle biopsy was taken and snap-frozen in liquid nitrogen. The biopsies were stored in liquid nitrogen until they were analyzed. The tolerability of the liquid supplement (supplement or placebo) was monitored throughout the study with biweekly telephone calls conducted by the study coordinator.

The primary end point was a comparison of the myocardial levels of taurine, carnitine, and coenzyme Q10 between placebo- and MyoVive-fed patients. The secondary end points were (1) the safety and tolerability of supplementation (assessed by biochemical measurements and symptomatic questionnaire) and (2) left ventricular end-diastolic (LVEDV) and end-systolic volume (LVESV) and EF as assessed by radionuclide ventriculography.

Method of radionuclide ventriculography

Left ventricular (LV) function was assessed with radionuclide ventriculography. Studies were acquired in the anterior, 45-degree left anterior oblique and 70-degree left anterior oblique with multigated acquisition of 32 frames per cardiac cycle after in vitro labeling of red blood cells with 30 mCi of technetium-99m. The technologist was unaware of treatment assignment. Global LVEF was calculated with the use of a semiautomated method for definition of end-diastolic and end-systolic regions, with calculation of background from the left paraventricular region of interest. LV activities were calculated from a region of interest manually drawn around the LV perimeter at end diastole. LV time-activity curves were generated from counts within the region of interest from the 32 frames of the summed cardiac cycle corrected for decay and attenuation. A 2-mL blood sample was withdrawn during the gated left anterior oblique image and counted on the camera for volume calculation. LV volumes were attenuation-corrected by taking a geometric measurement of the LV depth with a point source marker and the camera and applying an attenuation coefficient of 0.15/cm. Decay correction of the radioisotope was made on the basis of the law of radioactive decay. LV curve plotted from the LV region of interest of the gated left anterior oblique images yielded cardiac parameters, such as EDV and ESV.

Myocardial analysis for coenzyme Q10, taurine, and carnitine

These analyses have all been standardized before measurement, and their precision and accuracy were checked by performing repeated measurements and comparing the measurements with published data. The reproducibility of our biochemical analysis was found to be 8.3% for coenzyme Q10, 3% for taurine, and 7% for carnitine. In instances in which hamster data are not available, human data obtained in our laboratory are almost identical to published values.

Coenzyme Q10 levels

Myocardial biopsies were prepared for the determination of coenzyme Q10 concentration by use of high-performance liquid chromatography.[20]

Taurine

Taurine was analyzed by high-performance liquid chromatography with the pico-tag method.[21] Briefly, weighed tissue was homogenized in cold 0.1 N HCl. After a short centrifugation, the supernatant went through an ultrafiltration process. The filtrate was diluted 1:1 with methionine sulfone (internal standard). Twenty-five microliters of the resulting sample and known concentrations of taurine standard were dried in separate tubes. The samples were redried with a solution containing methanol, sodium acetate, and triethylamine. The dried material was derivatized with phenylisothiocyanate to produce phenylthiocarbamyl amino acids. These amino acid derivatives were analyzed by high-performance liquid chromatography with a specific Pico-Tag Column (Waters Co, Mississauga, Ontario, Canada) and a gradient system. The concentration of taurine was calculated from the peak area ratios of the sample and the taurine standard.

Carnitine

Carnitine was measured by spectrophotometric enzymatic assay, which measures the formation of 5-thio-2-nitrobenzoate from CoAsh and 5,5-dithiobis-2-nitrobenzoate in the presence of carnitine acetyl transferase.[22] The formed 5-thio-2-nitrobenzoate is proportional to the amount of carnitine present in the sample. Briefly, tissues were homogenized in cold high-performance liquid chromatography–grade water with a ground glass homogenizer. Free carnitine was determined by mixing fixed volumes of 1 M potassium hydroxide (KOH) in methanol, 10% phosphoric acid, and saturated potassium phosphate monobasic with a sample of the tissue homogenate. The mixture was spun down, and the supernatant was assayed for free carnitine.

Total carnitine was determined by mixing a sample of the tissue homogenate with alcoholic KOH. The mixture was heated for 1 hour at 65°C to hydrolyze the acylcarnitines. After cooling the sample to room temperature, 10% phosphoric acid and saturated potassium phosphate were added to the sample. The sample was spun down, and the supernatant was analyzed for total carnitine.

To analyze the samples for carnitine, the spectrophotometer was heated electronically to 37°C, and a cuvette was placed in the sample compartment to equilibrate to 37°C. A fixed volume of sample and reaction mixture was pipetted into the cuvette and incubated for 1 minute followed by the addition of carnitine acetyl transferase solution. The absorbance readings were taken at 412 nm and at fixed time intervals. A standard curve was prepared with different concentrations of L-carnitine, the same way as described above.

Statistical analysis

Continuous variable data are presented as means and the corresponding standard deviation, and the categoric data are

Table II. Baseline characteristics of patients

Variable	MyoVive (n = 20)	Placebo (n = 18)	P
Age (y)	62 ± 11	69 ± 5	.03
Male (%)	19 (90.5)	18 (100)	.99
Weight (kg)	87.4 ± 17.2	94.9 ± 36.4	.62
Height (cm)	171.2 ± 7.7	172.5 ± 5.9	.6
Cardiac history (%)			
Previous MI	15 (75)	16 (88.9)	.41
Previous thrombolysis	7 (38.9)	3 (17.7)	.26
Previous PTCA	3 (15)	3 (16.7)	.99
Previous CABG	0	1 (5.6)	.47
Valve disease	3 (15)	2 (11.1)	.99
Clinical history (%)			
Hypertension	15 (75)	10 (55.6)	.3
Diabetes	9 (45)	6 (33.3)	.52
Family history	15 (75)	8 (44.4)	.05
Current smoker	7 (35)	8 (47.1)	.44
Peripheral vascular disease	3 (16.7)	5 (29.4)	.44
Hyperlipidemia	14 (73.7)	11 (64.7)	.72
CCS class (%)			.38
I	2 (10)	4 (22.2)	
II	12 (60)	6 (33.3)	
III	5 (25)	7 (38.9)	
IV	1 (5)	1 (5.6)	
NYHA class (%)			.22
II	5 (25)	10 (50)	
III	12 (60)	7 (38.9)	
IV	3 (15.0)	1 (5.6)	
Radionuclide ventriculography	n = 19	n = 18	
Ejection fraction (%)	42.8 ± 12.2	44.6 ± 3.6	.69
End-diastolic volume (mL)	170.5 ± 50.0	178.9 ± 61.8	.78
End-systolic volume (mL)	99.8 ± 42.5	105 ± 55.9	.84
Medications (%)			
Angiotensin-converting enzyme inhibitor	15 (75)	10 (55.6)	.3
Aspirin	18 (90)	15 (83.3)	.65
β-Blocker	17 (85)	17 (94.4)	.6
Calcium-channel blocker	6 (30)	11 (61.1)	.1
Digoxin	7 (35)	0	.008
Diuretics	6 (30)	6 (33.3)	.99
Nitroglycerin	8 (40)	12 (66.7)	.11
Vitamin supplements	8 (40)	6 (33.3)	.74

presented as frequencies and percentages. Comparison between the 2 groups for continuous variables was performed with the nonparametric Wilcoxon rank sum test. Comparison between the 2 treatment groups for categoric variables was performed with the Pearson χ^2 test or the Fisher exact test.

Results

Fifty-three patients were approached for the study. Twelve refused to participate—4 because it was too difficult to travel to the hospital and 8 because they did not want to be randomly assigned to receive the placebo. Forty-one patients were recruited from St Michael's Hospital. At baseline, the patients were well matched. Minor exceptions included greater digoxin use in the MyoVive group and younger age (Table II). The compliance was 93% and 99% (P = .0104) for the MyoVive and placebo groups, respectively. The number of days patients took the supplement at the preoperative visit was 29.7 ± 10.2 days and 30.2 ± 9.6 days (P = not significant [NS]) for the MyoVive and placebo groups, respectively. The biopsies were conducted after 34.1 ± 12.7 days and 34.9 ± 8.4 days (P = NS) of supplementation in the MyoVive and placebo groups, respectively.

After supplementation, the MyoVive group had a significantly higher myocardial content of coenzyme Q10, taurine, and total carnitine, by 144%, 66%, and 40%, respectively, compared with the placebo group (Table III). The mean LVEDV in the MyoVive group fell by a significant amount from the baseline to the preoperative assessment (170.5 ± 50 mL vs 158.9 ± 51 mL; $P < .05$) (Table IV), and the paired data also showed a significant reduction in the preoperative LVEDV compared with placebo (−7.5 ± 22 mL vs

Table III. Heart muscle biopsy results

Variable	MyoVive	Placebo	P
Total carnitine (nmol/g wet weight)	1735.4 ± 798.5	1237.6 ± 343.1	.0569
Coenzyme Q10 (nmol/g wet weight)	138.17 ± 39.87	56.67 ± 23.08	.0006
Taurine (μmol/g wet weight)	13.12 ± 4.00	7.91 ± 2.81	.0016

Table IV. Radionuclide ventriculography results

	MyoVive			Placebo		
Variable	Baseline (n = 19)	Preoperative (n = 19)	Change prebaseline (paired data)	Baseline (n = 18)	Preoperative (n = 17)	Change prebaseline (paired data)
EF (%)						
Mean ± SD	42.8 ± 12.2	43.7 ± 12.5	0.9 ± 4.7	44.6 ± 13.6	46.7 ± 13.4	1.6 ± 4.0
Median (Q1, Q3)	43 (31, 53)	46 (33, 56)	0.0 (−3, 5)	44.5 (36, 56)	47 (42, 57)	2 (−1, 4)
Min to max	26-59	22-60	−7-7	19-70	23-70	−5-8
EDV (mL)						
Mean ± SD	170.5 ± 50	158.9 ± 51*	−7.5 ± 22†	178.9 ± 61.8	179.9 ± 52.8	10 ± 19.8
Median (Q1, Q3)	161 (140, 206)	158.5 (126, 171)	−7.5 (−18, 5)	172 (117, 224)	168 (145, 220.5)	8.5 (−8.5, 28)
Min to max	98-306	79-295	−48, 43	88-306	101-293	−17-40
ESV (mL)						
Mean ± SD	99.8 ± 42.5	94.2 ± 46.4	−4.7 ± 18.1	105 ± 55.9	101 ± 52.7	1.4 ± 17.2
Median (Q1, Q3)	91.2 (61.7, 124)	78.1 (64.7, 132)	−5.4 (−14, 4)	105 (64, 138)	88.3 (63, 121.4)	−0.5 (−14, 4)
Min to max	48-211	43.6-203.6	−39.3-40.8	26.4-247.9	33.9-225.6	−22.3-29.4

*$P < .05$ vs baseline.
†$P < .05$ vs placebo.

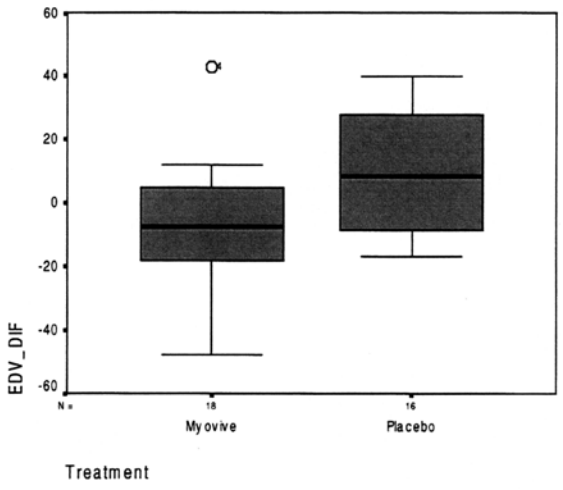
10 ± 19.8 mL; $P < .05$) (Figure 1). The difference between the baseline and preoperative LVEDV in the MyoVive group was still significant ($P = .0311$) when adjusted for calcium-channel blockers and angiotensin-converting enzyme inhibitors. In the placebo group, there was no significant change in the mean LVEDV from baseline to the preoperative assessment (178.9 ± 61.8 mL vs 179.9 ± 52.8 mL) (Table IV). There was a trend toward a reduction in the mean LVESV in the MyoVive-fed patients from baseline to the preoperative assessment (99.8 ± 42.5 mL vs 94.2 ± 46.4 mL) (Table IV), and the paired data also showed a trend toward a reduction compared with placebo (−4.7 ± 18.1 mL vs 1.4 ± 17.2 mL) (Figure 2). In the placebo group, there was no significant difference in the mean data from baseline LVESV to the preoperative assessment (105 ± 55.9 mL vs 101 ± 52.7 mL) (Table IV). There was no significant change in the EF in MyoVive and placebo groups, and the paired measurements between the MyoVive and placebo groups were not different (Figure 3 and Table IV).

One patient in the MyoVive group had nausea and 1 had a single episode of vomiting. Two patients developed diarrhea and 1 of them dropped out of the study as a result. None of the other patients dropped out because of these symptoms. None of the patients in the placebo group had any adverse symptoms (Table V). The difference between groups was not significant.

The administration of supplement compared with placebo did not influence blood biochemistry, with the exception of significantly higher creatinine levels at the preoperative assessment. However, the blood urea nitrogen was not increased (Table VI).

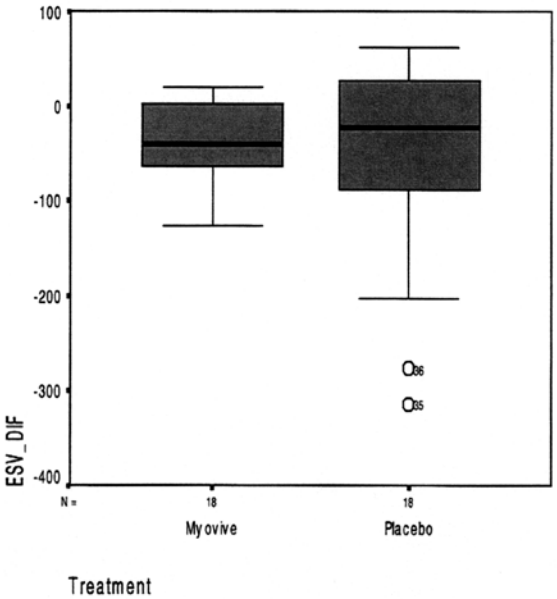
Clinically significant adverse events were few. One patient from each group had a preoperative myocardial infarction; both of these patients went on to surgery successfully. One patient who had a body mass index of 42 in the placebo group developed sepsis after the operation and spent 3 weeks in the intensive care unit before being transferred to the floor. One patient in the MyoVive group developed pneumonia and had a 6-day stay in the intensive care unit. Clinically significant renal failure developed after surgery in 2 of the patients in the placebo group, both secondary to retention caused by prostate obstruction. In the MyoVive group, 3 patients developed clinically significant renal failure, 1 patient had renal artery stenosis, 1 patient had renal failure related to diabetes, and the final patient had multiple medical problems after the

Figure 1



Box-whisker plot of the paired difference between baseline and preoperative left ventricular end-diastolic volume (*EDV DIF*) for the MyoVive group (−7.5 ± 22 mL) and the placebo group (10 ± 19.8 mL; $P < .05$).

Figure 2



Box-whisker plot of the paired difference between baseline and preoperative left ventricular end-systolic volume (*ESV DIF*) for the MyoVive group (−4.7 ± 18.1 mL) and the placebo group (1.4 ± 17.2 mL; P = NS).

operation with a wound infection, urinary tract infection, and atrial fibrillation.

Only 1 patient was lost to observation (MyoVive group). Two patients died during the study, 1 from each group: the patient in the MyoVive group developed coagulopathy after the operation, and the patient in the placebo group developed severe mitral regurgitation, which was complicated by cardiogenic shock and CHF, after the operation and died during a second operation. Four patients dropped out of the study, 2 from each group. Of the 2 patients in the MyoVive group, 1 dropped out after developing diarrhea and the other patient dropped out after developing renal failure caused by renal artery stenosis. Of the patients who dropped out of the placebo group, 1 dropped out after family members had concerns about the patient being enrolled in the study and the other patient dropped out after developing atrial fibrillation after the operation.

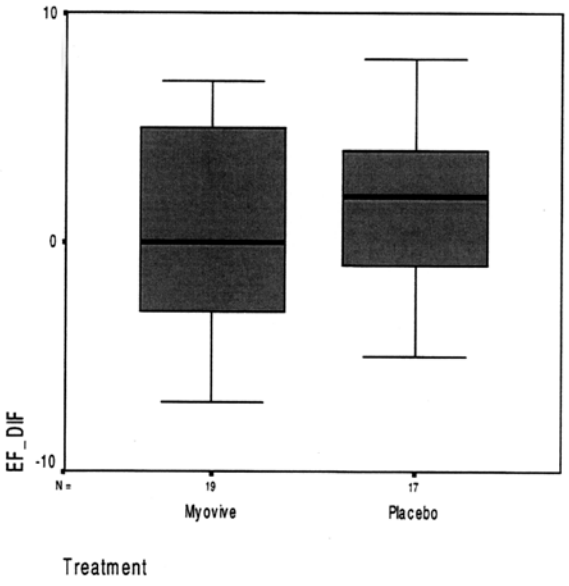
Discussion

The patients were selected on the basis of the LV function assessed by either cardiac catheterization with LV angiogram or echocardiography conducted by the referring institution. Our baseline radionuclide angiography results indicated that the average EF was 42.8% ± 12.2% and 44.6% ± 13.6% (P = NS) for the MyoVive and placebo groups, respectively. It is accepted that radionuclide ventriculography results often show a higher EF than echocardiography and LV angiogram. In any case, the groups were well matched for baseline EF.

Overall, the patients tolerated MyoVive well and compliance was better than in most studies. There was a significant increase in creatinine in the MyoVive group from the baseline assessment to the preoperative assessment. However, there was no rise in blood urea nitrogen. In our study, there was no clinical significance to this rise in creatinine, and the rise in creatinine did not affect the outcome in the patients who were given MyoVive. In MyoVive, 50% of the creatine is in the form of creatinine, which can raise blood creatinine levels without a change in creatinine clearance. However, measuring the creatinine clearance in future studies with MyoVive will be important to confirm this hypothesis.

The physiologic functions of coenzyme Q10, carnitine, and taurine have been well described. Ubiquinone or coenzyme Q10 plays a pivotal role as a rate-limiting carrier for the flow of electrons through the first stages of the mitochondrial respiratory chain and is an important endogenous antioxidant.[23] Taurine is a unique amino acid that has no role as a component of

Figure 3



Box-whisker plot of the paired difference between baseline and preoperative ejection fraction (*EF DIF*) for the MyoVive group (0.9 ± 4.7 mL) and the placebo group (1.6 ± 4.0 mL; *P* = NS).

Table V. Tolerability data

	Preoperative		
Variable	**MyoVive**	**Placebo**	***P***
Adverse symptoms (%)			
Cramps	0	0	–
Diarrhea	2 (9.5)	0	.4899
Fullness	0	0	–
Nausea	1 (4.8)	0	.5385
Reflux	0	0	–
Vomiting	1 (4.8)	0	.5385
Total	4	0	.0519

protein synthesis or as a substrate for metabolism; it is, however, the most plentiful amino acid in the myocyte, and it plays a critical role in intracellular calcium homeostasis.[24] Carnitine is essential for long-chain fatty acid transport from the cytoplasm to the mitochondrial matrix; it also plays an important role in the balance between glycolysis and glucose oxidation.[25] Therefore, these nutrients are essential for normal cell and mitochondrial function and calcium homeostasis. This study has shown that feeding patients a mixture of carnitine, coenzyme Q10, and taurine resulted in higher myocardial levels of these components. To date, the only available data on supplementation and the subsequent increase in myocardial levels are with coenzyme Q10.[9] A study of endomyocardial biopsies taken from patients with predominantly dilated cardiomyopathy measured coenzyme Q10 levels.[9] Five of the 43 patients in this study subsequently received supplementation with coenzyme Q10 and underwent biopsy again.[9] The results showed a 20% to 85% increase of myocardial coenzyme Q10.[9] Our study is the only randomized, double-blinded, placebo-controlled trial that has been conducted specifically to demonstrate that supplemen-tation with carnitine, coenzyme Q10, and taurine results in higher myocardial levels of these components. The finding that feeding these components, which have a potential to improve myocardial function, results in higher myocardial levels is important. If myocardial levels were not higher with oral supplements, then the validity of such supplements could be questioned.

In addition to the increases in myocardial levels of carnitine, coenzyme Q10, and taurine, an improvement in LV dimensions in the form of a significantly reduced LVEDV and a trend toward a smaller LVESV was noted. These changes are important because LVEDV has been shown to be an independent prognostic factor in patients with advanced heart failure.[26] In addition, the risk of death in patients undergoing surgical revascularization[27] is related to the preoperative LVEDV. Therefore, a preoperative reduction of the LVEDV could potentially reduce the risk of surgical revascularization. Furthermore, a reduction in LV volume has resulted in improved prognosis in several drug trials in patients with heart failure.[28-31] The mechanistic question is whether one or more of carnitine, coenzyme Q10, or taurine improve function, or the action of other constituents in MyoVive improves function. As previously mentioned, on the basis of previous observations, protein-calories and the standard vitamin-micronutrients in this formulation should not influence cardiac function. MyoVive also contains creatine, but this constituent, although improving skeletal muscle function, does not improve cardiac function in patients with CHF[32] or in animals.[33] However, larger studies are required to determine whether creatine supplementation does not have an effect on cardiac function. Finally, MyoVive contains a high dose of vitamin E. However, a recent controlled clinical trial of vitamin E supplementation found no reduction in oxidative stress in patients with heart failure.[34] Therefore, it is likely that improvement in function can be ascribed to the independent or synergistic action of 1 or more of carnitine, coenzyme Q10, or taurine, and not to the other constituents of MyoVive. A possible physiologic basis for these data is the recent observation (unpublished data) that the action of a combination of

Table VI. Safety data

	Baseline			Preoperative		
Variable	**MyoVive**	**Placebo**	***P***	**MyoVive**	**Placebo**	***P***
ALP (μ/L)	77.2 ± 17.2	81.3 ± 25.8	.9883	78.5 ± 20.4	78.9 ± 22.5	.8367
ALT (μ/L)	33.1 ± 15.5	27.1 ± 9.8	.3036	29 ± 9.3	26.6 ± 10	.3485
AST (μ/L)	26.0 ± 11.0	25.4 ± 6.6	.7261	26.8 ± 15.4	25.3 ± 8.7	.8306
BUN (mmol/L)	7.2 ± 3.3	7.1 ± 2.0	.7065	7.2 ± 2.9	7.3 ± 2.7	.6189
Creatinine (mmol/L)	108.1 ± 37.6	101.4 ± 22	.9442	153.3 ± 60.1	108.2 ± 28.7	.0196

taurine, carnitine, and coenzyme Q10 in vitro on isolated mitochondria from cardiomyopic hamsters is similar to that seen with β-blockers.[35] Perhaps it is the reduction in the rate of oxygen consumption, which results from this combination of nutrients, that subsequently causes a match between limited oxygen delivery caused by ischemia and the consumption rate. This so-called flow match created by MyoVive supplementation would benefit the ischemic patient and result in improved cardiac function.

The potential limitations of our study are the small sample size and short duration of observation. Our hypothesis would need to be confirmed in larger studies with a longer follow-up period.

In summary, oral supplementation of carnitine, taurine, and coenzyme Q10 results in higher myocardial levels of these constituents, which are known to influence myocardial function. In addition, there is a reduction of LVEDV, which is an important marker of prognosis in a variety of cardiac conditions. The findings of this study support the potential role of these components in the management of patients with ventricular dysfunction. Larger clinical trials of these supplements need to be performed to evaluate their effect in improving cardiac function and outcome.

References

1. Freeman L, Roubenoff R. The nutrition implications of cardiac cachexia. Nutr Rev 1994;52:340-7.
2. Pittman JG, Cohen P. The pathogenesis of cardiac cachexia. N Engl J Med 1964;271:403-9.
3. Anker SD, Ponikowski P, Varney S, et al. Wasting as independent risk factor for mortality in chronic heart failure. Lancet 1997;349: 1050-3.
4. Heymsfield SB, Casper K. Congestive heart failure: clinical management by use of continuous nasogastric feeding. Am J Clin Nutr 1989;50:539-44.
5. Broqvist M, Arnqvist H, Dahlstrom U, et al. Nutritional assessment and muscle energy metabolism in severe chronic congestive heart failure: effects of long-term dietary supplementation. Eur Heart J 1994;15:1641-50.
6. Quigley AF, Kapsa RMI, Esmore D, et al. Mitochondrial respiratory chain activity in idiopathic dilated cardiomyopathy. J Card Fail 2000;6:47-55.
7. Regitz V, Shug AL, Fleck E. Defective myocardial carnitine metabolism in congestive heart failure secondary to dilated cardiomyopathy and coronary, hypertensive and valvular heart diseases. Am J Cardiol 1990;65:755-60.
8. Masumura Y, Kobayashi A, Yamazaki N. Myocardial free carnitine and fatty acylcarnitine levels in patients with chronic heart failure. Jpn Circ J 1990;54:1471-6.
9. Folkers K, Vadhanavikit S, Mortensen SA. Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q10. Proc Natl Acad Sci USA 1985;82: 901-4.
10. Folkers K. Heart failure is a dominant deficiency of coenzyme Q10 and challenges for future clinical research on CoQ10. Clin Invest Med 1993;71:S51-4.
11. Suleiman MS, Fenando HC, Dihmis WC. A loss of taurine and other amino acids from ventricles of patients undergoing bypass surgery. Br Heart J 1993;69:241-5.
12. Jeejeebhoy KN, Sole MJ. Nutrition and the heart. Clin Nutr. 2001; 20(1 Suppl):181-6.
13. Rizos I. Three-year survival of patients with heart failure caused by dilated cardiomyopathy and L-carnitine administration. Am Heart J 2000;139:S130-3.
14. Morisco C, Trimarco B, Condorelli M. Effect of coenzyme Q10 therapy in patients with congestive heart failure: a long-term multicenter randomized study. Clin Invest Med 1993;71:S134-6.
15. Iliceto S, Scrutinio D, Bruzzi P, et al. Effects of L-carnitine administration on left ventricular remodeling after acute anterior myocardial infarction: the L-carnitine Ecocardiografia Digitalizzata Infarto Miocardico (CEDIM) Trial. J Am Coll Cardiol 1995;26:380-7.
16. Hofman-Bang C, Rehnqvist N, Swedberg K, et al. Coenzyme Q10 as an adjunctive in the treatment of chronic congestive heart failure. The Q10 Study Group. J Card Fail 1995;1:101-7.
17. Khatta M, Alexander BS, Krichten CM, et al. The effect of coenzyme Q10 in patients with congestive heart failure. Ann Intern Med 2000;132:636-40.
18. Azari J, Brumbaugh P, Barbeau A, et al. Taurine decreases lesion severity in the hearts of cardiomyopathic hamsters. Can J Neurol Sci 1980;7:435-40.
19. Witte KK, Clark AL, Cleland JG. Chronic heart failure and micronutrients. J Am Coll Cardiol 2001;37:1765-74.
20. Okamoto T, Fukui K, Nakamoto M, et al. High performance liquid chromatography of coenzyme Q-related compounds and its application to biological materials. J Chromatogr 1985;342:35-46.
21. Cohen SA, Strydom DJ. Amino acid analysis utilizing phenylisothiocyanate derivatives. Anal Biochem 1988;174:1-16.
22. Maeda J, Dudrick SJ. Rapid spectrophotometric determination of

plasma carnitine concentrations. J Parenter Enteral Nutr 1990;14: 527-32.
23. Littaru GP. Energy and defense. In: Facts and perspectives on coenzyme Q10 in biology and medicine. Rome: Casa Editrice Scientifica Internazionale; 1995. p. 1-91.
24. Azuma J, Swanamura A, Awata N. Usefulness of taurine in chronic congestive heart failure and its prospective application. Jpn Circ J 1992;56:95-9.
25. Lopaschuk GD, Belke DD, Gamble J, et al. Regulation of fatty acid oxidation in the mammalian heart in health and disease. Biochim Biophys Acta 1994;1213:263-76.
26. Koelling TM, Semigran MJ, Mijller-Ehmsen J, et al. Left ventricular end-diastolic volume index, age, and maximum heart rate at peak exercise predict survival in patients referred for heart transplantation. J Heart Lung Transplant 1998;17:278-87.
27. De Carlo M, Milano A, Borzoni G, et al. Predicting outcome after myocardial revascularization in patients with left ventricular dysfunction. Cardiovasc Surg 1998;6:58-66.
28. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling-concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. J Am Coll Cardiol 2000;35:569-82.
29. Cohn JN, Johnson G, Ziesche S, et al. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. N Engl J Med 1991;325:303-10.
30. Cohn JN, Johnson G, Zshabetai R, et al. Ejection fraction, peak exercise oxygen consumption, cardiothoracic ratio, ventricular arrhythmias and plasma norepinephrine as determinants of prognosis in heart failure. Circulation 1993;87(6 Suppl):5-16.
31. Pfeffer MA, Braunwald E, Moye LA, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the Survival and Ventricular Enlargement Trial. N Engl J Med 1992;327:669-77.
32. Gordon A, Hultman E, Kaijser L, et al. Creatine supplementation in chronic heart failure increases skeletal muscle creatine phosphate and muscle performance. Cardiovasc Res 1995;30:413-8.
33. Horn M, Remkes H, Dienesch C, et al. Chronic high-dose creatine does not attenuate left ventricular remodeling in rat hearts post-myocardial infarction. Cardiovasc Res 1999;43:117-24.
34. Keith M, Jeejeebhoy KN, Langer A, et al. A controlled clinical trial of vitamin E supplementation in patients with congestive cardiac failure. Am J Clin Nutr 2001;73:219-24.
35. Katyare SS, Rajan RR. Altered energy coupling in rat heart mitochondria following in vivo treatment with propanolol. Biochem Pharmacol 1991;42:617-23.

The following article is an AHJ Online Exclusive.
Full text of this article is available at no charge at our website:
www.mosby.com/ahj.

No variants in the cardiac actin gene in Finnish patients with dilated or hypertrophic cardiomyopathy

Satu Kärkkäinen, MD, Keijo Peuhkurinen, MD, Pertti Jääskeläinen, MD, Raija Miettinen, MSc, Päivi Kärkkäinen, MSc, Johanna Kuusisto, MD, and Markku Laakso, MD ***Kuopio, Finland***

Background Dilated and hypertrophic cardiomyopathies are primary myocardial diseases that cause considerable morbidity and mortality. Although these cardiomyopathies are clinically heterogeneous, genetic factors play an important role in their etiology and pathogenesis. The defects in the cardiac actin (ACTC) gene can cause both cardiomyopathies. The aim of our study was to screen for variants in the ACTC gene in patients with dilated or hypertrophic cardiomyopathy from Eastern Finland.

Materials and Methods Altogether, 32 patients with dilated and 40 patients with hypertrophic cardiomyopathy were included in the study. Commonly approved diagnostic criteria were applied, and secondary cardiomyopathies were carefully excluded. All 6 exons of the ACTC gene were amplified with polymerase chain reaction and screened for variants with single-strand conformation polymorphism analysis.

Results and Conclusion We did not find any new or previously reported variants. Our results indicate that defects in the ACTC gene do not explain dilated cardiomyopathy or hypertrophic cardiomyopathy in subjects from Eastern Finland and confirm earlier results that the ACTC gene does not play an important role in the genetics of dilated or hypertrophic cardiomyopathies. (Am Heart J 2002;143:e6.)

4/90/122514

SPECIAL COMMENTARY

Conditioned nutritional requirements and the pathogenesis and treatment of myocardial failure

Michael J. Sole and Khursheed N. Jeejeebhoy

The majority of symptomatic patients with congestive heart failure have been shown to be significantly malnourished. Myocardial and skeletal muscle energy reserves are also diminished. Total daily energy expenditure in these patients is less than that in control individuals, and high protein–calorie feeds do not reverse the abnormalities; thus, the wasting that occurs in patients with congestive heart failure is metabolic rather than because of negative protein–calorie balance. Several specific deficiencies have been found in the failing myocardium: a reduction in the content of L-carnitine, coenzyme Q_{10}, creatine and thiamine, nutrient cofactors that are important for myocardial energy production; a relative deficiency of taurine, an amino acid that is integral to the modulation of intracellular calcium levels; and an increase in myocardial oxidative stress, and a reduction of both endogenous and exogenous antioxidant defences. In addition, these processes may influence skeletal muscle metabolism and function. Cellular nutritional requirements conditioned by metabolic abnormalities in heart failure are important considerations in the pathogenesis of the skeletal and cardiac muscle dysfunction. A comprehensive restoration of adequate myocyte nutrition would seem to be essential to any therapeutic strategy designed to benefit patients suffering from this disease. Curr Opin Clin Nutr Metab Care 3:417–424.

Divisions of Cardiology and Gastroenterology, Department of Medicine, University of Toronto, Toronto, Ontario, Canada

Correspondence to Khursheed N. Jeejeebhoy, 3-035 Queen Wing, St. Michael's Hospital, 30 Bond Street, Toronto, ON M5B 1W8, Canada
Tel: +416 864 5388; fax: +416 864 5882; e-mail: khush.jeejeebhoy@utoronto.ca

Current Opinion in Clinical Nutrition and Metabolic Care 2000, 3:417–424

Abbreviations

ADP	adenosine diphosphate
ATP	adenosine triphosphate
CoQ_{10}	coenzyme Q_{10}
PCr	creatine phosphate

1363-1950

Introduction

Congestive heart failure has emerged as a major health problem during the past 3 decades [1,2]. In spite of our advances, no presently available therapeutic intervention has been shown to improve substantially the long-term survival of patients who suffer from this disease. The modern pharmacological therapy of heart failure has focused on the amelioration of fluid overload, haemodynamic abnormalities and inappropriate neurohormonal stimulation. Attention is now being turned to the evaluation and management of aetiological factors that lead to progressive, long-term myocardial damage, particularly factors that result in myocyte loss or dysfunction [3].

Energy demands are increased and energy production is diminished in the overloaded myocytes of the failing heart [4]. Whether basal myocardial function is impaired by a chronic energy deficit is controversial; however, it has become clear that energy reserve is markedly limited [5,6]. Several metabolic abnormalities are important contributors to the energy deficit and the pathogenesis of myocyte deterioration. In particular, there is a gradual accumulation of calcium in myocytes, which results in mitochondrial calcium overload, decreased myocyte energy production, increased oxidative stress and protease activation; this cascade culminates in myocyte dysfunction and death.

These processes may also adversely impact on skeletal muscle metabolism and function, which are the primary determinants of functional capacity of patients with chronic heart failure [7]. Muscle wasting or cachexia is common; 50–68% of patients with heart failure have been reported in surveys to be malnourished [8,9].

Although metabolic rate is commonly regarded as increased in patients with heart failure, recent studies [10] have shown that daily energy expenditure is decreased, particularly in cachectic patients, suggesting that hypermetabolism cannot account for the cachexia. In addition, a recent controlled trial [11] showed that high protein–calorie feeds did not reverse the abnormalities in skeletal muscle observed in patients with severe congestive heart failure. Thus, the wasting and malnutrition in patients with congestive heart failure is metabolic rather than due to negative protein–calorie

balance. There also appears to be a significant correlation between the loss of lean body mass in these patients and increases in circulating catabolic neuroendocrine factors, such as the catecholamines and cortisol, and cytokines [12]. These findings indicate that correction of malnutrition in patients with cardiac failure is not simply a matter of feeding protein and energy; it also depends on correcting metabolic abnormalities that interfere with the maintenance of cardiac and skeletal muscle structure and function.

Nutrient factors that influence myocardial energetics

Myocardial energy production is aerobic and dependent on a continual and adequate flow of nutrients. Attainment of this metabolic need requires the assistance of a number of cofactors. Reductions in the levels of these [i.e. carnitine, which is critical for the transport of long-chain fatty acid substrate and the maintenance of adequate glucose oxidation; coenzyme Q_{10} (CoQ_{10}), which is critical for the transport of electrons through the initial portion of the mitochondrial respiratory chain and is an important endogenous antioxidant; thiamine, which is important as a coenzyme in carbohydrate metabolism; and creatine, which is important as a storage reservoir and shuttle of high energy phosphate] have been described in heart failure.

L-Carnitine

Carnitine and metabolism

L-Carnitine, an amino-acid derivative (3-hydroxy-4-*N*-trimethylaminobutyric acid), is essential for the transport of long-chain fatty acids from the cytoplasm into the sites of β-oxidation within the mitochondrial matrix [13]. In addition to promoting the entry of fat into the mitochondria, carnitine binds acyl groups and releases free coenzyme A. This benefits the myocyte by removing toxic, short-chain acyl groups to form acylcarnitines, which can freely diffuse out of the cell and be eliminated through the urine. Carnitine also indirectly activates pyruvate dehydrogenase, the rate-limiting enzyme for glucose oxidation [13,14]; this in turn improves the coupling between glycolysis and glucose oxidation, reducing the lactate and hydrogen burden on the myocyte.

Body stores of L-carnitine are supplied by both diet and via endogenous biosynthesis from trimethyllysine. The concentration of carnitine in normal adult cardiac and skeletal muscle is approximately 8–15 nmol/mg noncollagen protein; plasma levels are approximately 35–50 μmol/l. Thus, plasma levels are not a good measure of tissue concentrations. A 20–50:1 intracellular:extracellular carnitine gradient is maintained by a sodium-dependent plasma membrane transport system. Carnitine transport can be stimulated by β-adrenergic agonists or dibutryl-cyclic adenosine monophosphate. After oral administration, peak plasma concentration occurs at 3 h and decays with a half-time of 3–4 h. The turnover of endogenous muscle carnitine has not been evaluated in humans, but data from the rat suggest that it is in the order of several days [15].

Carnitine and heart failure

Genetically determined carnitine deficiency is associated with the development of cardiomyopathy and skeletal muscle dysfunction [16]; this can be ameliorated by L-carnitine administration. Evaluation of carnitine metabolism in several cardiac pathologies has led to the realization that carnitine deficiency may also be acquired and organ selective. Failing myocardium generally exhibits a marked depletion (up to 50%) of both free and total carnitine [13,15].

The administration of L-carnitine (3–6 g in divided doses) or of one of its analogues (e.g. proprionyl-carnitine) has been reported [13,15,17] to result in haemodynamic improvement and an overall benefit in the functional capacity of animals and patients with myocardial dysfunction. A recent multicentre, randomized, placebo-controlled, double-blind clinical trial [18] showed a significant beneficial effect, including a reduction in adverse cardiac remodelling, when L-carnitine is taken for 12 months after myocardial infarction.

Ubiquinone or coenzyme Q_{10}

Ubiquinone and metabolism

CoQ_{10} or ubiquinone (2,3-dimethoxy-5-methyl-6-decaprenyl benzoquinone) plays a vital role as a rate-limiting carrier for the flow of electrons through complexes I, II and III of the mitochondrial respiratory chain. It is also a major endogenous lipophilic antioxidant. The molecule is sited within the inner mitochondrial membrane, but it is also associated with the membranes of other intracellular organelles, where it is important for the maintenance of redox activity [19] and electron flow across membranes [20]. The importance of membrane effects are supported by studies that have shown that incubation of hepatocytes with adriamycin induces loss of respiration and mitochondrial potential, but concomitant incubation with CoQ_{10} completely protects both respiration and potential despite the fact that there is no cell uptake of CoQ_{10} [21]. Finally, it is also an important component of circulating low-density lipoprotein particles, protecting low-density lipoprotein from oxidation [22].

Ubiquinone is actively biosynthesized with the cells. The quinone ring is synthesized from the amino acid tyrosine and the polyisoprenoid side chain is formed through the acetyl coenzyme A–mevalonate pathway. The latter pathway is under the control of the enzyme

hydroxymethylglutaryl coenzyme A reductase, which is also used for cholesterol synthesis [22]. Inhibition of this pathway using hydroxymethylglutaryl coenzyme A reductase inhibitors, drugs that decrease plasma cholesterol, also results in a parallel decrease in plasma ubiquinone [22,23] and may also reduce tissue ubiquinone levels [24].

Ubiquinone is widespread throughout all food groups, and thus body stores may also be partially supplied by diet. The concentration of ubiquinone in normal cardiac muscle is approximately 0.4–0.5 μg/mg dry weight, slightly less in skeletal muscle and 0.6–1.3 μg/ml in plasma. Oral absorption is slow and markedly enhanced in the presence of lipid; plasma levels peak at 5–10 h and decay with a half-time of 34 h [25]. There is a large hepatic first pass effect so that only 2–5% of an oral dose is taken up by the myocardium. Available formulations show a very broad range of absorption.

Coenzyme Q_{10} and heart failure

Because the heart depends on aerobic oxidation for its energy needs, CoQ_{10}, which is critically necessary for oxidative energy production, should be very important for cardiac function. Unfortunately the role of CoQ_{10} in cardiac disease is indirect and unclear. Significantly reduced (up to 50%) levels of myocardial ubiquinone are well documented in heart failure both in animal models and humans [26,27], but it is not clear whether the degree of reduction impairs mitochondrial oxidation or increases cardiac damage through increased oxidative stress or alterations in myocyte membrane structure and function.

Oral CoQ_{10} therapy has been reported [28,29] to affect beneficially the course of heart disease in a wide variety of animal paradigms. The results of trials in patients with heart failure [30–33] are mixed. A meta-analysis of published reports (up to 1997) [34] supports a haemodynamic benefit. The studies that support a benefit [30,31] are small or have significant methodological problems. The negative studies [32,33] utilized doses of CoQ_{10} of 100 mg/day or less; it has been suggested that at least 150–200 mg/day of a highly bioavailable formulation may be required. The data currently available do not warrant the claims of proved benefit that are being disseminated by advocates of CoQ_{10}.

Thiamine

Thiamine and metabolism

Thiamine, or vitamin B_1, status may be compromised in heart failure because of a variety of causes. Thiamine is a water-soluble vitamin that functions as a coenzyme in a variety of enzyme systems, especially those that are related to carbohydrate energy metabolism. Thiamine is stored in very small quantities; thus thiamine requirements, which are related to daily energy expenditure and carbohydrate intake, must be met daily. Therefore, patients with poor intakes may be at increased risk for deficiency during acute illness [35]. Necropsy studies [36] indicated that thiamine deficiency is underdiagnosed in life. The classical deficiency signs of beri beri are often absent or not recognized.

Thiamine and heart failure

The potential for thiamine deficiency to contribute to myocyte loss and the evolution of heart failure may be gleaned from the literature that pertains to the central nervous system. Hakim and coworkers [37,38] showed that thiamine deficiency in rats results in a decrease in glucose utilization, focal acidosis, loss of calcium homeostasis, upregulation of the tumor-suppressor p53 (an important component of the apoptosis signalling pathway) and initiation of apoptosis in the brain.

Thiamine intake in patients with heart disease has been examined in only one study [39], which used a semiquantitative food frequency questionnaire focusing on foods that are high in thiamine. Nutrient analysis indicated a low overall intake of thiamine of 0.966 mg/day, with 33% of patients not meeting the recommended dietary allowance of 0.8 mg/day for thiamine.

There is evidence from both animal and human studies [40,41] that the use of diuretics, especially loop diuretics such as frusemide, causes increased urinary losses of thiamine, adversely affecting thiamine status. The incidence of thiamine deficiency in patients with heart failure is reported to be between 13 and 91%, depending on the dose of frusemide and the population studied [39,41,42].

Thiamine supplementation is reported to be of benefit in patients with heart failure, but the trials that evaluate this therapy are small and methodologically marginal [41,43]. Further prospective studies in patients with heart failure, documenting both the significance of thiamine deficiency and the possible benefits of treatment, are needed.

Creatine

Creatine and metabolism

Creatine phosphate (PCr) is the primary high-energy phosphate reservoir of the heart and skeletal muscle. Phosphocreatine is generated in the mitochondria and diffuses to the myofibrils; high-energy phosphate is transferred from PCr to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) through catalysis by creatine kinase:

$$\text{PCr} + \text{ADP} + \text{hydrogen} \leftrightarrow \text{ATP} + \text{creatine}$$

Muscle creatine stores are maintained through biosynthesis from endogenous precursors (arginine, glycine, and

methionine in the liver, pancreas and kidneys) and through the ingestion of meat and fish. The concentration of total creatine in normal adult human myocardium or skeletal muscle is approximately 140 μmol/g protein; creatine phosphate constitutes about 65–80% of the total creatine under aerobic conditions [44]. Creatine is accumulated by muscle against a large concentration gradient from the blood; the transporter is probably driven by the extracellular/intracellular sodium electrochemical potential. There is evidence that increased adrenergic drive (a characteristic of heart failure) can decrease myocardial creatine and creatine kinase [45].

Creatine and heart failure

Creatine has been shown [46] to directly benefit calcium homeostasis and survival of dystrophic myocytes in culture. Conversely, creatine depletion in animals results in structural, metabolic and functional abnormalities in muscle [44]. Myocardial and, to a lesser degree, skeletal muscle creatine content is reduced with the expected concomitant reduction in PCr in a wide variety of animal paradigms and humans with heart failure [5,6,45,47]. Recently, it was reported [47] that the myocardial PCr:ATP ratio may be a better predictor of patient mortality in dilated cardiomyopathy than left ventricular ejection fraction or the patient's functional class. This ratio also correlates well with myocardial function in patients with mitral insufficiency [48]. It should be noted, however, that this ratio is not only related to cardiac creatine levels, but is also dependent on ATP synthesis rate, an index of mitochondrial function.

Creatine supplements will increase skeletal muscle creatine, but the benefit of increased muscle creatine is only seen as a resistance to fatigue during short-term intense exercise, where it reduces lactate accumulation [49,50]. The role of creatine supplementation may therefore not be observed in cardiac muscle under normal levels of performance. The short-term administration of creatine supplements to patients with heart failure does not appear to increase cardiac ejection fraction, but is reported to increase skeletal muscle PCr with small but significant improvements in muscle strength, endurance and metabolism, relative to placebo-treated control patients [51,52]. These latter trials were of short duration and did not provide an adequate assessment of the effect (if any) of creatine supplementation on the failing heart.

Regulating intracellular calcium

The failing myocardium exhibits an increase in calcium content and impaired movement of intracellular calcium. The activity of pyruvate dehydrogenase and therefore glucose oxidation is sensitive to cell calcium levels [53], and modulation of intracellular calcium is important in promoting glucose oxidation during ischemia–reperfusion. Appropriate regulation of intracellular calcium has also been shown [54] to be important for mitochondrial DNA transcription and translation. Thus, intracellular calcium plays a vital role in myocyte energy production. Calcium is also the critical biochemical coupler for muscle function. Impaired reuptake of calcium into the sarcoplasmic reticulum adversely affects diastolic relaxation, whereas the kinetics of trans-sarcolemmal calcium flux and calcium release by the sarcoplasmic reticulum is a principal determinant of systolic function. Chronic intracellular calcium overload is initially buffered by mitochondria, which are injured if overloaded, resulting in increased production of reactive oxygen intermediates, reduced cell respiration and ultimately cell death. Taurine, an amino acid that is critical to the maintenance of intracellular calcium homeostasis, appears inadequately maintained in the failing myocardium.

Taurine

Taurine and metabolism

Taurine (2-aminoethanesulphonic acid) is a unique amino acid that lacks a carboxyl group, and as such it does not enter into protein synthesis. It is an important amino acid for the modulation of cellular calcium levels, and it exhibits a remarkable biphasic action by increasing or decreasing calcium levels to maintain intracellular calcium homeostasis [55,56]. In the heart, taurine appears to do this by affecting several myocardial membrane systems. It is reported to enhance calcium-induced calcium release from the sarcoplasmic reticulum both directly and through inhibition of the enzyme phospholipid methyl transferase, influencing the phospholipid environment of the ryanodine-sensitive calcium channel. It also modulates cardiac calcium and sodium levels through the cardiac sarcolemmal sodium–calcium exchanger and a taurine–sodium exchanger. Taurine also has antioxidant properties [57] and reacts with a variety of potentially toxic intracellular aldehydes, including acetaldehyde and malonyldialdehyde [58].

Taurine is found in particularly high concentrations in the heart (15–25 μmol/g protein), representing approximately 60% of the free amino acid pool in small animals and 25–30% in humans [55,59]. Plasma levels are approximately 50–80 μmol/l.

Taurine is not an essential amino acid in humans because it can be synthesized from cysteine or methionine [59]; however, most taurine in humans is obtained directly through dietary sources, particularly from fish and milk. Biosynthetic capacity is maturation dependent, being almost nonexistent in the human fetus and newborn, and progressively increasing until adulthood [60]. Taurine uptake by the myocyte is an active process [61]. In the heart, taurine transport, like that of carnitine, can be stimulated by β-adrenergic agonists or dibutryl-

cyclic adenosine monophosphate; however, in other tissues cyclic guanosine monophosphate pathways seem to be important [59]. The taurine transporter of all tissues is regulated by the activation of two calcium sensitive enzymes: protein kinase C (which inhibits the transporter) and calmodulin (which stimulates transport) [61]. This reciprocal regulation of intracellular taurine levels by these two enzymes is consistent with a physiological role for taurine in the maintenance of intracellular calcium homeostasis.

Taurine and heart failure

Cardiac taurine concentrations are altered in heart disease. In myocardial ischaemia concentrations are reduced [62]. Taurine depletion has been shown to render the heart more susceptible to doxorubicin toxicity or to ischaemic damage [63]. Prolonged depletion of the myocardium has also been shown [64] to decrease contractile force through reduction of myofibrils. This finding is of interest because increased calcium levels in the myocyte can activate calcium-dependent proteinases, which in turn may breakdown myofibrils. Cats have very little taurine biosynthetic capacity, and may exhibit a taurine-deficient cardiomyopathy [65]. In other species nonischaemic myocardial hypertrophy and failure is associated with an increase in cardiac taurine concentration [55].

Cytokine activity, particularly that of tumour necrosis factor-α and interleukin-6, is increased in heart failure [66,67]. The infusion of tumour necrosis factor-α into experimental animals has been shown [68] to reduce the trans-sulphuration of dietary methionine to cysteine, and in consequence to decrease the levels of taurine and glutathione unless the animals are supplemented with cysteine. These findings suggest that the increased cytokine activity in heart failure may increase the need for cysteine and taurine. Because cysteine will replenish not only taurine but also glutathione, it may be an important supplement for replenishing both.

In animal models, orally administered taurine has been shown [55,69,70] to significantly reduce myocardial damage induced by the calcium paradox, doxorubicin or isoproterenol, or in hamster cardiomyopathy; it has also been reported [71] to increase the survival of rabbits with aortic regurgitation. As noted above, myocardial taurine is increased in nonischaemic forms of heart failure, and thus these studies suggest that the increase in taurine was inadequate relative to the intracellular calcium burden.

Studies of taurine administration in humans with congestive heart failure have been very limited and uncontrolled. However, taurine, given in an oral dose of 1 g three times per day, has been reported by one group of investigators [55] to be extremely well tolerated and to improve both haemodynamic state and functional capacity. No toxicity has been seen in either animal or human studies.

Reducing oxidative stress

In addition to modulating energy production, nutrients are also important in modulating the effect of free radicals produced in the mitochondria at complexes I and III. Antioxidants have an important role to play in protecting mitochondria and cells from reactive oxygen intermediates.

Until recently there was a reluctance to accept that oxidative stress is important in the pathogenesis of heart failure. However, recent investigations have shown that oxidative stress may be an important contributor to the deterioration of the hypertrophied or failing myocardium (for review [72]).

Peroxidative damage has been demonstrated [72,73] in the hearts of dogs and rats with heart failure due to pressure or volume overload. We have observed decreases in the levels of glutathione peroxidase and α-tocopherol and a concomitant increase in protein oxidation in the myocardium of cardiomyopathic hamsters during the late stages of hamster cardiomyopathy [74]. The administration of vitamin E appeared to normalize these findings completely [74].

Recently, we also demonstrated a significant increase in the plasma level of lipid peroxides and malonyldialdehyde (markers of oxidative stress) in patients suffering from congestive heart failure [67]. The increase in oxidative stress was related to the clinical severity of heart failure. These observations, and similar data from other laboratories [75,76], suggest that antioxidant supplements may be important additions to the therapy of heart failure. Vitamin E supplementation (400 IU) in 12 patients with heart failure has been reported [76] to decrease serum markers of oxidative stress. We have been unable to confirm these data in a double-blind, placebo-controlled, randomized trial of 1000 IU vitamin E in 50 patients with New York Heart Association class II–IV heart failure (unpublished data) [77]. Larger studies examining the role of antioxidant therapy in heart failure are now underway in several centres.

Nutritional supplementation as a new therapeutic strategy for heart failure

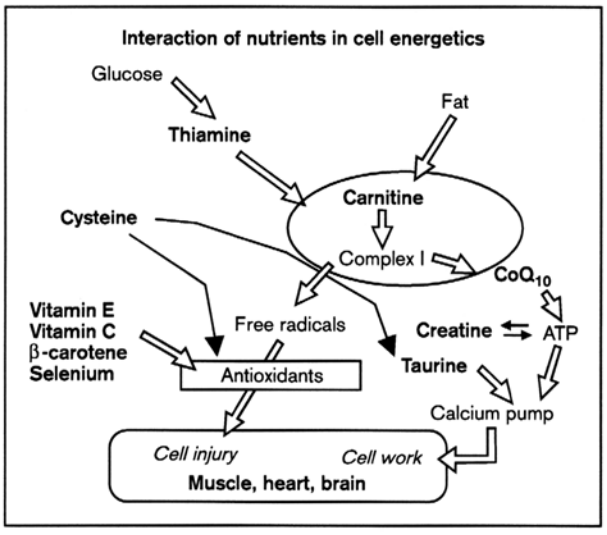
We believe that the recommended daily allowances for vitamins and related micronutrients established by federal nutritional authorities cannot be relied on for determining the nutritional needs of patients suffering from cardiac or other diseases. This is because, as described above, metabolic abnormalities or stress alter

the ability or requirements of nutrients to carry out their appropriate function. We have defined these nutritional demands of a given physiological or pathological state as 'conditioned nutritional requirements'.

The role of some nutrients in maintaining good health generally and in treating various disorders has long been recognized, particularly in the field of 'alternative medicine', which often emphasizes the use of 'natural' ingredients for these purposes. Failure to satisfy conditioned nutritional requirements as a (primary) cause of organ dysfunction and cell death has not been well recognized in either traditional or alternative medicines. The need for a given nutrient may not be readily detected, as its level in the blood may not reflect a deficiency or increased requirements in the diseased organ (e.g. carnitine, creatine, taurine or CoQ_{10} in myocardial failure). Even normal levels may be insufficient to maintain full functional status in the face of pathological metabolic demands. Furthermore, the replacement of only one nutritional constituent, in the traditional pharmacological paradigm, is unlikely to correct the cascade of interconnected abnormalities that is found in the failing myocardium (Fig. 1).

Finally, it should be noted that the population of myocytes within the failing heart is heterogeneous with respect to composition and structure. A 50% decrease in the concentration of a given factor in the whole heart reflects a distribution that includes only a minimal decrease in some cells, and a profound rate-limiting decrease in others. The failing heart deteriorates over a span of several years; thus, only a small minority of cells at any given time can be irreversibly injured. Hence, the vast majority of myocytes must be capable of at least partially responding to a therapeutic intervention with some recovery under the appropriate conditions.

Figure 1. Metabolic pathways with increased nutritional requirements in the decompensating myocyte



Conclusion

The failing myocardium exhibits both nutritional deficiencies and altered nutritional demands. These impair myocardial energy metabolism and calcium balance and increase oxidative stress. It is probable that skeletal muscle nutrition is similarly impaired, but to a lesser extent. Failure to satisfy these conditioned nutritional requirements appears to be an integral contributor to the myocyte dysfunction and loss, and possibly the fatigue and exercise intolerance seen in heart failure. Thus, restoring adequate myocyte nutrition would seem to be essential to any therapeutic strategy that is designed to benefit patients who suffer from this disease. Basic and clinical research in this area is sorely needed.

References

1 Kannel WB. Epidemiology of heart failure in the United States. In: Heart failure. Poole-Wilson PA, Colucci WS, Massie BM, Chatterjee K, Coats AJS (editors). New York: Churchill Livingstone; 1997. pp. 279–288.

2 Sutton GG, Cowie MR. Epidemiology of heart failure in Europe. In: Heart failure. Poole-Wilson PA, Colucci WS, Massie BM, Chatterjee K, Coats AJS (editors). New York: Churchill Livingstone;1997. pp. 289–296.

3 Sole MJ. Shifting the paradigm for the treatment of dilated cardiomyopathy. Eur Heart J 1995; 16(Suppl O):176–179.

4 Katz AM. The myocardium in congestive heart failure. Am J Cardiol 1989; 63:12A–16A.

5 Vogt AM, Kubler W. Heart failure: is there an energy deficit contributing to contractile dysfunction? Basic Res Cardiol 1998; 93:1–10.

6 Ingwall JS. Is cardiac failure a consequence of decreased energy reserve? Circulation 1993; 87(Suppl VII):VII-58–VII-62.

7 Clark AL, Sparrow JL, Coats AJS. Muscle fatigue and dyspnoea in chronic heart failure: two sides of the same coin? Eur Heart J 1995; 16:49–52.

8 Freeman L, Roubenoff R. The nutrition implications of cardiac cachexia. Nutr Rev 1994; 52:340–347.

9 Carr JG, Stevenson LW, Walden JA, Heber D. Prevalence and hemodynamic correlates of malnutrition in severe congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 1989; 63:709–713.

10 Toth MJ, Gottlieb SS, Goran MI, *et al.* Daily energy expenditure in freeliving heart failure patients. Am J Physiol 1997; 272:E469–E475.

11 Broqvist M, Arnqvist H, Dahlstrom U, *et al.* Nutritional assessment and muscle energy metabolism in severe chronic congestive heart failure: effects of long-term dietary supplementation. Eur Heart J 1994; 15:1641–1650.

12 Anker SD, Chua TP, Ponikowski P, *et al.* Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia. Circulation 1997; 96:526–534.

13 Arsenian MA. Carnitine and its derivatives in cardiovascular disease. Prog Cardiovasc Dis 1997; 40:265–286.

14 Schonekess BO, Allard MF, Lopaschuk GD. Propionyl L-carnitine improvement of hypertrophied heart function is accompanied by an increase in carbohydrate oxidation. Circ Res 1995; 77:726–734.

15 Pepine CJ. The therapeutic potential of carnitine in cardiovascular disorders. Clin Ther 1991; 13:2–18.

16 Engel AG. Carnitine deficiency syndromes and lipid storage myopathies. In: Myology, basic and clinical. Engel AG, Banker BQ (editors). Toronto: McGraw-Hill Book Co; 1986. pp. 1663–1696.

17 Whitmer JT. L-carnitine treatment improves cardiac performance and restores high-phosphate pools in cardiomyopathic Syrian hamster. Circ Res 1987; 61:396–408.

18 Iliceto S, Scrutinio D, Bruzzi P, *et al.* Effects of L-carnitine administration on left ventricular remodeling after acute anterior myocardial infarction: the L-Carnitine Ecocardiografia Digitalizzata Infarto Miocardico (CEDIM) Trial. Am Coll Cardiol 1995; 26:380–387.

19 Villalba JM, Navarro F, Cordoba F, *et al.* Coenzyme Q reductase from liver plasma membrane: purification and role in trans-plasma-membrane electron transport. Proc Natl Acad Sci USA 1995; 92:4887–4891.

20 Crane FL, Sun IL, Crowe RA, *et al.* Coenzyme Q10, plasma membrane oxidase and growth control. Mol Aspects Med 1994; 15(Suppl):s1–s11.

21 Lenaz G, Fato R, Castelluccio C, *et al.* An updating of the biochemical function of coenzyme Q in mitochondria. Mol Aspects Med 1994; 15(suppl):s29–s36.

22 Littaru GP. Energy and defence: facts and perspectives on coenzyme Q10 in biology and medicine. Rome: Casa Editrice Scientifica Internazionale; 1995. pp. 1–91.

23 Folkers K, Langsjoen P, Willis R, *et al.* Lovastatin decreases coenzyme Q levels in humans. Proc Natl Acad Sci USA 1990; 87:8931–8934.

24 Low P, Anderson M, Edlund C, Dallner G. Effects of mevinolin treatment on tissue dolichol and ubiquinone levels in the rat. Biochim Biophys Acta 1992; 1165:102–109.

25 Nylander M, Weiner J, Ruokonen I, Laakso J. Plasma levels of coenzyme Q10 before and after oral supplementation: a bioavailability study. CoQ Res Biol Med 1995; 3:25–32.

26 Folkers K, Vadhanavikit S, Mortensen SA. Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q10. Proc Natl Acad Sci USA 1985; 82:901–904.

27 Folkers K, Langsjoen P, Langsjoen PH. Therapy with coenzyme Q10 of patients in heart failure who are eligible or ineligible for a transplant. Biochem Biophys Res Commun 1992; 182:247–253.

28 Guarnieri C, Muscari C, Manfroni S, *et al.* The effect of treatment with coenzyme Q10 on the mitochondrial function and superoxide radical formation in cardiac muscle hypertrophied by mild aortic stenosis. J Mol Cell Cardiol 1987; 19:63–71.

29 Momomura S, Serizawa T, Ohtani Y, *et al.* Coenzyme Q10 attenuates the progression of cardiomyopathy in hamsters. Jpn Heart J 1991; 32:101–110.

30 Langsjoen PH, Vadhanavikit S, Folkers K. Response of patients in classes III and IV of cardiomyopathy to therapy in a blind and crossover trial with coenzyme Q10. Proc Natl Acad Sci USA 1985; 82:4240–4244.

31 Langsjoen P, Langsjoen PH, Folkers K. Long-term efficacy and safety of coenzyme Q10 therapy for idiopathic dilated cardiomyopathy. Am J Cardiol 1990; 65:521–523.

32 HofmanBang C, Rehnqvist N, Swedberg K, *et al.*, for the Q10 Study Group. Coenzyme Q10 as an adjunctive in the treatment of chronic congestive heart failure. J Card Fail 1995; 1:101–107.

33 Watson PS, Scalia GM, Galbraith A, *et al.* Lack of effect of coenzyme Q on left ventricular function in patients with congestive heart failure. J Am Coll Cardiol 1999; 33:1549–1552.

34 Soja AM, Mortenson SA. Treatment of congestive heart failure with coenzyme Q10 illuminated by meta-analysis of clinical trials. Mol Aspects Med 1997; 18(Suppl):S159–S168.

35 O'Keeffe ST, Tormey WP, Glasgow R, Lavan JN. Thiamine deficiency in hospitalized elderly patients. Gerontology 1994; 40:18–24.

36 Harper CG, Giles M, Finlay-Jones R. Clinical signs in the Wernicke–Korsakoff complex: a retrospective analysis of 131 cases diagnosed at necropsy. J Neurol Neurosurg Psychiatry 1986; 49:341–345.

37 Hakim AM, Pappius HM. The sequence of metabolic, clinical and histological reversibility of thiamine deficiency. Ann Neurol 1983; 13:365–375.

38 Matsushima K, McManus JP, Hakim AM. Apoptosis is restricted to the thalamus in thiamine-deficient rats. Neuroreport 1997; 8:867–870.

39 Brady JA, Rock CL, Horneffer MR. Thiamine status, diuretic medications and the management of congestive heart failure. J Am Diet Assoc 1995; 95:541–544.

40 Yui Y, Itokawa Y, Kawai C. Furosemide induced thiamine deficiency. Cardiovasc Res 1980; 14:537–540.

41 Seligmann H, Halkin H, Rauchfleisch S, *et al.* Thiamine deficiency in patients with congestive failure receiving long-term furosemide therapy: a pilot study. Am J Med 1991; 91:151–155.

42 Pfitzenmeyer P, Guilland JC, d'Athis Ph, *et al.* Thiamine status of elderly patients with cardiac failure including the effects of supplementation. Int J Vit Nutr Res 1994; 64:113–118.

43 Shimon I, Almong S, Vered Z, *et al.* Improved left ventricular function after thiamine supplementation in patients with congestive heart failure receiving long-term furosemide therapy. Am J Med 1995; 98:485–490.

44 Wyss M, Walliman T. 1-4 Creatine metabolism and the consequences of creatine depletion in muscle. Mol Cell Biochem 1994; 133/134:51–66.

45 Nascimben L, Ingwall JS, Pauletto P, *et al.* Creatine kinase system in failing and nonfailing human myocardium. Circulation 1996; 94:1894–1901.

46 Pulido SM, Passaquin AC, Leijendekker WJ, *et al.* Creatine supplementation improves intracellular Ca^{2+} handling and survival in *mdx* skeletal muscle cells. FEBS Lett 1998; 439:357–362.

47 Neubauer S, Horn M, Cramer M, *et al.* In patients with cardiomyopathy the myocardial phosphocreatine/ATP ratio predicts mortality better than ejection fraction or NYHA class. Circulation 1997; 96:2190–2196.

48 Conway MA, Bottomley PA, Ouwerkerk R, *et al.* Mitral regurgitation: impaired systolic function eccentric hypertrophy and increased severity are linked to lower phosphocreatine/ATP ratios in humans. Circulation 1998; 97:1716–1723.

49 Balsom PD, Soderlund K, Ekblom B. Creatine in humans with special reference to creatine supplementation. Sports Med 1994; 18:268–280.

50 Casey A, Constantin-Teodosiu D, Howell S, *et al.* Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. Am J Physiol 1996; 271:E31–E37.

51 Gordon A, Hultman E, Kaijser L. Creatine supplementation in chronic heart failure increases skeletal muscle creatine phosphate and muscle performance. Cardiovasc Res 1995; 30:413–418.

52 Andrews R, Grenhaff P, Curtis S, Perry A, Cowley AJ. The effect of dietary creatine supplementation on skeletal muscle metabolism in congestive heart failure. Eur Heart J 1998; 19:617–622.

53 Itoi T, Lopaschuk GD. Calcium improves mechanical function and carbohydrate metabolism following ischemia in isolated bi-ventricular working hearts from immature rabbits. J Mol Cell Cardiol 1996; 28:1501–1514.

54 Joyal JL, Hagen T, Aprille JR. Intramitochondrial protein synthesis is regulated by matrix adenine nucleotide content and requires calcium. Arch Biochem Biophys 1995; 319:322–330.

55 Azuma J, Sawamura A, Awata N. Usefulness of taurine in chronic congestive heart failure and its prospective application. Jpn Circ J 1992; 56:95–99.

56 Satoh H, Sperelakis N. Review of some actions of taurine on ion channels of cardiac muscle cells and others. Gen Pharmacol 1998; 30:451–463.

57 Cunningham C, Tipton KF, Dixon HBF. Conversion of taurine into N-chlorotaurine (taurine chloramine) and sulphoacetaldehyde in response to oxidative stress. Biochem J 1998; 330:939–945.

58 Ogasawara M, Nakamura T, Koyama I, *et al.* Reactivity of taurine with aldehydes and its physiological role. In: Taurine in health and disease. Huxtable R, Michalk DV (editors). Adv Exp Med Biol 1994; 359:71–78.

59 Huxtable RJ, Chubb J, Azari J. Physiological and experimental regulation of taurine content in the heart. Fed Proc 1980; 39:2685–2690.

60 Sturman JA. Taurine in development. Physiol Rev 1993; 73:119–147.

61 Ganapathy V, Leibach FH. Expression and regulation of the taurine transporter in cultured cell lines of human origin. In: Taurine in health and disease. Huxtable R, Michalk DV (editors). Adv Exp Med Biol 1994; 359:51–57.

62 Crass MF III, Lombardini JB. Loss of cardiac muscle taurine after acute left ventricular ischemia. Life Sci 1977; 21:951–958.

63 Schaffer SW, Allo S, Harada, Mozaffari M. Potentation of myocardial ischemic injury by drug- induced taurine depletion. In: The biology of taurine. Huxtable RJ, Franconi F, Giotti A (editors). New York: Plenum Press; 1987. pp. 151–158.

64 Lake N. Alterations of ventricular contractility and myofibrillar loss in taurine deficient hearts. In: Taurine in health and disease. Huxtable R, Michalk DV (editors). Adv Exp Med Biol 1994; 359:335–342.

65 Pion PD, Kittleson MD, Rogers QR, Morris JG. Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. Science 1987; 237:764–768.

66 Ferrari R, Bachetti T, Confortini R, *et al.* Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure. Circulation 1995; 92:1379–1382.

67 Keith M, Geranmaygan A, Sole MJ, *et al.* Increased oxidative stress in patients with congestive heart failure. J Am Coll Cardiol 1998; 31:1352–1356.

68 Grimble RF, Jackson AA, Persaud C, *et al.* Cysteine and glycine supplementation modulate the metabolic response to tumor necrosis factor alpha in rats fed a low protein diet. J Nutr 1992; 122:2066–2073.

69 Kramer JH, Chovan JP, Schaffer SW. The effect of taurine on calcium paradox and ischemic heart failure. Am J Physiol 1981; 240:H238–H246.

70 Azari J, Brumbaugh P, Barbeau A, Huxtable R. Taurine decreases lesion severity in the hearts of cardiomyopathic Syrian hamsters. Can J Neurol Sci 1980; 7:435–440.

71 Takihara K, Azuma J, Awata N. Beneficial effect of taurine in rabbits with chronic congestive heart failure. Am Heart J 1986; 112:1278–1284.

72 Ball AMMM, Sole MJ. Oxidative stress and the pathogenesis of heart failure. Cardiol Clinics 1998; 16:665–675.

73 Singh N, Dhalla AK, Singal PK. Oxidative stress and heart failure. Mol Cell Biochem 1995; 147:77–81.

74 Li R-K, Sole MJ, Mickle DAG, *et al.* Vitamin E and oxidative stress in the heart of the cardiomyopathic Syrian hamster. Free Radical Biol Med 1997; 24:252–258.

75 Diaz-Velez CR, Garcia-Castineiras S, Mendoza-Ramos E, Hernandez Lopez E. Increased malondialdehyde in peripheral blood of patients with congestive heart failure. Am Heart J 1996; 131:146–152.

76 Chatak A, Brar MJ, Agarwal A, *et al.* Oxy free radical system in heart failure and therapeutic role of vitamin E. Int J Cardiol 1996; 57:119–127.

77 Keith ME, Jeejeebhoy KN, Langer A, *et al.* A controlled clinical trial of vitamin E supplementation in patients with congestive cardiac failure. Am J Clin Nutr; in press.

BioFactors 25 (2005) 137–145
IOS Press

Coenzyme Q_{10} improves contractility of dysfunctional myocardium in chronic heart failure

Romualdo Belardinelli[a,*], Andi Muçaj[a], Francesca Lacalaprice[a], Maridia Solenghi[b], Federica Principi[c], Luca Tiano[c] and Gian Paolo Littarru[c]
[a]*Lancisi Heart Institute, Department of Cardiology and Cardiac Surgery, Ancona, Italy*
[b]*Clinical Chemistry Lab, Umberto I° Hospital, Ancona, Italy*
[c]*Institute of Biochemistry, Polytechnic University of the Marche, Ancona, Italy*

Abstract. *Background:* There is evidence that plasma CoQ_{10} levels decrease in patients with advanced chronic heart failure (CHF).

Objective: To investigate whether oral CoQ_{10} supplementation could improve cardiocirculatory efficiency in patients with CHF.

Methods: We studied 21 patients in NYHA class II and III (18M, 3W, mean age 59 ± 9 years) with stable CHF secondary to ischemic heart disease (ejection fraction 37 ± 7%), using a double-blind, placebo-controlled cross-over design. Patients were assigned to oral CoQ_{10} (100 mg tid) and to placebo for 4 weeks, respectively.

Results: CoQ_{10} supplementation resulted in a threefold increase in plasma CoQ_{10} level ($P < 0.0001$ vs placebo). Systolic wall thickening score index (SWTI) was improved both at rest and peak dobutamine stress echo after CoQ_{10} supplementation (+12.1 and 15.6%, respectively, $P < 0.05$ vs placebo). Left ventricular ejection fraction improved significantly also at peak dobutamine (15% from study entry $P < 0.0001$) in relation to a decrease in LV end-systolic volume index (from 57 ± 7 mL/m^2 to 45 mL/m^2, $P < 0.001$). Improvement in the contractile response was more evident among initially akinetic (+33%) and hypokinetic (+25%) segments than dyskinetic ones (+6%). Improvement in SWTI was correlated with changes in plasma CoQ_{10} levels ($r = -0.52$, $P < 0.005$). Peak VO_2 was also improved after CoQ_{10} as compared with placebo (+13%, < 0.005). No side effects were reported with CoQ_{10}.

Conclusions: Oral CoQ_{10} improves LV contractility in CHF without any side effects. This improvement is associated with an enhanced functional capacity.

Keywords: Coenzyme Q_{10}, chronic heart failure, left ventricular contractility, functional capacity

1. Introduction

Coenzyme Q_{10}, first isolated from beef heart mitochondria, [5] is an essential component of the mitochondrial respiratory chain, and also has antioxidant properties [6]. However, its role in chronic heart failure is not well defined. The rationale for CoQ_{10} supplementation in chronic heart failure lies in at least two factors. One is the well-known role of CoQ_{10} in myocardial bioenergetics, and the second is its antioxidant properties. Its bioenergetic effect is believed to be of fundamental importance,

*Address for correspondence: Romualdo Belardinelli, MD, FESC, Lancisi Heart Institute, Via Conca, 71, Torrette di Ancona, Italy. Tel.: +39 71 596 5344; Fax: +39 71 36819; E-mail: r.belardinelli@fastnet.it.

particularly in cells with high metabolic demand such as cardiac myocytes. Previous reports have shown that CoQ_{10} concentration is decreased in myocardial tissue [18,19] in chronic heart failure, and the greater its deficiency the more severe is the cardiocirculatory impairment [23]. Plasma CoQ_{10} levels are also decreased in severe cardiocirculatory dysfunction [23] as well as in conditions of high oxidative stress, such as diabetes and liver disease [30]. Moreover, it has been hypothesized that an improvement in LV function may be obtained by raising plasma CoQ_{10} availability. However, in advanced heart failure and ischemic heart disease, oral CoQ_{10} supplementation, at doses close to 100 mg/die, improved left ventricular systolic function in some studies [2,10,11,13,16,20,22,27], but not in others [12,29]. A possible explanation may be that CoQ_{10} exerts biological effects when it reaches at least 3 times the normal plasma range, and that oral doses used in previous studies were too low [14]. Other explanations for these contrasting results could be concomitant medications, such as statins [8], and/or the choice of insufficiently accurate techniques.

Another important abnormality in chronic heart failure is endothelial dysfunction, which contributes to functional impairment. We speculate that oral CoQ_{10} may improve the endothelium-dependent relaxation of coronary as well as peripheral arteries in chronic heart failure, and that this benefit may be associated with enhanced functional capacity [9].

The objective of the present study was to determine whether, in patients with stable moderate chronic heart failure, oral CoQ_{10} supplementation may enhance left ventricular contractile dysfunction and left ventricular systolic function, and whether these improvements may translate into a greater functional capacity.

2. Methods

We studied 21 patients with chronic heart failure secondary to ischemic heart disease (Table 1). All patients had documented coronary artery disease and had performed a coronary angiography in the last 6 months. Inclusion criteria were NYHA II and III chronic heart failure clinically stable in the previous 3 months, i.e. no need to change medications in the last 3 months and no hospitalizations for acute heart failure, and ability to exercise. Exclusion criteria were a recent acute coronary syndrome and/or coronary interventions of revascularization (PTCA, CABG), renal insufficiency (serum creatinine > 2.5 mg/dL), liver abnormalities, uncontrolled hypertension, habitual use of antioxidants (vitamin C, E, A or coenzyme CoQ_{10}), orthopedic and/or neurological limitations. None of our patients regularly took multivitamin/mineral tablets or relevant amounts of food particularly rich in antioxidants.

2.1. Study design

The protocol was approved by the local Ethical Committee. After a run-in period of 1 week, during which patients signed an informed written consent, were visited by a cardiologist and underwent a familiarization cardiopulmonary exercise test, they started a scheme composed of two consecutive treatments (each one lasting four weeks), according to a double-blind, placebo-controlled cross over design: oral CoQ_{10} supplementation (100 mg t.i.d., Q-absorb-100- Jarrow Formulas, Los Angeles, CA), and placebo (t.i.d.). The sequence of the two different treatments was randomised. On study entry and at the end of each phase, all patients underwent a symptom-limited cardiopulmonary exercise testing, a blood chemistry assessment and a low-dose dobutamine stress echocardiographic study. Medications were not changed throughout the study period. Nine out of 21 patients were taking statins, which they discontinued one month before starting the present study.

Table 1
Population study

N/Sex (M/F)	21 (18/3)
Age, years	59 ± 9
Diagnosis, n (%)	
– Ischemic cardiomyopathy	21 (100%)
– Previous PTCA	5 (21.7%)
– Previous CABG	9 (39%)
Coronary Risk Factors, n (%)	
– Hypertension	4 (17.4)
– Hypercholesterolemia	8 (34.7)
– Diabetes Mellitus	6 (26)
– Cigarette smoking	5 (22)
LV Ejection Fraction, %	37 ± 7
NYHA Functional Class, n (II/III)	16/5
Medications, n (%)	
– Nitrates	5 (21.7)
– ACEI	14 (60.8)
– ATA-II	8 (34.7)
– BB	12 (61)
– Digitalis	6 (26)
– Diuretics	7 (30)
– ASA	15 (65)
– Warfarin	7 (30)

2.2. *Cardiopulmonary exercise testing*

After a familiarization test, a symptom-limited cardiopulmonary exercise test was performed on an electronically-braked cycle ergometer using a ramp increase in work rate. Expired gases and volumes were analyzed, breath-by-breath, with a metabolic cart (Sensormedics 2900 Z, Yorba Linda, CA). Heart rate and blood pressure were measured every minute during increasing work rate exercise and recovery. A 12-lead ECG was recorded every minute. The exercise test was stopped when one or more of the following criteria were present: predicted heart rate, fatigue, dyspnea, excessive systemic blood pressure increase ($\geqslant$ 230/130 mmHg), $\geqslant$ 2 mm ST depression in at least 2 adjacent leads and/or angina. The anaerobic threshold was measured by the V-slope method [3]. Peak oxygen uptake was the average oxygen uptake during the last 15 seconds of exercise.

2.3. *Dobutamine stress echocardiography*

Under continuous ECG monitoring, dobutamine was infused into a peripheral antecubital vein at an incremental regimen of 5 μg/kg/min every 3 minutes until a maximum of 20 μg/kg/min. In fact, we focused on the contractile response of viabile myocardium, which can be determined with doses equal or below 20 μg/kg/min. Moreover, we wanted to avoid myocardial ischemia that is habitually induced by higher doses of dobutamine. Echocardiographic studies were performed with the patients supine in the left lateral position. Two dimensional echo images were continuously acquired from the parasternal long-axis, short axis and apical four- and two-chamber views using a wide-angle mechanical scanner (2.5 MHz, Challenge, ESAOTE, Italy).

2.4. *Measurements*

A 16-segment model was used for LV contractility analysis. Each segment was visually graded using a semiquantitative scoring system, where 1 = normal, 2 = hypokinetic, 3 = akinetic and 4 = dyskinetic.

Systolic wall thickening score index was calculated at rest and at each stage of dobutamine infusion. A 20% reduction in systolic wall thickening represents the 95% CI, discriminating a significant difference between normal and abnormal contractile response to low-dose dobutamine by two-dimensional echocardiography in our laboratory [4].

2.5. Data analysis

All studies were analyzed with an off-line system equipped with digital processing (Panasonic AG 7700). Representative cycles of rest and peak dobutamine dose images in comparable views were digitized and positioned side by side on a quad screen format. The echocardiographic images were evaluated in a blinded manner by two independent, experienced observers who adopted the same assessment criteria. Disagreement between the two observers occurred in 7% of studies. Differences in interpretation were resolved by a third independent cardiologist. In any of the following examinations, an improvement in the contractile response to dobutamine, compared with the initial study, was considered a reduction in SWTI by $\geqslant 1$ at peak infusion and/or a $\geqslant 20\%$ reduction in systolic wall thickening in at least two adjacent segments.

2.6. Blood chemistry

Coenzyme Q_{10} (CoQ_{10}) assay.

CoQ_{10} was determined by HPLC using a direct extraction method [7]. Normal values for plasma CoQ_{10}, in the Italian region where the study was conducted, were 0.78 ± 0.2 μg/ml, and are in agreement with previous findings [28]. So far no influence of aging on this range has been found.

2.7. Statistical analysis

Data were entered into a commercially available statistical software package (SPSS 6.1 version) for analysis. Factor analysis was used. Comparisons were made using multiple ANOVA with Bonferroni correction. Regression analysis was also performed and a correlation coefficient was expressed. Statistical significance was considered at $P < 0.05$. Data are mean $\pm$ SD.

3. Results

Of 23 patients enrolled, 21 completed the protocol. One patient dropped out after 2 months for reasons related to work. Another one had an orthopedic injury that limited his ability to exercise. Data reported are relative only to the subset of 21 patients who completed the protocol. There were no side effects attributed to CoQ_{10} or untoward events during training sessions in patients who completed the protocol.

3.1. CoQ_{10} vs placebo

CoQ_{10} supplementation resulted in a threefold increase in plasma CoQ_{10} level from study entry (from 0.82 ± 0.5 μg/mL to 3.25 ± 1.5 μg/mL, $P < 0.001$), while there was no change from study entry with placebo (0.83 ± 0.4 μg/mL). As shown in Table 2(a) and (b), after CoQ_{10} supplementation, there were relevant improvements, as compared with study entry, in peak VO_2 (+13%), ventilatory threshold (+11%), ventilation (+39%), O_2pulse (+13.6%,), $\Delta VO_2/\Delta W$ (+11%,) and peak workload (+13.9%,):

Table 2
(a) Cardiopulmonary Exercise Testing

	Study Entry	Q_{10}	Placebo
Peak VO_2, mL/kg/min	17.35 ± 3.6	19.6 ± 4.8	17.9 ± 3.8
AT VO_2, mL/kg/min	9.6 ± 2.3	13.5 ± 3.8	9.9 ± 2.6
Ventilation, L/min	49.5 ± 15	68.9 ± 14	46.4 ± 15
Peak O_2 pulse, mL/beat	9.5 ± 1.5	10.8 ± 1.6	8.9 ± 2.2
$\Delta VO_2/\Delta W$, mL/min/W	7.5 ± 0.8	8.3 ± 0.7	7.4 ± 1.0
Peak Work Rate, Watts	108 ± 21	123 ± 20	98 ± 19
Resting Heart Rate, beats/min	74.1 ± 11	76.5 ± 12	76 ± 13
Peak Heart Rate, beats/min	130 ± 20	137 ± 19	129 ± 20
Peak Systolic Blood Pressure, mmHg	148 ± 22	173 ± 25	142 ± 23

Twenty-one patients completed the four treatment phases.

(b) Results of statistically significant comparisons (significance $P < 0.05$ in normal characters; $P < 0.001$ in bold characters)

Variables	Treatment	
	Q_{10}	Placebo
Peak VO_2	**study entry**	Q_{10}
	Placebo	
AT VO_2	**study entry**	Q_{10}
	Placebo	
Ventilation	**study entry**	Q_{10}
	Placebo	
Peak O_2 pulse	study entry	Q_{10}
	Placebo	
$\Delta VO_2/\Delta W$	study entry	Q_{10}
	Placebo	
Peak Work Rate	**study entry**	Q_{10}
	Placebo	
Peak Heart Rate	–	–
Peak Systolic Blood Pressure	**study entry**	Q_{10}
	Placebo	

all these changes were highly significant. No changes were observed in resting and peak exercise heart rate. However, systolic blood pressure at peak exercise was significantly higher than baseline, reflecting improved cardiocirculatory function.

Resting left ventricular ejection fraction significantly increased from study entry after CoQ_{10} supplementation, while it returned to baseline levels after placebo (Table 3a and b). Left ventricular ejection fraction improved significantly also at peak dobutamine (15% from study entry $P < 0.0001$) in relation to a decrease in LV end-systolic volume index (from 57 ± 7 mL/m^2 to 45 mL/m^2, $P < 0.001$). Systolic wall thickening score index had similar improvements as ejection fraction both at rest and at peak dobutamine (12.1 and 15.6%, respectively). These improvements were related to changes in regional contractility. Of 195 segments with resting wall motion abnormalities, 125 demonstrated improved contractility ($P < 0.001$ vs initial), and these changes were evident during the first 5 minutes of dobutamine infusion. Improvement in the contractile response was more evident among initially akinetic (+33%) and hypokinetic (+25%) segments than dyskinetic ones (+6%). There were no changes in contractility after placebo. Improvement in SWTI was correlated with changes in plasma CoQ_{10} levels ($r = -0.52$, $P < 0.005$). Patients with plasma CoQ_{10} levels above 2.4 μg/mL showed the highest improvement in SWTI at peak dobutamine (Fig. 1) ($P = 0.04$).

Table 3

(a) Left ventricular ejection fraction and systolic wall thickening score index after CoQ_{10} and placebo during low-dose dobutamine

	Study Entry	Q_{10}	Placebo
Resting Ejection Fraction, %	37 ± 8.3	43 ± 8.7	37.9 ± 8
Peak Ejection Fraction, %	46.7 ± 8.4	53.9 ± 9.4	44.5 ± 8.3
Resting SWTI	2.23 ± 0.3	1.96 ± 0.4	2.19 ± 0.3
Peak SWTI	1.86 ± 0.3	1.57 ± 0.3	1.87 ± 0.3

SWTI = Systolic wall thickening score index.

(b) Results of statistically significant comparisons (significance: $P < 0.05$ in normal characters; $P < 0.001$ in bold)

Variables	Treatment	
	Q_{10}	Placebo
Resting Ejection Fraction	**study entry**	Q_{10}
	Placebo	
Peak Ejection Fraction	**study entry**	Q_{10}
	Placebo	
Resting SWTI	**study entry**	Q_{10}
	Placebo	
Peak SWTI	**study entry**	Q_{10}
	Placebo	

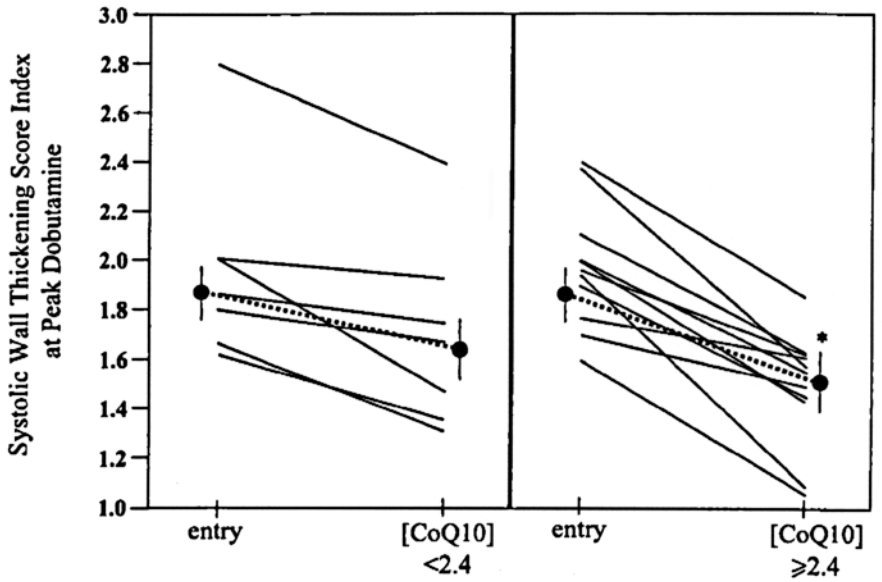


Fig. 1. Systolic wall thickening score index (SWTI)at peak dobutamine infusion after CoQ_{10} supplementation. In patients with plasma $CoQ_{10} > 2.4$ mg/mL (n = 14, Group B) the decrease in SWTI was statistically significant as compared with patients with plasma $CoQ_{10} < 2.4$ mg/mL (n = 7, group A). Dotted line equals the average. $*P = 0.04$ B vs A.

4. Discussion

The results of the present study demonstrate that, in patients with NYHA class II and III chronic heart failure secondary to ischemic heart disease, oral CoQ_{10} supplementation significantly improved left ventricular contractility, LV ejection fraction and peak VO_2. Improvement in SWTI was correlated

with changes in plasma CoQ_{10} levels ($r = -0.52$, $P < 0.005$).

Plasma CoQ_{10} concentration depends on the metabolic demand in various tissues, and plasma levels of 0.6–1.0 μg/mL are considered as normal [28]. In the present study, however, plasma levels of CoQ_{10} at study entry were within the normal range (0.82 ± 0.5 μg/mL), probably because of mild to moderate cardiocirculatory dysfunction in our population (peak VO_2 17.4 ± 3.6 mL/kg/min). The addition of high oral doses of CoQ_{10} tripled its plasma level, which correlated with improved LV function. Previous studies provided conflicting results about the level that CoQ_{10} should reach in plasma in order to elicit benefits in heart failure patients. Langsjoen postulated a threshold of 2.5 μg/mL, above which marked effects can be observed [14].

This level cannot be reached with low oral doses, and this "threshold hypothesis" helps to explain why results obtained with 100 mg/day dosages, in advanced heart failure, were not univocal regarding the effects on left ventricular function. In the present study, the oral dose of CoQ_{10} was three times higher and raised plasma CoQ_{10} levels well above the postulated threshold (3.25 ± 1.5 μg/mL). In the light of our results doses of 200–300 mg/day should therefore be preferred. During the trial none of the patients received statins. Acting as HMGCoA reductase inhibitors, these drugs lower the production of mevalonate, a critical precursor for both cholesterol and coenzyme Q_{10} synthesis. Extensive work has established the impact of statin treatment on blood and tissue levels of CoQ_{10} [15,24]. Even though the effect of statin treatment on tissue levels of CoQ_{10} is still debated [26], there is no doubt that statins have a dose-related lowering effect on plasma CoQ_{10} [15]. Since the aim of the present study was to investigate the relationship between CoQ_{10} treatment, CoQ_{10} plasma levels and cardiac function, we chose to exclude patients on statin treatment, in order to avoid a possible bias. On the basis of the known pleiotropic effects of statins we cannot exclude that the addition of statins to our therapeutic schemes could have generated an even better result.

A second important element is the antioxidant activity of CoQ_{10} [6]. CoQ_{10} may improve nitric oxide bioactivity by decreasing superoxide generation and by interacting with superoxide generation and free radicals [18,21]. Moreover, CoQ_{10} supplementation was found to upregulate guanylyl cyclase, the receptor for nitric oxide, in human skeletal muscle [17]. In conditions of high oxidative stress, such as chronic heart failure and multiple coronary risk factors, the inactivation rate of nitric oxide to peroxynitrite by superoxide anions may be reduced by CoQ_{10}, and this may be one possible explanation for the improvement in functional capacity. Moreover, the high plasma levels of CoQ_{10} were not associated with side effects. Both the improved ATP production and the antioxidant properties may be involved in explaining these benefits. We found a significant improvement in left ventricular contractility in dysfunctional segments located in non-infarcted areas served by stenotic arteries, where hibernation and/or chronic stunning is likely to occur. The upregulation of contractile function after CoQ_{10} suggests that chronic post-ischemic stunned cells improve or normalize their metabolism and function [1]. This effect translates into mechanical efficiency and contributes to reduce left ventricular dysfunction. It is noteworthy that this effect was obtained without any change in heart rate, differently from traditional inotropic substances.

We used low-dose dobutamine to detect changes in myocardial contractility after CoQ_{10} in humans with ischemic cardiomyopathy, because it shows good agreement with thallium imaging as well as PET scanning, and also because the good accuracy and reproducibility in our laboratory [7].

In conclusion, in patients with ischemic cardiomyopathy and chronic heart failure in NYHA functional class II and III, oral supplementation with CoQ_{10} at doses that increase plasma CoQ_{10} levels threefold from study entry was safe and determined significant improvements in left ventricular contractility and functional capacity when compared with placebo. In the light of our results doses of 200–300 mg/day should therefore be preferred. These potential benefits were not accompanied by side effects.

References

[1] D. Atar, S.A. Mortensen, H. Flachs and W.R. Herzog, Coenzyme Q_{10} protects ischemic myocardium in an open-chest swine model, *Clin Invest* **71** (1993), s103–s111.

[2] E. Baggio, R. Gandini, A.C. Plancher, M. Passeri and G. Carmosino, Italian multicenter study on the safety and efficacy of Coenzyme Q_{10} as adjunctive therapy in heart failure, *Molec Aspects Med* **15** (1994), s287–s294.

[3] W.L. Beaver, W. Wasserman and B.J. Whipp, A new method for detecting the anaerobic threshold by gas exchange, *J Appl Physiol* **60** (1986), 2020–2027.

[4] R. Belardinelli, D. Georgiou and A. Purcaro, Low dose dobutamine echocardiography predicts improvement in functional capacity after exercise training in patients with ischemic cardiomyopathy: Prognostic implication, *J Am Coll Cardiol* **31** (1998), 1027–1034.

[5] F.L. Crane, Y. Hatefi, P.L. Lester and C. Widmer, Isolation of a quinone from beef heart mitochondria, *Biochim Biophys Acta* **25** (1957), 220–221.

[6] L. Ernster and P. Forsmark-Andrée, Ubiquinol: an endogenous antioxidant in aerobic organisms, *Clin Investig* **71** (1993), S60–S65.

[7] F. Mosca, D. Fattorini, S. Bompadre and G.P. Littarru, Assay of coenzyme Q(10) in plasma by a single dilution step, *Anal Biochem* **305** (2002), 49–54.

[8] K. Folkers, P. Langsjoen, R. Willis, P. Richardson, L. Xia et al., Lovastatin decreases coenzyme Q levels in humans, *Proc Nat Acad Sci USA* **87** (1990), 8931–8934.

[9] R. Hambrecht, E. Fiehn, C. Weigl et al., Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure, *Circulation* **98** (1998), 2709–2715.

[10] C. Hoffman-Bang, K. Swedberg, N. Renhnqvist and H. Astrom, Coenzyme Q_{10} as an adjunctive in treatment of congestive heart failure, *J Cardiac Failure* **1** (1995), 101–107.

[11] T. Kamikawa, A. Kobayashi, T. Yamashita, H. Hayashi and N. Yamazaki, Effects of coenzyme Q_{10} on exercise tolerance in stable angina pectoris, *Am J Cardiol* **56** (1985), 247–251.

[12] M. Khatta, B.S. Alexander, C.M. Krichten, M.L. Fisher, R. Freudenberger, S.W. Robinson and S.S. Gottlieb, The effect of Coenzyme Q in patients with congestive heart failure, *Ann Int Med* **132** (2000), 636–640.

[13] P. Langsjoen and A. Langsjoen, Overview of the use of CoQ_{10} in cardiovascular disease, *BioFactors* **9** (1999), 273–284.

[14] P. Langsjoen, Lack of effect of Coenzyme Q on left ventricular function in patients with congestive heart failure, *J Am Coll Cardiol* **35** (2000), 816–817.

[15] P.H. Langsjoen and A.M. Langsjoen, The clinical use of HMG CoA-reductase inhibitors and the associated depletion of coenzyme Q_{10}. A review of animal and human publications, *BioFactors* **18** (2003), 101–112.

[16] P.H. Langsjoen, P.H. Langsjoen and K. Folkers, Long-term efficacy and safety of coenzyme Q_{10} therapy for idiopathic dilated cardiomyopathy, *Am J Cardiol* **65** (1990), 521–523.

[17] A. Linnane, G. Kopsidas, C. Zhang, N. Yarovaya, S. Kovalenko, P. Papakostopoulos, H. Eastwood, S. Graves and M. Richardson, Cellular redox activity of coenzyme Q_{10}: effect of CoQ_{10} supplementation on human skeletal muscle, *Free Radical Research* **36** (2002), 445–453.

[18] G.P. Littarru, L. Ho and K. Folkers, Deficiency of coenzyme Q_{10} in human heart disease – part I, *Int J Vit Nut Res* **42** (1972), 291–305.

[19] G.P. Littarru, L. Ho and K. Folkers, Deficiency of Coenzyme Q_{10} in human heart disease – part II, *Int J Vit Nutr Res* **42** (1972), 413–434.

[20] C. Mazzola, E.E. Guffanti, A. Vaccarella, M. Meregalli et al., Noninvasive assessment of coenzyme Q_{10} in patients with chronic stable effort angina and moderate heart failure, *Curr Therap Res* **6** (1987), 923–928.

[21] M.F. McCarty, Coenzyme Q versus hypertension: does CoQ decrease endothelial superoxide generation? *Med Hypothesis* **53** (1999), 300–304.

[22] C. Morisco, B. Trimarco and M. Condorelli, Effect of coenzyme Q_{10} therapy in patients with congestive heart failure: a long-term multicenter randomized study. K. Folkers, S.A. Mortensen, G.P. Littarru, T. Yamagami and G. Lenaz, eds, *Clin Invest* **71** (1993), s134–s136.

[23] S.A. Mortensen, S. Vadhanavikit, U. Baandrup and K. Folkers, Long-term coenzyme Q_{10} therapy: a major advance in the management of resistant myocardial failure, *Drugs Under Exptl & Clin Res* **11** (1985), 581–593.

[24] S. Passi, A. Stancato, E. Aleo, A. Dmitrieva and G.P. Littarru, Statins lower plasma and lymphocyte ubiquinol/ubiquinone without affecting other antioxidants and PUFA, *BioFactors* **18** (2003), 113–124.

[25] R. Petrucci, E. Giorgini, E. Damiani, P. Carloni, G. Marrosu, A. Trazza, G.P. Littarru and L. Greci, A study on the interactions between Coenzyme Q_{10} and superoxide anion. Could ubiquinones mimic superoxide dismutase (SOD)? *Res Chem Intermed* **26** (2000), 269–282.

[26] W.H. Schaefer, J.W. Lawrence, A.F. Loughlin, D.A. Stoffregen, L.A. Mixson, D.C. Dean, C.E. Raab, N.X. Yu, G.R. Lankas and C.B. Fredrick, Evaluation of ubiquinone concentration and mitochondrial function relative to cerivastatin-induced skeletal myopathy in rats, *Toxicology and Applied Pharmacology* **194** (2004), 10–23.

[27] A.M. Soja and S.A. Mortensen, Treatment of congestive heart failure with coenzyme Q_{10} illuminated by meta-analyses of clinical trials, *Molecular Aspects of Medicine* **18** (1997), s159–s168.

[28] M. Tomasetti, R. Alleva, M.D. Solenghi and G.P. Littarru, Distribution of antioxidants among blood components and lipoproteins: significance of lipids/CoQ_{10} ratio as a possible marker of increased risk for atherosclerosis, *BioFactors* **9** (1999), 231–240.

[29] P.S. Watson, G.M. Scalia, A. Galbraith, D.J. Burstow, N. Bett and C.N. Aroney, Lack of effect of coenzyme Q on left ventricular function in patients with congestive heart failure, *J Am Coll Cardiol* **33** (1999), 1549–1552.

[30] Y. Yamamoto and S. Yamashita, Plasma ubiquinone to ubiquinol ratio in patients with hepatitis, cirrhosis and hepatoma, and in patients treated with percutaneous transluminal coronary reperfusion, *BioFactors* **9** (1999), 241–246.

The Effect of Coenzyme Q_{10} in Patients with Congestive Heart Failure

Meenakshi Khatta, MS, CRNP;
Barbara S. Alexander, MD, PhD;
Cathy M. Krichten, MS, CRNP; Michael L. Fisher, MD;
Ronald Freudenberger, MD; Shawn W. Robinson, MD;
and Stephen S. Gottlieb, MD

Background: Coenzyme Q_{10} is commonly used to treat congestive heart failure on the basis of data from several unblinded, subjective studies. Few randomized, blinded, controlled studies have evaluated objective measures of cardiac performance.

Objective: To determine the effect of coenzyme Q_{10} on peak oxygen consumption, exercise duration, and ejection fraction.

Design: Randomized, double-blind, controlled trial.

Setting: University and Veterans Affairs hospitals.

Patients: 55 patients who had congestive heart failure with New York Heart Association class III and IV symptoms, ejection fraction less than 40%, and peak oxygen consumption less than 17.0 mL/kg per minute (or <50% of predicted) during standard therapy were randomly assigned. Forty-six patients completed the study.

Intervention: Coenzyme Q_{10}, 200 mg/d, or placebo.

Measurements: Left ventricular ejection fraction (measured by radionuclide ventriculography) and peak oxygen consumption and exercise duration (measured by a graded exercise evaluation using the Naughton protocol) with continuous metabolic monitoring.

Results: Although the mean (±SD) serum concentration of coenzyme Q_{10} increased from 0.95 ± 0.62 μg/mL to 2.2 ± 1.2 μg/mL in patients who received active treatment, ejection fraction, peak oxygen consumption, and exercise duration remained unchanged in both the coenzyme Q_{10} and placebo groups.

Conclusion: Coenzyme Q_{10} does not affect ejection fraction, peak oxygen consumption, or exercise duration in patients with congestive heart failure receiving standard medical therapy.

Ann Intern Med. 2000;132:636-640.

For author affiliations, current addresses, and contributions, see end of text.

There are numerous reasons to believe that deficiency of coenzyme Q_{10} (ubiquinone) may exacerbate the poor contractility of myocardial cells in patients with heart failure. Not only does coenzyme Q_{10} play a central role in mitochondrial oxidative phosphorylation (1), but it may also act as an antioxidant scavenger (2). Because the myocardium of patients with congestive heart failure demonstrates oxidative stress (3) and coenzyme Q_{10} prevents lipid peroxidation (4), this substance conceivably could prevent myocardial destruction. Furthermore, the concentration of coenzyme Q_{10} is decreased in myocardial cells of patients with advanced heart failure (5), and the extent of myocardial coenzyme Q_{10} deficiency correlates with the clinical severity of heart failure (5, 6).

It is thus not surprising that nutritional supplementation with coenzyme Q_{10} has been proposed as a treatment for congestive heart failure, that it is extensively advertised, and that it is commonly used by patients with this condition. Many small studies have been published, but most were uncontrolled and unblinded. Approximately 31 Japanese clinical reports describe favorable effects with intravenous or oral coenzyme Q_{10} (7). The studies involved only a small number of patients with heart failure and tended to include patients with cardiac disease of various causes. Nevertheless, in 1974 the Japanese government approved marketing of coenzyme Q_{10} for the treatment of heart failure.

The few U.S. and European studies have had conflicting results. Some controlled studies showed no effect (8, 9), but their limitations make the results inconclusive. Other trials noted improvement (10–13), but concerns about end points, small numbers of patients, and the lack of blinding have limited the acceptance of these studies. With such conflicting data, randomized, controlled, and blinded studies are needed to test the hypothesis that patients with advanced heart failure are deficient in coenzyme Q_{10} and that oral supplementation with coenzyme Q_{10} results in clinical improvement. We therefore evaluated the effects of coenzyme Q_{10} supplementation on left ventricular ejection fraction and exercise tolerance in patients with symptomatic heart failure despite standard medical therapy.

Methods

We performed a randomized, double-blind, placebo-controlled trial to compare the effects of oral coenzyme Q_{10} (200 mg/d) and placebo. The two primary end points were change in ejection fraction, as assessed by nuclear ventriculography, and change in peak oxygen consumption. The study protocol

was approved by the human volunteers committee of the University of Maryland School of Medicine.

Inclusion and Exclusion Criteria

Patients with New York Heart Association functional class III or IV disease were eligible for inclusion in this study. All patients had ejection fractions less than 40% (documented by radionuclide ventriculography) and maximal oxygen consumption less than 17.0 mL/kg of body weight per minute or less than 50% of the predicted value. These criteria were used to select symptomatic patients who would have the potential to improve. The mean peak oxygen consumption in our patients was 13.1 mL/kg per minute. In comparison, the peak oxygen consumption criterion for cardiac transplantation is generally considered to be less than 14.0 mL/kg per minute, and the mean peak oxygen consumption in nonexercising normal elderly persons (mean age, 67 years) has been reported to be 19.0 mL/kg per minute (14). Patients were required to have been receiving an unchanged medical regimen for at least 1 month. Patients who had previously taken coenzyme Q_{10} were excluded.

Baseline Testing

At baseline, three procedures were performed. First, a graded symptom-limited cardiopulmonary exercise test using the Naughton protocol was conducted to assess maximal oxygen consumption. The test was performed by the same operator and was repeated until the maximum oxygen consumption measures on two consecutive test results were within 15% of each other. The final test was considered to be the baseline test with which to assess change during therapy. Second, radionuclide ventriculography was performed by using standard techniques. Third, serum concentration of coenzyme Q_{10} was measured as described elsewhere (15). Three patients did not have concentrations obtained at baseline or follow-up.

Intervention

Patients were randomly assigned to receive 200 mg of coenzyme Q_{10} per day or placebo. Randomization was performed by using a random-number generator. All patients and study personnel were blinded to study group assignment until all data were final. The dosage was chosen to minimize the chance of inadequate treatment. Previous studies reporting benefit with coenzyme Q_{10} supplementation have generally used daily dosages of 100 or 150 mg (6, 7, 9–13, 16–18).

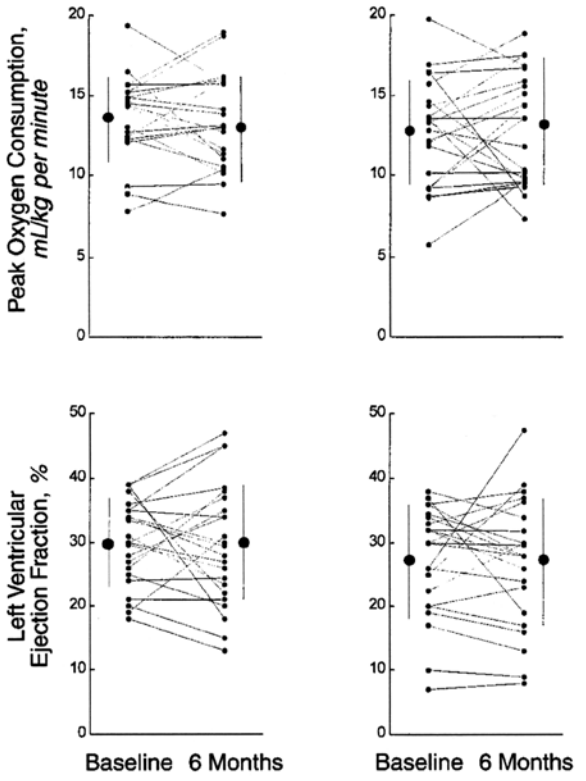


Figure 1. Ejection fraction and peak oxygen consumption before and after the treatment period for each patient who received placebo (*left*) and coenzyme Q_{10} (*right*). Coenzyme Q_{10} had no overall effect. The mean ± SD is shown for each time point.

Final Assessment

After 6 months, all baseline procedures were repeated. At that time, patients were asked whether their symptoms were improved, worse, or the same.

Statistical Analysis

The change in values of primary and secondary end points were compared by using an unpaired Student *t*-test. All values are given as the mean ± SD. For significance, a *P* value less than 0.05 was required. The study was planned to have 80% power to detect a difference of 2.8 mL/kg per minute in the peak oxygen consumption, with a *P* value of 0.05. This assumed a mean oxygen consumption of 13.0 ± 4.0 mL/kg per minute. We used StatMost, version 3.5 (Dataxiom Software, Inc., Los Angeles, California), for all statistical analyses.

Results

Fifty-five patients were randomly assigned. Nine patients did not finish the study: 5 in the coenzyme Q_{10} group and 4 in the placebo group. One patient (who was randomly assigned to receive coenzyme Q_{10}) was withdrawn from the study before repeated

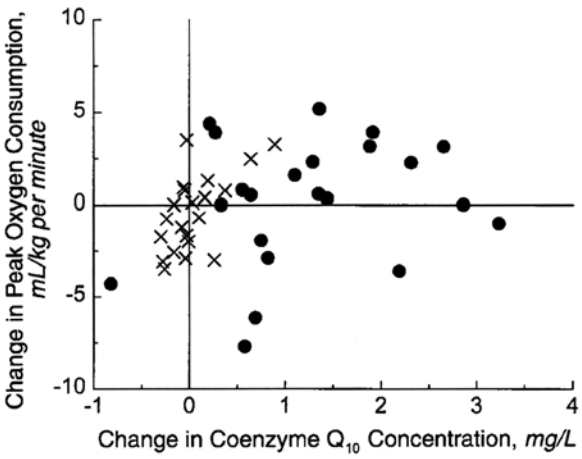


Figure 2. The change in coenzyme Q_{10} concentration compared with the change in peak oxygen consumption. Circles represent patients who received active treatment, and crosses represent patients who received placebo. The study drug clearly increased serum concentrations of coenzyme Q_{10}. However, there was no relation between the change in serum concentration and the change in peak oxygen consumption.

assessments and unblinding because of error in enrollment criteria. Three patients died: One patient assigned to the placebo group died of progressive heart failure, and 2 patients assigned to the coenzyme Q_{10} group died of myocardial infarction and sudden death, respectively. Four patients did not complete the study because of conditions that prevented them from exercising (esophageal cancer, uncontrolled ventricular tachycardia, foot amputation, and pulmonary edema). One patient randomly assigned to receive coenzyme Q_{10} withdrew from the study.

Baseline characteristics did not differ between the two groups. Twenty-three patients in each group completed the study. The study sample consisted of 39 men and 7 women, and the mean age in both groups was 64 years. Twenty-seven patients had known ischemic heart disease. Forty-two patients were categorized as being in New York Heart Association class III and 4 were in class IV. All patients were receiving digoxin and angiotensin-converting enzyme inhibitors or other vasodilators. Eighteen patients in each group were receiving β-blockers, and 22 patients in each group were receiving diuretics. No adverse reactions were attributed to the study drug, and no gastrointestinal side effects occurred.

Maximal Oxygen Consumption

After 6 months of blinded therapy, maximal oxygen consumption did not improve in the placebo or coenzyme Q_{10} group (**Figure 1**). Maximal oxygen consumption increased by 0.21 ± 3.4 mL/kg per minute (95% CI, -1.25 to 1.68 mL/kg per minute) in the patients who received coenzyme Q_{10} and decreased by 0.49 ± 2.4 mL/kg per minute (CI, -1.54 to 0.55 mL/kg per minute) in the patients who received placebo. The difference between groups was not significant. The respiratory quotient was 1.01 ± 0.07 at baseline and 0.99 ± 0.07 after treatment. Exercise duration did not change significantly in either group. In the coenzyme Q_{10} recipients, mean exercise duration was 8.5 ± 3.2 minutes before treatment and 9.1 ± 3.4 minutes after treatment. In the placebo recipients, exercise duration was 7.7 ± 3.2 minutes before treatment and 7.5 ± 2.9 minutes after 6 months.

Radionuclide Ventriculography

Coenzyme Q_{10} had no effect on left ventricular ejection fraction (**Figure 2**). Ejection fraction decreased minimally (0.3 ± 8 percentage points [CI, -3.7 to 3.1 percentage points]) in the patients who received coenzyme Q_{10} and decreased by 0.2 ± 8.6 percentage points (CI, -4.0 to 3.6 percentage points) in the patients who received placebo. Mean left ventricular ejection fraction was 27% before and after treatment in the patients who received coenzyme Q_{10} and was 30% before and after treatment in the patients who received placebo. Right ventricular ejection fraction decreased from $39\% \pm 14\%$ to $37\% \pm 8\%$ in the placebo group. In patients receiving coenzyme Q_{10}, right ventricular ejection fraction was $35\% \pm 13\%$ before treatment and $35\% \pm 11\%$ after 6 months.

Symptoms

One patient in each group had improved symptoms, as indicated by New York Heart Association classification. Almost three quarters of the patients classified themselves as neither improved nor worse after 6 months of treatment (18 patients receiving placebo and 16 patients receiving coenzyme Q_{10}). Six patients in the coenzyme Q_{10} group believed that their symptoms had improved even minimally, and one patient believed that symptoms had deteriorated. Two patients in the placebo group reported improvement in symptoms and 3 patients reported increased severity of symptoms.

Coenzyme Q_{10} Serum Concentrations

Before randomization, coenzyme Q_{10} serum concentrations were similar in both groups. With treatment, concentrations increased in the intervention group from 0.95 ± 0.62 μg/mL to 2.2 ± 1.2 μg/mL; in the placebo group, concentrations did not change (0.92 ± 0.34 μg/mL before treatment and 0.96 ± 0.45 μg/mL after 6 months). The difference between groups was highly significant ($P < 0.001$). Among patients who received the treatment, serum concentrations increased in 21 of the 22 patients in whom this value was measured (**Figure 2**). Among patients who received placebo, concentrations increased

slightly in only 8 of the 21 patients in whom this value was measured. The increase in patients who received placebo can be explained by small fluctuations in serum concentration or laboratory variability.

No association was seen between change in serum concentration of coenzyme Q_{10} and change in peak exercise oxygen consumption (**Figure 2**) or ejection fraction. This was true for patients in both groups.

Discussion

In this blinded, randomized, placebo-controlled trial, we detected no objective benefit from coenzyme Q_{10} administration in patients with heart failure. Cardiac performance (measured by ejection fraction) and maximal exercise (evaluated with oxygen consumption and test duration) did not change with coenzyme Q_{10}.

The use of coenzyme Q_{10} for the treatment of heart failure has been advocated by both physicians and nonphysicians. Patients use this over-the-counter nutritional supplement extensively, often without the knowledge of their physicians. However, the studies cited to support its use have had major limitations. In addition to open-label studies with obvious susceptibility to unintentional bias (7, 11), some studies have based their conclusions on evaluations of minimally symptomatic and inadequately treated patients (9) or on studies with noncomparable controls, subjective end points, poor statistics, or too few patients (12, 13). The few controlled studies have been contradictory. Our study was blinded and controlled and evaluated moderately and severely ill patients with heart failure who were receiving appropriate standard medical therapy. In addition, the end points were objective and relevant. Our findings suggest that coenzyme Q_{10} should not be recommended for treatment of heart failure.

Evaluations of the effects of coenzyme Q_{10} on ejection fraction have been contradictory. In contrast to our study, one double-blind crossover study of 19 patients with class III and IV heart failure receiving 100 mg of coenzyme Q_{10} per day reported that ejection fraction improved (16). However, the ejection fractions were derived by using echocardiography, not radionuclide ventriculography. In a larger study of 79 patients, resting or exercise ejection fraction did not improve according to radionuclide measurement (10). In that study, a minimal effect on ejection fraction was seen only during volume loading, raising the question of statistical irrelevance. Another blinded crossover trial also did not detect an effect of coenzyme Q_{10} on ejection fraction (9). At present, there is little reason to believe that coenzyme Q_{10} improves ventricular function.

Few studies have evaluated the effect of coenzyme Q_{10} on maximal exercise. These studies present contradictory data; for example, maximal workload was reported to increase slightly in one study (10) but was unchanged in an investigation of minimally impaired patients (9). Our study examined peak oxygen consumption in a randomized, blinded fashion. We found no trend toward an improvement in peak oxygen consumption or exercise duration; thus, it is unlikely that coenzyme Q_{10} improves maximal exercise performance in patients with heart failure.

Many open-label uncontrolled studies have shown a subjective improvement in clinical measures of heart failure (17). Morisco and colleagues' large randomized, blinded trial (18) detected a lower rate of hospitalization for heart failure among patients receiving coenzyme Q_{10}. However, this study also reported high rates of pulmonary edema and cardiac asthma in these patients and used vague definitions. The effect on symptoms in other studies have been inconsistent (8, 10). In our trial, most patients reported no change in symptoms.

We studied patients who were receiving standard therapy for heart failure, including β-blockers for most patients (19). Consequently, our findings should be applicable to the contemporary treatment of heart failure.

In our study, coenzyme Q_{10} supplementation clearly increased serum concentrations in the patients who received active treatment. The increase was dramatic (more than doubling the baseline concentration) and proves that patients took their medication. The lack of correlation between change in coenzyme Q_{10} concentration and change in ejection fraction or peak oxygen consumption supports the conclusion that coenzyme Q_{10} exerted no clinical benefit.

Because of the relatively small size of our study, we cannot definitively say that coenzyme Q_{10} has no effect in patients with heart failure. However, the lack of any trend and the relatively narrow confidence intervals make it unlikely that this nutritional supplement exerts clinically important effects in patients already receiving well-titrated standard medication. The dose given was similar to that given in previous studies that reported positive results. With a documented increase in serum concentrations and a 6-month duration of therapy, the lack of effect cannot be ascribed to inadequate treatment.

In conclusion, our study shows no benefit to adding coenzyme Q_{10} to the standard treatment of heart failure. Chronic illnesses motivate patients to seek out alternative therapy, and it is not surprising that people have been willing to buy an expensive and unproven drug. However, patients should be made aware that coenzyme Q_{10} has been studied in randomized, blinded, and controlled studies and that these studies have found no detectable benefit.

From University of Maryland School of Medicine and Veterans Affairs Medical Center, Baltimore, Maryland.

Acknowledgments: The authors thank Dr. S. Mortensen, Technical University of Denmark, for measuring serum concentrations of coenzyme Q_{10} and Dr. W. Herzog and Matthew Metcalf for their help in performing this study.

Grant Support: Supported in part by grant P60AG12583 from the National Institute of Aging, Claude D. Pepper Older Americans Independence Center, Bethesda, Maryland. Coenzyme Q_{10} and matching placebo were provided by PharmaNord, Sadlmadervej, Denmark.

Requests for Single Reprints: Stephen S. Gottlieb, MD, Division of Cardiology, University of Maryland School of Medicine, 22 South Greene Street, Baltimore, MD 21201.

Requests To Purchase Bulk Reprints (minimum, 100 copies): Barbara Hudson, Reprints Coordinator; phone, 215-351-2657; e-mail, bhudson@mail.acponline.org.

Current Author Addresses: Ms. Khatta, Drs. Alexander, Fisher, Freudenberger, Robinson, and Gottlieb, and Ms. Krichten: Division of Cardiology, University of Maryland School of Medicine, 22 South Greene Street, Baltimore, MD 21201.

Author Contributions: Conception and design: M. Khatta, B. Alexander, C.M. Krichten, M. Fisher, R. Freudenberger, S.W. Robinson, S. Gottlieb.

Analysis and interpretation of the data: M. Khatta, B. Alexander, C.M. Krichten, M. Fisher, R. Freudenberger, S.W. Robinson, S. Gottlieb.

Drafting of the article: M. Khatta, B. Alexander.

Critical revision of the article for important intellectual content: M. Khatta, C.M. Krichten, M. Fisher, R. Freudenberger, S.W. Robinson, S. Gottlieb.

Final approval of the article: M. Khatta, B. Alexander, C.M. Krichten, M. Fisher, R. Freudenberger, S.W. Robinson, S. Gottlieb.

Provision of study materials or patients: B. Alexander, M. Fisher, R. Freudenberger, S.W. Robinson, S. Gottlieb.

Statistical expertise: S. Gottlieb.

Collection and assembly of data: M. Khatta, C.M. Krichten.

References

1. **Crane FL, Hatefi Y, Lester RL, Widmer G.** Isolation of a quinone from beef heart mitochondria. Biochim Biophys Acta. 1957;25:220-1.
2. **Greenberg S, Frishman WH.** Co-enzyme Q_{10}: a new drug for cardiovascular disease. J Clin Pharmacol. 1990;30:596-608.
3. **Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, et al.** Increased oxidative stress in patients with congestive heart failure. J Am Coll Cardiol. 1998;31:1352-6.
4. **Marubayashi S, Dohi K, Yamada K, Kawasaki T.** Changes in the levels of endogenous coenzyme Q homologs, alpha-tocopherol, and glutathione in rat liver after hepatic ischemia and reperfusion, and the effect of pretreatment with coenzyme Q_{10}. Biochim Biophys Acta. 1984;797:1-9.
5. **Folkers K, Vadhanavikit S, Mortensen SA.** Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q_{10}. Proc Natl Acad Sci U S A. 1985;82:901-4.
6. **Mortensen SA.** Perspectives on therapy of cardiovascular disease with coenzyme Q_{10} (ubiquinone). Clin Investig. 1993;71:S116-S123.
7. **Tanaka J, Tominaga R, Yoshitoshi M, Matsui K, Komori M, Sese A, et al.** Coenzyme Q_{10}: the prophylactic effect on low cardiac output following cardiac valve replacement. Ann Thorac Surg. 1982;33:145-51.
8. **Watson PS, Scalia GM, Galbraith A, Burstow DJ, Bett N, Aroney CN.** Lack of effect of coenzyme Q on left ventricular function in patients with congestive heart failure. J Am Coll Cardiol. 1999;33:1549-52.
9. **Permanetter B, Rossy W, Klein G, Weingartner F, Seidl KF, Blömer H.** Ubiquinone (coenzyme Q_{10}) in the long-term treatment of idiopathic dilated cardiomyopathy. Eur Heart J. 1992;13:1528-33.
10. **Hofman-Bang C, Rehnqvist N, Swedberg K, Wiklund I, Astrom H.** Coenzyme Q_{10} as an adjunctive in the treatment of chronic congestive heart failure. The Q_{10} Study Group. J Card Fail. 1995;1:101-7.
11. **Langsjoen PH, Vadhanavikit S, Folkers K.** Long-term efficacy and safety of coenzyme Q_{10} therapy for idiopathic dilated cardiomyopathy. Am J Cardiol. 1990;65:521-3.
12. **Soja AM, Mortsensen SA.** Treatment of congestive heart failure with coenzyme Q_{10} illuminated by meta-analyses of clinical trials. Mol Aspects Med. 1997;18:S159-S168.
13. **Judy WV, Hall JH, Toth PD, Folkers K.** Double blind-double crossover study of coenzyme Q_{10} in heart failure. In: Folkers KA, Yamamura Y, eds. Biomedical and Clinical Aspects of Coenzyme Q. v 5. Amsterdam: Elsevier Science Publishers; 1986.
14. **Blumenthal JA, Emery CF, Madden DJ, Coleman RE, Riddle MW, Schniebolk S, et al.** Effects of exercise training on cardiorespiratory function in men and women older than 60 years of age. Am J Cardiol. 1991;67:633-9.
15. **Edlund PO.** Determination of coenzyme Q_{10}, α-tocopherol and cholesterol in biological samples by coupled-column liquid chromatography with coulometric and ultraviolet detection. J Chromatogr. 1988;425:87-97.
16. **Langsjoen PH, Vadhanavikit S, Folkers K.** Response of patients in classes III and IV of cardiomyopathy to therapy in a blind and crossover trial with coenzyme Q_{10}. Proc Natl Acad Sci U S A. 1985;82:4240-4.
17. **Baggio E, Gandini R, Plancher AC, Passeri M, Carmosino G.** Italian multicenter study on the safety and efficacy of coenzyme Q_{10} as adjunctive therapy in heart failure. CoQ_{10} Drug Surveillance Investigators. Mol Aspects Med. 1994;15:S287-S294.
18. **Morisco C, Trimarco B, Condorelli M.** Effect of coenzyme Q_{10} therapy in patients with congestive heart failure: a long-term multicenter randomized trial. Clin Investig. 1993;71:S134-S136.
19. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure (MERIT-HF). Lancet. 1999;353:2001-7.

Clin. Cardiol. 27, 295–299 (2004)

Coenzyme Q10 in Patients with End-Stage Heart Failure Awaiting Cardiac Transplantation: A Randomized, Placebo-Controlled Study

MARIUS BERMAN, M.D., ARIE ERMAN, PH.D.,* TUVIA BEN-GAL, M.D.,† DAN DVIR, M.D., GEORGIOS P. GEORGHIOU, M.D., ALON STAMLER, M.D., YAFFA VERED, PH.D.,‡ BERNARDO A. VIDNE, M.D., DAN ARAVOT, M.D.

Department of Cardiothoracic Surgery, Heart-Lung Transplant Unit; *Institute of Nephrology and Hypertension; †Department of Cardiology, Echocardiographic Unit, Rabin Medical Center, Beilinson Campus, Potah Tikva; ‡Clinical Biochemistry Laboratory Tel-Aviv Souraski Medical Center, Tel Aviv; both affiliated with the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Summary

Background: The number of patients awaiting heart transplantation is increasing in proportion to the waiting period for a donor. Studies have shown that coenzyme Q10 (CoQ10) has a beneficial effect on patients with heart failure.

Hypothesis: The purpose of the present double-blind, placebo-controlled, randomized study was to assess the effect of CoQ10 on patients with end-stage heart failure and to determine if CoQ10 can improve the pharmacological bridge to heart transplantation.

Methods: A prospective double-blind design was used. Thirty-two patients with end-stage heart failure awaiting heart transplantation were randomly allocated to receive either 60 mg U/day of Ultrasome™—CoQ10 (special preparation to increase intestinal absorption) or placebo for 3 months. All patients continued their regular medication regimen. Assessments included anamnesis with an extended questionnaire based partially on the Minnesota Living with Heart Failure Questionnaire, 6-min walk test, blood tests for atrial natriuretic factor (ANF) and tumor necrosis factor (TNF), and echocardiography.

Results: Twenty-seven patients completed the study. The study group showed significant improvement in the 6-min walk test and a decrease in dyspnea, New York Heart Association (NYHA) classification, nocturia, and fatigue. No significant changes were noted after 3 months of treatment in echocardiography parameters (dimensions and contractility of cardiac chambers) or ANF and TNF blood levels.

Conclusion: The administration of CoQ10 to heart transplant candidates led to a significant improvement in functional status, clinical symptoms, and quality of life. However, there were no objective changes in echo measurements or ANF and TNF blood levels. Coenzyme Q10 may serve as an optional addition to the pharmacologic armamentarium of patients with end-stage heart failure. The apparent discrepancy between significant clinical improvement and unchanged cardiac status requires further investigation.

Key words: coenzyme Q10, heart failure, heart transplantation

Introduction

Coenzyme Q10 (CoQ10), first identified in 1940,[1] is a fat-soluble quinone with characteristics common to vitamins. It is found in the organs of various animal species,[2,3] with highest concentrations in the heart, liver, kidney, and pancreas.[4] In 1957, Crane *et al.*[5] demonstrated that CoQ10 plays an important role as a redox carrier in the mammalian respiratory transport chain, as it has a direct regulatory role on succinyl and reduced nicotinamide adenine dinucleotide (NADH) dehydrogenases. The relatively high concentration of CoQ10 in healthy human myocardium[6] has led to the assumption that a myocardial deficiency of CoQ10 is detrimental to cardiac function. This was supported by studies showing low levels of CoQ10 in myocardial biopsies from patients with various cardiac diseases.[7–10] Lower than normal levels of CoQ10 have also been found in blood samples from patients with cardiovascular diseases compared with levels in healthy human subjects.[11] Oral CoQ10 was first used in 1965 in the treatment of cardiovascular disease,[12] and several years later Folkers *et al.*[7] presented the rationale for using CoQ10 in treating congestive heart failure (CHF). Since then, clinical reports worldwide have described favorable effects of both intravenous and oral CoQ10 in patients with CHF of various etiologies.[13–15] Baggio *et al.*,[16] in a multicenter study in Italy (173 centers, 2,664 pa-

Address for reprints:

Dr. Marius Berman
Department of Cardiothoracic Surgery
Rabin Medical Center, Beilinson Campus
Petah-Tikva 49100, Israel
e-mail: mariusby@yahoo.com

Received: September 5, 2002
Accepted with revision: May 8, 2003

tients), demonstrated that CoQ10 was safe and effective as an adjunctive therapy in heart failure. Hofman-Bang *et al.*,[17] in a double-blind, cross-over, placebo-controlled multicenter study in Sweden and Denmark, found CoQ10 treatment to have a small but significant effect on exercise capacity, quality of life, and ejection fraction in patients with moderate to severe CHF.

In 1992, Folkers *et al.*[18] reported on 11 heart transplant candidates treated with CoQ10. All showed improvement in New York Heart Association (NYHA) functional class, and some required no conventional drugs and no life-style restrictions after treatment.

Today, both the number of patients awaiting heart transplantation and the waiting period for donors are increasing steadily. Despite their receipt of medical treatment and support, candidates suffer a general decline in their quality of life. The purpose of the present double-blind, placebo-controlled, randomized study was to assess the effect of CoQ10 on patients with end-stage heart failure and to determine whether CoQ10 can improve the pharmacologic bridge to heart transplantation.

Materials and Methods

Patients

Patients with end-stage heart failure were selected for the study from the candidates for heart transplant on our center's waiting list. Inclusion criteria were NYHA functional class 3 or 4, left ventricular ejection fraction < 25%, cardiopulmonary exercise test (CARPET) with maximal O_2 consumption < 14 ml/kg/h, and evident symptoms of heart failure such as nocturia, dyspnea, and paroxysmal nocturnal dyspnea. Patients with United Network for Organ Sharing (UNOS) Status One, or with moderate renal failure with creatinine levels > 2.5 mg/dl were excluded. The endpoints in the study for those patients who had begun treatment were (1) heart transplantation and (2) hospitalization because of clinical deterioration, thereby preventing the regular intake of the CoQ10 capsule.

The final study sample consisted of 32 patients (28 men, 4 women aged 40 to 67 years, mean 54.6), 25 of whom had ischemic cardiomyopathy and 7 had dilated cardiomyopathy. All had been stable for at least 1 month. The waiting time for a heart transplant ranged from 2 to 67 months (mean 26 months). All patients were receiving some combination of the following conventional medications: diuretics (76%), antiarrhythmic agents (45%), beta blockers (52%), digitalis (85%), afterload reducing agents (65%), angiotensin-converting enzyme inhibitors (45%), and anticoagulants (19%). All provided informed consent to participate in the study.

The study was approved by the Institutional Helsinki Committee.

Test Drug

Ultrasome™ coenzyme Q10 is a novel, patented technology developed by Herbamed Ltd. (Science Park Kiryat Weizmann, Rehovot, Israel) for improving the oral bioavailability of lipophilic nutriceutical and pharmaceuticals. The Ultrasome lipid particles are "hybrid" liposomes and oil in water emulsions. Their matrix is comprised of a hydrophobic core as in emulsion, but surrounded by phospholipid bilayer. Ultrasome coenzyme Q10 (U-CoQ10) is a spray-dried, lipid-based, powdered formulation of CoQ10 in capsule form. It has shown high drug-trapping efficiency and enhanced oral delivery, as indicated by better intestinal absorption and improved performance.

Randomization

The patients were randomly divided into two groups according to age and gender. Group allocation was done by a third (external) party. Patients were given a personal addressed sealed envelope containing the words "code A" or "code B". They were instructed to give the envelope to the pharmacist in return for a 3-month supply of capsules of either U-CoQ10 60 mg/day, or corn flour-based placebo. The capsules for the two groups were externally identical.

Assessment Parameters

All patients underwent a detailed anamnesis before and after the trial, using a survey based in part on the Minnesota Living with Heart Failure Questionnaire[19] to determine the quality of life and the severity of heart failure symptoms subjectively. Also assessed were echocardiography findings (aortic diameter, left atrium diameter, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, posterior wall thickness, interventricular septal width, shortening fraction), results on the 6-min walk test, and blood levels of atrial natriuretic factor (ANF) and tumor necrosis factor (TNF) alpha.

Six weeks into the trial, we measured CoQ10 blood levels to assess absorption and compliance. Compliance was also checked at the end of the study by interviewing the participants and by a count of the remaining capsules by pharmacy personnel.

Sample Preparation and Analyses

Blood was collected in the morning, after an 8-h fast. Coenzyme Q10 blood levels were analyzed by the method described by Grossi *et al.*[20] Our modification was that we used 2 cc instead of 1 cc of plasma due to the low Q10 levels in serum.

Atrial natriuretic factor: Plasma ANF determination was performed by radioimmunoassay kit (Peninsula Laboratories, Belmont, Calif., USA). One ml of blood was drawn into test tubes containing 50 µl of 0.01M solution of K_3EDTA to which aprotinin 500 kIU/ml was added. The plasma was extracted on C-18 columns (200 mg) using 1% trifluoroacetic acid and 60% acetonitrite in 1% trifluoroacetic acid as solvents. The extract was evaporated to dryness. One ml of phosphate buffer was added, and the tubes were kept frozen at −20°C until assay. Results were expressed as pg/ml.

Tumor necrosis factor alpha: Plasma TNF-alpha determination was performed using a solid phase, two-sided chemiluminescent enzyme immunometric assay (immulite auto-

mated analyzer) kit (DPC, Los Angeles, Calif., USA). The plasma samples were kept at −20°C until assay. Results were expressed as pg/ml.

Statistical Analysis

Statistical analysis was performed with the BMDP statistical software (Stonehill Corporate, Saugus, Mass., USA), as follows: (1) Repeated measure for time 0 and time 3 months with one nested variable (two groups) performed for all variables, and (2) analysis of variance with data screening.

Categorical variables are described as frequencies and percentages, and continuous variables as the means (standard deviation [SD]). A p value of < 0.05 was considered statistically significant.

Results

Five patients failed to complete the study because of death, need for heart transplantation, drug-induced intestinal upset,[16] inconvenient travel connections, and lack of compliance (one patient each). Gastrointestinal upset was the only side effect of CoQ10 reported.

Anamnesis

The placebo group showed increased frequency of nocturia (2.1–2.63 times per night), increased severity of fatigue (median score, from 2.9 to 3.54), decreased severity in dyspnea (median score, from 3.68 to 3.5), and no change in NYHA classification (from median 3.68 to 3.6). Findings for the study group were as follows: decrease in nocturia (1.7–1.46 times per night), decrease in fatigue (2.4–1.96), decrease in dyspnea (2.9–2.1), and decrease in NYHA functional class (from 3.1 to 2.4). The findings for all four parameters in the study group were significant ($p < 0.01$, $p < 0.001$, $p = 0.04$, and $p = 0.01$, respectively; Figs. 1–4).

Six-Minute Walk Test

The most significant result was noted for the 6-min walk test. The patients treated with U-CoQ10 showed a significant improvement in performance, from 269.5 to 382.2 m ($p < 0.0001$), whereas the placebo group deteriorated, from 254 to 177 m (Fig. 5).

The study group, as expected, showed an increase in blood levels of CoQ10 at 6 weeks, from a median of 0.22 to 0.83 mg/l. Levels in the placebo group measured 0.18 mg/l at onset of the study and 0.178 mg/l at 6 weeks.

Echocardiography

In neither group there was significant change in cardiac chamber contractility between the beginning and the end of the study, and there were no significant differences between the groups at either time point.

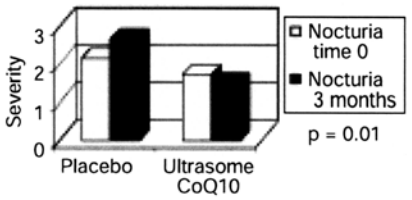


FIG. 1 Nocturia at entry and 3 months later in the placebo and Ultrasome coenzyme Q10 groups ($p = 0.01$).

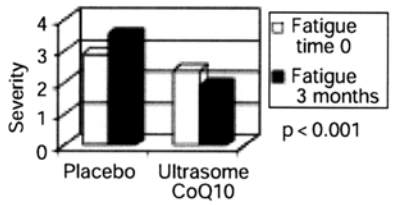


FIG. 2 Fatigue at entry and 3 months later in the placebo and Ultrasome coenzyme Q10 groups ($p < 0.001$)

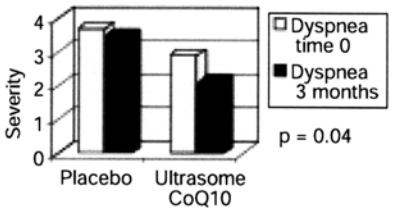


FIG. 3 Dyspnea at entry and 3 months later in the placebo and Ultrasome coenzyme Q10 groups ($p = 0.04$).

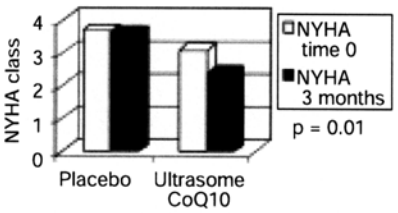


FIG. 4 New York Heart Association (NYHA) class at entry and 3 months later in the placebo and Ultrasome coenzyme Q10 groups ($p = 0.01$).

Atrial natriuretic and tumor necrosis factor blood levels: The study group had baseline ANF mean levels of 231 ± 22.5 pg/ml, which decreased to 185.2 ± 21.6 pg/ml at the end of the study. The placebo group had mean levels of 307.33 ± 29.4 pg/ml, which decreased to 260.22 ± 23.8 pg/ml at the end of the study. The results were not significant.

The study group had baseline TNF mean levels of 8.25 ± 2.3 pg/ml, which increased to 10.47 ± 3.1 pg/ml at the end of the study. The placebo group had mean levels of 15.57 ± 2.9

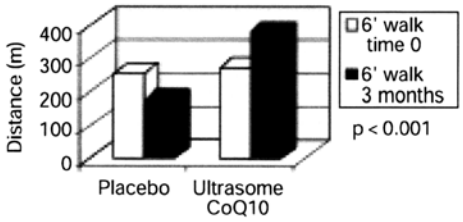


FIG. 5 Six-minute walk test at entry and 3 months later in the placebo and Ultrasome coenzyme Q10 groups ($p < 0.0001$).

pg/ml, increasing to 16.1 ± 3.5 pg/ml at the end of the study. The results were not significant.

Discussion

The study of the mechanism of action of CoQ10 has highlighted its importance in the diastolic phase of the cardiac cycle, when cellular energy is highest, in preparation for systolic contraction.[21] Coenzyme Q10 deficiency may be due to the primary progression of myocardial failure leading to excessive utilization of the enzyme. It may also be secondary to intake of 3-hydroxy-3-methylglutaryl (HMG) coenzyme A, which lowers blood cholesterol levels by blocking cholesterol biosynthesis, and thereby CoQ10 biosynthesis.[22] In both cases, myocardial cell function and structure are impaired. External correction of CoQ10 levels can presumably restore the mitochondrial bioenergetics[23] and exert an antioxidant effect, which increases the oxygen delivery to the striated skeletal muscle. The findings of the present study support the efficacy of CoQ10 treatment on symptoms in patients with end-stage heart failure. Unlike those in the placebo group, the patients given CoQ10 showed a significant reduction in fatigue during activities of daily living, in addition to significant improvements in nocturia and dyspnea and in results of the 6-min walk test. Although there was no change in the medical regimen, the study group showed an improvement of 0.7 in NYHA class, whereas the placebo group did not. Accordingly, in an Italian multicenter study, Baggio *et al.*[16] demonstrated that the use of adjunctive CoQ10 induces clinical improvement in patients with mild to moderate heart failure. These authors noted a better outcome in patients in NYHA class 2, with over 90% showing improvement, and a reduction in peripheral resistance resulting in a significant decrease in blood pressure,[24] perhaps via the inhibitory effect on plasma catecholamine levels in heart failure. They therefore suggest that treatment is better started sooner, when there are more living myocytes and less fibrous tissue and oxidative damage. Furthermore, Langsjoen *et al.*[23] claimed that empty CoQ10 apoenzyme-binding sites may be occupied within a day or two of CoQ10 administration, providing some benefit to cardiac function as early as 1 week. The improvement that occurs over a longer period may be due to the slowly increasing levels of CoQ10 apoenzymes because of their increased synthesis and degradation. In another crossover, blinded multicenter trial, Hofman-Bang *et al.*[17] reported that in patients with moderate to severe congestive heart failure, oral treatment with CoQ10 has a small but significant beneficial effect on exercise capacity, quality of life, and ejection fraction at volume load. This conclusion was supported by Mortensen.[25] Morisco *et al.*[26] reported that the addition of CoQ10 to conventional treatment significantly reduced hospitalization for worsening heart failure and serious complications (pulmonary edema, arrhythmia, and cardiac asthma) over a 1-year period. However, our results are not comparable as none of the patients in either group required hospitalization and the study period was much shorter.

We found no difference between the groups in cardiac chamber dimensions or contractility, and no change in either group in this parameter over time. The data on this subject in the literature are controversial. Watson *et al.*[27], using half the CoQ10 dose we used, also found no differences in cardiac chamber volumes. Langsjoen *et al.*[21] noted a significant change only in patients with dilated cardiomyopathy with low fractional shortening at baseline; they improved significantly with the addition of CoQ10. However, in their patients with both ischemic and dilated cardiomyopathy, left ventricular wall thickness decreased and diastolic function improved after CoQ10 treatment. We believe that echocardiography findings should be interpreted with caution in this patient population. In ischemic cardiomyopathy, the myocytes may suffer from free-radical burden, and CoQ10 is known to be a free-radical scavenger.[1] However, a patient's predominantly dilated cardiomyopathy may benefit less because of progressive replacement of myocytes by fibrocytes, which are insensitive to exogenous CoQ10. In our study, 25 patients had predominantly ischemic cardiomyopathy and only 7 had dilated cardiomyopathy, and therefore comparing them was statistically unfeasible.

Atrial natriuretic factor is a 28-amino acid peptide, produced mainly in the heart atria and to a lesser extent in the ventricles; it is released into the circulation during atrial distension.[28] In patients with heart failure, plasma ANF concentrations rise concomitantly with the increase in atrial pressure and proportionately to the severity of heart failure.[29] At the kidney level,[30] high ANF level induces natriuresis and suppression of the renin–aldosterone axis. In our study, the starting ANF level in both groups was very high, corresponding to the reported levels in patients with heart failure.[31] Three months later, both groups showed a median decrease of 50 pg/ml.

Several studies have reported an increase in measurable TNF in the presence of heart failure.[32, 33] Anker *et al.*[34] suggested that heart failure is associated with mesenteric stasis which leads to an increase in enteric permeability, bacteria passage out of the intestinal lumen, activation of the immune system, and ultimately a rise in TNF.

Our candidates for heart transplantation had mean basal TNF levels > 8.25 pg/ml (ten-fold the normal value), which showed no significant change 3 months later in either group.

The absence of changes in echocardiography parameters and in ANF and TNF blood levels has several possible explanations:

1. The dosage of CoQ10 was too low for the gravity of the disease; some trials describe daily doses of 600 mg/day.[16]

2. The trial period was long enough to show clinical improvement but too short to show cardiovascular anatomic and hormonal reorganization.
3. Echocardiographic changes were lacking due to progressive replacement of myocytes rich in Q10 reserve by fibrotic tissue, which is not contractible, low in Q10 reserve, and unresponsive to external administration of it.
4. The ANF levels corresponded to distended heart atrium failure.

Coenzyme Q10 is known to be virtually free of side effects;[16] only 1 of our 32 patients complained of drug-induced gastrointestinal symptoms. Coenzyme Q10 is structurally related to vitamin K and possesses a procoagulant effect that may result in diminishing the effect of warfarin therapy.[35]

In our study, despite clinical improvement, we did not decrease or alter the patients' ongoing conventional treatment because of the fragile steady state. However, it may be possible to decrease the intake of cardiovascular drugs in heart transplant candidates receiving CoQ10 gradually under close monitoring,[24] and thereby improve their quality of life (some of these patients take 20–25 pills per day). Further studies are needed to determine feasibility and the timing of gradual drug diminution.

Conclusion

The heart transplant candidates who took CoQ10 showed significant improvement in functional status, clinical symptoms, and quality of life. However, there were no objective changes in echo measurements or TNF and ANF blood levels. We suggest that CoQ10 should be considered as an optional addition to a regular medical regimen for the management of end-stage heart failure. The apparent discrepancy between the significant clinical improvement and the lack of change in cardiac parameters requires further investigation.

Acknowledgment

The authors thank Gloria Ginzach for her editorial assistance.

References

1. Lehninger AI: Oxidation–reduction enzymes and electron transport. In *Biochemistry*, 2nd edition, p. 493–497. New York: Worth Publishers, 1975
2. Lowe JS, Mortom RA, Vernon J: Unsaponifiable constituents of kidney in various species. *Biochem J* 1957;67:228–234
3. Cunningham NF, Morton RA: Unsaponifiable constituents of liver: Ubiquinone and substance SC in various species. *Biochem J* 1959;72:92–99
4. Linn BO, Page AC, Wong EI Wong AL, Gale PH, Shunk GH, Folkers K: Isolation and distribution of coenzyme Q10 in animal tissues. *Am Chem Soc* 1959;81:4007–4010
5. Crane FL, Hatefi Y, Lester RL, Widmer G: Isolation of a quinone from beef heart mitochondria. *Biochem Biophys Acta* 1957;25:220–221
6. King TE: Ubiquinone proteins and cardiac mitochondria. In *Function of Quinones in Energy Conserving Systems* (Ed. Trumpower BL), p. 3–25. New York: Academic Press, 1982
7. Folkers K, Littaru GP, Ho L, Runge TM, Havanonda S, Cooley D: Evidence for a deficiency of coenzyme Q10 in human heart disease. *Int J Vitamin Res* 1970;40:380–390
8. Littaru GP, Ho L, Folkers K: Deficiency of coenzyme Q10 in human heart disease, Part 1. *Int J Vitamin Res* 1972;42:291–305
9. Littaru GP, Ho L, Folkers K: Deficiency of coenzyme Q10 in human heart disease, Part 2. *Int J Vitamin Res* 1972;42:413–434
10. Mortensen SA: Perspectives on therapy of cardiovascular diseases with coenzyme Q10 (ubiquinone). *Clin Invest* 1993;71S:116–123
11. Folkers K, Baker L, Richardson PC, Shizukuishi S, Takamura K, Drzewoski J, Lewandowski J, Ellis JM: *Biomedical and Clinical Aspects of Coenzyme Q*, Vol. 2 (Eds. Yamamura Y, Folkers K, Ito Y), p. 449. Amsterdam: Elsevier/North Holland, 1980
12. Yamamura Y, Ishiyama T, Yamogami T, Morita Y, Ishio I, Kashiwamura S, Terader A, Tsukamoto N, Toyama S, Nakajima Y, Wada N: Clinical use of coenzyme Q for treatment of cardiovascular disease. *Jpn Circ J* 1967;31:168–169
13. Yamamura Y: A survey of the therapeutic uses of coenzyme Q10. In *Coenzyme Q Biochemisty, Bioenergetics and Clinical Applications of Ubiquinone* (Ed. Lenaz G), p. 479–507. New York: Wiley-Interscience, 1985
14. Mortensen SA, Vadhanavikit S, Baandrup U, Folkers K: Long-term coenzyme Q10 therapy: A major advance in the management of resistant myocardial failure. *Drug Exp Clin Res* 1985;8:581–593
15. Langsjoen PH, Vadhanavikit S, Folkers K: Effective treatment with coenzyme Q10 of patients with chronic myocardial disease. *Drug Exp Clin Res* 1985;8:577–579
16. Baggio E, Gandini R, Plancher AC, Passeri M, Carmosino G: Italian multicenter study on the safety and efficacy of coenzyme Q10 as adjunctive therapy in heart failure. *Molec Aspects Med* 1994;15s:287–294
17. Hofman-Bang C, Rehnqvist N, Swedberg K, Wiklund I, Astrom H: Coenzyme Q10 as an adjunctive in treatment of congestive heart failure. The Q10 study group. *J Cardiac Fail* 1995;1(2):101–107
18. Folkers K, Langsjoen P, Langsjoen PH: Therapy with coenzyme Q10 of patients in heart failure who are eligible or ineligible for a transplant. *Biochem Biophys Res Comm* 1992;182:247–253
19. Rector J, Cohn JN: Assessment of patient outcome with the Minnesota Living with Heart Failure Questionnaire—Reliability and validity during a randomized, double-blind, placebo-controlled trial of pimobendan. Pimobendan Multicenter Research Group. *Am Heart J* 1992;124:1017–1025
20. Grossi G, Bargossi AM, Fiorella PL, Piazzi S: Improved high-performance liquid chromatographic method for the determination of coenzyme Q10 in plasma. *J Chromat* 1992;593:217–226
21. Langsjoen H, Langsjoen P, Langsjoen P, Willis R, Folkers K: Usefulness of coenzyme Q10 in clinical cardiology: A long term study. *Molec Aspects Med* 1994;15:165–175
22. Folkers K, Langsjoen P, Willis R, Richardson P, Xia LJ, Ye CQ, Tamagawa H: Lovastatin decreases coenzyme Q levels in humans. *Proc Natl Acad Sci USA* 1990;87:8931–8934
23. Langsjoen PH, Vadhanavikit S, Folkers K: Response of patients in classes 3 and 4 of cardiomyopathy to therapy in a blind and crossover trial with coenzyme Q10. *Proc Natl Acad Sci USA* 1985;82:4240–4244
24. Digiesi V, Cantini F, Brodbeck B: Effect of coenzyme Q10 on essential arterial hypertension. *Curr Ther Res Clin Exp* 1990;47:841–845
25. Mortensen SA: Coenzyme Q10 as an adjunctive therapy in patients with congestive heart failure. *J Am Coll Cardiol* 2000;36:304–305
26. Morisco C, Trimarco B, Condorelli M: Effect of coenzyme Q10 therapy in patients with congestive heart failure: A long-term multicenter randomized study. *Clin Invest* 1993;71(8 suppl):s134–136
27. Watson PS, Scalia GM, Galbraith A, Burstow DJ, Bett N, Aroney CN: Lack of effect of coenzyme Q on left ventricular function in patients with congestive heart failure. *J Am Coll Cardiol* 1999;33:1549–1552
28. Boland DJ, Abraham WT: Natriuretic peptides in heart failure. *Cong Heart Fail* 1998;4:23–33
29. Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, Ogawa H, Okumura K, Mukoyama M, Nakao K: Localisation and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation* 1994;90:195–203
30. Kim JK, Summer SN, Durr J, Schrier RW: Enzymatic and binding effects of atrial natriuretic factor in glomeruli and nephrons. *Kidney Int* 1989;35:799–805
31. Steele IC, McDowell G, Moore A, Campbell NP, Shaw C, Buchanan KD, Nicholls DP: Responses of atrial natriuretic peptide and brain natriuretic peptide to exercise in patients with chronic heart failure and normal control subjects. *Eur J Clin Invest* 1997;27:270–276
32. Zhao SP, Zeng LF: Elevated plasma levels of tumor necrosing factor in chronic heart failure with cachexia. *Int J Cardiol* 1997;58:257–261
33. Milani RV, Mehra MR, Endres S, Eigler A, Cooper ES, Lavie CJ Jr, Ventura HO: The clinical relevance of circulating tumor necrosis factor alpha in acute decompensated chronic heart failure without cachexia. *Chest* 1996;110(4):992–995
34. Anker SD, Egerer KR, Volk HD, Kox WJ, Poole-Wilson PA, Coats AJ: Elevated soluble CD14 receptors and altered cytokines in chronic heart failure. *Am J Cardiol* 1997;79(10):1426–1430
35. Landbo C, Almdal TP: Interaction between warfarin and coenzyme Q10. *Ugeskr Laeger* 1998;160:3226–3227

Hypertension
Journal of the American Heart Association

High Dietary Taurine Reduces Apoptosis and Atherosclerosis in the Left Main Coronary Artery: Association With Reduced CCAAT/Enhancer Binding Protein Homologous Protein and Total Plasma Homocysteine but not Lipidemia

Anthony Zulli, Eza Lau, Bagus P.P. Wijaya, Xin Jin, Komang Sutarga, Grace D. Schwartz, Jonathon Learmont, Peter J. Wookey, Angelo Zinellu, Ciriaco Carru and David L. Hare

Hypertension. 2009;53:1017-1022; originally published online April 27, 2009;
doi: 10.1161/HYPERTENSIONAHA.109.129924
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563

High Dietary Taurine Reduces Apoptosis and Atherosclerosis in the Left Main Coronary Artery

Association With Reduced CCAAT/Enhancer Binding Protein Homologous Protein and Total Plasma Homocysteine but not Lipidemia

Anthony Zulli, Eza Lau, Bagus P.P. Wijaya, Xin Jin, Komang Sutarga, Grace D. Schwartz, Jonathon Learmont, Peter J. Wookey, Angelo Zinellu, Ciriaco Carru, David L. Hare

Abstract—We sought to determine whether taurine could specifically protect against coronary artery disease during an atherogenic diet and whether taurine affects the lipid profile, metabolites of methionine, and endothelial atherogenic systems. Rabbits were fed one of the following diets for 4 weeks: (1) control diet; (2) 0.5% cholesterol+1.0% methionine; or (3) 0.5% cholesterol+1.0% methionine+2.5% taurine. Endothelial function was examined, and the left main coronary artery atherosclerosis was quantified by stereology and semiquantitative immunohistochemistry to determine the endothelial expression of proteins related to the NO, renin-angiotensin, endoplasmic reticulum, and oxidative stress systems, as well as apoptosis. Taurine normalized hyperhomocysteinemia ($P<0.05$) and significantly reduced hypermethioninemia ($P<0.05$) but not lipidemia. The intima:media ratio was reduced by 28% ($P=0.034$), and atherosclerosis was reduced by 64% ($P=0.012$) and endothelial cell apoptosis by 30% ($P<0.01$). Endothelial cell CCAAT/enhancer binding protein homologous protein was normalized ($P<0.05$). Taurine failed to improve hyperlipidemia, endothelial function, or endothelial proteins related to the NO, renin-angiotensin, and oxidative stress systems. Taurine reduces left main coronary artery wall pathology associated with decreased plasma total homocysteine, methionine, apoptosis, and normalization of CCAAT/enhancer binding protein homologous protein. These results elucidate the antiapoptotic and antiatherogenic properties of taurine, possibly via normalization of endoplasmic reticulum stress. (***Hypertension.*** **2009;53:1017-1022.)**

Key Words: cholesterol ■ homocysteine ■ methionine ■ atherosclerosis ■ plaque ■ CHOP

Cardiovascular disease deaths have decreased in some developed countries but increased in low- to middle-income countries.[1] Coronary heart disease remains the most common cause of death throughout the world and is predicted to remain so in higher-income countries and will become so in lower-income countries by the year 2030.[2]

The prevention and treatment of atherosclerotic cardiovascular disease have used many interventional modalities. One of the most successful has been the use of statin therapy, which decreases plasma low-density lipoprotein (LDL) cholesterol, has modest effects on raising plasma high-density lipoprotein (HDL) cholesterol, and has pleiotropic effects, of which the clinical importance remains uncertain.[3] Although statin therapy has a potent effect on reducing cardiovascular events, all of the randomized clinical trials still indicate a significant residual risk of events in the statin intervention arm of the studies. It is currently unclear whether this is purely from some patients not reaching low enough LDL levels or that additional therapeutic modalities are required.

Populations with higher fish intake have lower cardiovascular death rates than populations with high meat intake.[4] Taurine is found in high concentrations in fish, the major human source of taurine.[5] Increased taurine intake is inversely related to the incidence of coronary heart disease[6] and has been associated with reduced insulin resistance,[7] whereas taurine deficiency has been associated with increased obesity.[8]

Methionine, which is metabolized to homocysteine, is coingested with cholesterol in meat. Increased levels of plasma total homocysteine (tHcy) have consistently been associated with increased atherosclerotic burden in animal models[9,10] and also clinical cardiovascular events. However, simplistic approaches to reduce plasma tHcy by small amounts using B vitamins and folic acid have a possible beneficial role for the reduction of stroke,[11–13] but this is not the case for myocardial causes of clinical events.[11] Because taurine might affect methionine absorption,[14] we postulated

Received January 29, 2009; first decision February 4, 2009; revision accepted March 26, 2009.

From the Departments of Cardiology (A.Z., E.L., B.P.P.W., X.J., K.S., G.D.S., J.L., P.J.W., D.L.H.) and Medicine (A.Z., D.L.H.), University of Melbourne, Austin Health, Heidelberg, Australia; and the Department of Biomedical Sciences (A.Z., C.C.), University of Sassari Viale S Pietro, Sassari, Italy.

Correspondence to Anthony Zulli, Vascular Laboratory, Department of Cardiology, Austin Health, Heidelberg, Australia. E-mail azulli@unimelb.edu.au

Hypertension **is available at http://hyper.ahajournals.org** **DOI: 10.1161/HYPERTENSIONAHA.109.129924**

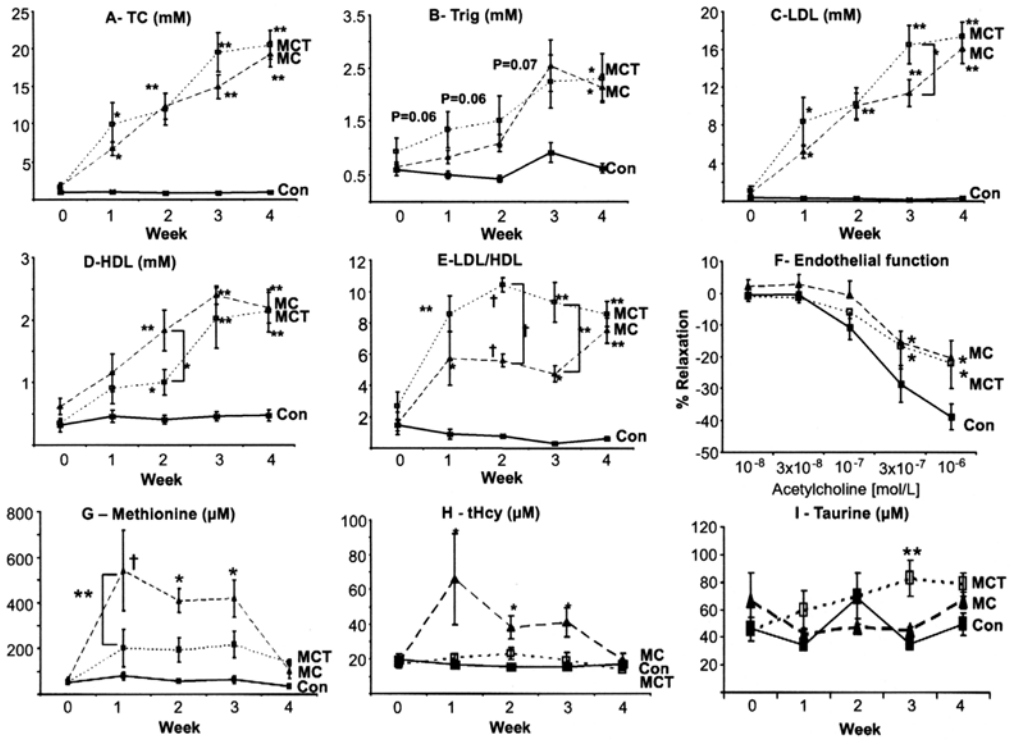


Figure 1. Blood lipids. A-TC, Total plasma cholesterol significantly increased in both experimental groups. B-Trig, Total plasma triglyceride also rose during the dietary regimen. C-LDL, Total plasma LDL (as determined by the Friedewald formula) significantly increased in both experimental groups. D-HDL, Total plasma HDL significantly increased in both experimental groups after the second week. E-LDL/HDL, Total plasma LDL:HDL ratio significantly increased in both experimental groups after the first week and continued to rise until sacrifice. F-Endothelial function, Endothelial dysfunction was present in the MC group, and taurine failed to restore this effect to normal ($P<0.05$). G-Methionine, Plasma methionine significantly increased in MC; however, the addition of taurine to the diet significantly reduced the plasma methionine level. H-Homocysteine, tHcy significantly increased only in the MC group after the first week and normalized at the fourth week. I, Plasma taurine was only increased in MCT at the third week ($P<0.001$). *$P<0.05$; **$P<0.01$; †$P<0.001$.

that a diet high in taurine, while impairing the development of atherosclerosis, might also reduce dietary-induced hyperhomocysteinemia.

Although clinical trials of simple, oral antioxidant therapies, eg, vitamin E or combinations of vitamins C, E, and β-carotene, have focused on the absorption of the oxygen radical,[15,16] the hypochlorite anion has not as yet been targeted. The hypochlorite anion (OCl^-/H^+) is a powerful oxidant that is able to oxidize both HDL and LDL into hypochlorite-modified atherogenic forms (hypochlorous LDL[17] and hypochlorous HDL[18]). Hypochlorous LDL particles are recognized by the scavenger receptor class B type I, which also impairs reverse cholesterol transport from cells.[19] Taurine removes the oxidant hypochlorite and, thus, might impair the hypochlorite modification of LDL to hypochlorous LDL. Furthermore, taurine has several potentially beneficial cardiovascular effects, which involve regulating the NO system and endothelial function,[20–23] the renin-angiotensin system (RAS),[24–27] the oxidative stress system, and apoptosis,[28–38] as well as the endoplasmic reticulum (ER) stress system.[39,40]

Thus, we hypothesized that reduction of circulating HOCl by taurine[41] during an atherogenic diet aimed at increasing both tHcy and LDL[9,10] would impair the formation of plasma hypochlorous LDL and endothelial cell apoptosis and that high dietary taurine would be associated with beneficial changes in the NO system, renin-angiotensin system, oxidative stress system, and ER stress system in the endothelial layer of the left main coronary artery.

Methods

Male New Zealand white rabbits at 3 months of age were randomized into 3 groups and fed one of the following diets for 4 weeks: (1) control (n=5); (2) a normal rabbit chow diet supplemented with 0.5% cholesterol+1.0% methionine+5.0% peanut oil (n=5; MC); or (3) a normal rabbit chow diet supplemented with 0.5% cholesterol+1.0% methionine + 2.5% taurine+5.0% peanut oil (n=5; MCT). The animals were housed in individual cages and maintained at a constant temperature of ≈21°C. Food and water were supplied ad libitum. The experiments were carried out according to the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The animals were then euthanized by an overdose IV injection of ketamine and xylazine via the main ear vein, as described previously in our laboratory.[9,10] The aorta and heart were then excised. The aorta was cleaned of connective tissue and fat and used for isometric tension studies, and an apical section of the heart, which included the left main coronary artery, was cut and immersed in freshly prepared 4% paraformaldehyde solution in 1×PBS (pH 7.4).

For detailed methods relating to isometric tension studies, left main coronary artery analysis, apoptosis detection by single-strand DNA (ssDNA), methionine, taurine, plasma thiols, lipids, and homocysteine, please see the online data supplement at http://hyper.ahajournals.org.

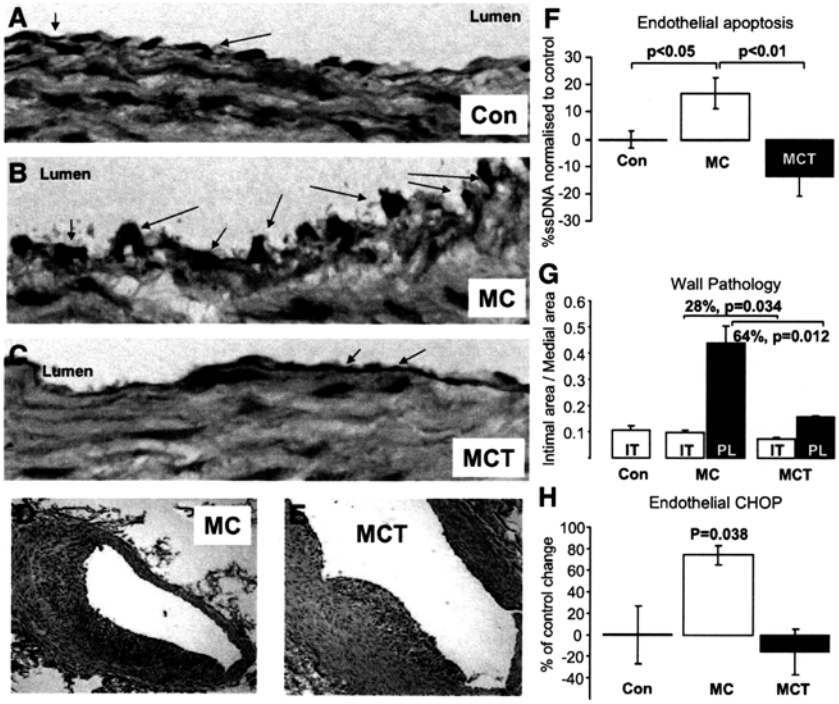


Figure 2. Immunostaining of ssDNA in the left main coronary artery using a commercially available monoclonal antibody (A through C and quantified in F). Neointimal formation and plaque formation in the left main coronary artery in MCT group (E) were significantly reduced compared with those in the MC group (D), as quantified in G. IT indicates intimal thickening; PL, plaque. Endothelial CHOP was normalized to control (H).

Data Analysis

Comparisons between MC and MCT were performed by Student *t* test for single comparisons. A 1-way ANOVA followed by Newman-Keuls posthoc test was performed for comparison among the control, MC, and MCT groups. A 2-way ANOVA followed by Bonferroni posthoc test was performed for comparison of weekly effects. In all of the cases, $P<0.05$ was accepted as significant. All of the data are expressed as mean±SEM.

Results

Blood total cholesterol (TC) in both MC and MCT significantly increased after the first week of the diet and continued to increase until week 4 (Figure 1A, TC). There was no significant difference in TC between the MC and MCT groups. As well, plasma triglycerides also increased over the dietary period in both the MC and MCT groups, but again without difference between groups (Figure 1B, Trig). When LDL was calculated using the Friedewald formula, LDL remained higher throughout the dietary protocol in the MCT group, and this result was significant at the third week ($P<0.05$; Figure 1C, LDL). However, plasma HDL did remain lower throughout the dietary protocol in the MCT group, and this decrease was significant at the 2-week point ($P<0.05$; Figure 1D, HDL). The overall worse lipid profile is illustrated by the TC:HDL (second and third weeks; $P<0.01$; figure not shown) and LDL:HDL (second and third weeks; $P<0.01$; Figure 1E, LDL/HDL) ratios. After 4 weeks of dietary manipulation, endothelial dysfunction was evident in the MC group ($P<0.05$), but taurine did not improve endothelial function in the abdominal aorta, which remained significantly impaired compared with the control ($P<0.05$; Figure 1F). Taurine markedly inhibited the increase in plasma methionine (Figure 1G) and completely inhibited the increase in the tHcy level observed in the MC group ($P<0.05$; Figure 1H). Plasma taurine was only increased in the third week in MCT versus MC (Figure 1I). Interestingly, there was no significant change in plasma methionine or tHcy at the end of the regimen.

Apoptotic endothelial cells, as detected by ssDNA, were present in the left main coronary artery of the control group (arrows, Figure 2A). However, the MC group showed a 17% increase in endothelial ssDNA staining ($P<0.05$; Figure 2B). The addition of taurine significantly reduced this staining to −13% below control levels (P value not significant versus control; $P<0.01$ versus MC; Figure 2C). Plaque cells also showed positive ssDNA cells in both MC (Figure 2D) and MCT (Figure 2E) groups, and apoptosis is quantified in Figure 2F. Despite the worse lipid profile, wall pathology (intima:media ratio) was also decreased by the addition of taurine to the diet. Intimal thickening, as determined by a neointima devoid of macrophages, decreased by 28% in the taurine-treated group compared with the MC group (Figure 2D and 2E and quantified in Figure 2G; $P<0.05$). As well, atheroma in the left main coronary artery was also reduced by 64% (Figure 2D and 2E and quantified in Figure 2G). ER stress, as measured by endothelial CCAAT/enhancer binding protein homologous protein (CHOP), increased by 74% in MC ($P<0.05$), and this was normalized by taurine treatment (Figure 2H).

For analysis of plasma cysteinylglycine, glutathionine, and cysteine please see Figure S1. For analysis of NO synthase (NOS) proteins (total eNOS, peNOS-S1177, peNOS-T495, and caveolin-1), for RAS (angiotensin-converting enzyme 2, angiotensin II type 2 receptor, angiotensin-converting enzyme, and angiotensin II type 1 receptor), and for oxidative stress system (heat shock protein 70, heme-oxygenase-1, myeloperoxidase, nitrotyrosine, inducible NOS), please see Figure S1. For Western blot analysis of hypochlorous LDL, plaque hypochlorous LDL, correlation

between plasma homocysteine, and hypochlorous acid LDL, please see Figure S2.

Discussion

This is the first comprehensive study examining the effect of high dietary taurine supplementation on the left main coronary artery. The major findings of this investigation are as follows: (1) taurine supplementation inhibited the development of hyperhomocysteinemia and hypermethioninemia and temporal effects of diet on plasma tHcy and methionine levels; (2) taurine supplementation inhibited endothelial cell apoptosis possibly by reduction in ER stress; (3) taurine supplementation reduced left main coronary artery atherosclerosis; and (4) taurine supplementation did not significantly affect the endothelial level of proteins associated with the NOS, RAS, or oxidative stress systems.

The reduction in tHcy by dietary taurine presented in this study was not attributed to increased metabolism of homocysteine to cysteine or other sulfur-containing amino acids, nor the reduced formation of homocysteine from methionine. Indeed, we observed that high dietary taurine significantly impaired the increase in plasma methionine compared with the untreated group, indicating that other possible routes of methionine metabolism are upregulated by taurine or that taurine can impair the absorption of methionine. Indeed, this latter hypothesis is supported by a recent study in cultured CaCo-2 cells, whereby methionine transport across the apical membrane of Caco-2 cells was affected by extracellular pH and taurine.[14] Thus, it appears that taurine can impair the absorption of methionine and, thus, provide a novel way to reduce plasma tHcy. These results might have implications in nutrition. As the popularity of processed fast foods high in methionine is increasing and has been linked to increased tHcy,[42] the addition of taurine to the diet might help stem the increase in tHcy and, thus, reduce cardiovascular disease risk. Further research to determine whether these results hold true in humans is warranted.

Furthermore, impaired methionine transport across the intestinal epithelia because of other factors could be causing the temporal effect on tHcy and methionine after the first dietary week. Indeed, we first eluded to this temporal effect in a similar study in rabbits on a 3-month dietary protocol.[10] It is unclear why this phenomenon occurs; however, it is possible that both gut Na+-dependent and Na+-independent mechanisms[14] are involved. As well, these results suggest that, if these effects hold true in humans, plasma methionine or tHcy might not be a reflection of dietary methionine intake.

In the study presented here, taurine inhibited apoptotic coronary endothelial cells even on a background of a worse lipid profile. Apoptosis could be inhibited by a reduction in ER stress, as measured by a normalization of CHOP protein. In vitro research suggests that homocysteine causes ER stress, and this stimulates CHOP mRNA in human umbilical vein endothelial cells[43] and apoptosis in cultured endothelial cells.[44,45] Our study confirms this theory, because CHOP protein was significantly increased in the atherogenic group, which also had higher plasma tHcy levels, and, thus, a reduction in tHcy would impair apoptosis.

Furthermore, novel insights into the mechanisms involved in homocysteine-induced cellular damage include homocysteinylation of proteins. Both HDL[46] and the intracellular atheroprotective enzyme metallothionein[47,48] can become dysfunctional via homocysteinylation. For example, Barbato et al[47] found that homocysteinylation of metallothionein impairs its zinc binding function, thus impairing its superoxide scavenging properties and possibly amplifying oxidative stress in endothelial cells. Thus, targeting a reduction in both tHcy and cellular homocysteine to reduce protein homocysteinylation could be a novel avenue for the treatment of homocysteine-induced vascular damage.

The decreased intimal thickening and reduced atherosclerosis in the left main coronary artery of this model during taurine treatment could be attributed to the impaired increase in tHcy. Although clinical trials involving the reduction of tHcy by vitamin supplementation have failed to significantly reduce myocardial events,[11] our studies in rabbits[9,10] and others in mice[49,50] show that hyperhomocysteinemia on a hyperlipidemic background does enhance the development of atherosclerotic plaque burden in animal models. The human studies only managed small reductions (eg, 2.4 μmol/L) in plasma tHcy, using an intervention that would reduce tHcy by increasing methionine, which might not be the most advantageous way of reducing tHcy. In addition, it is possible that the role of hyperhomocysteinemia might be more important in the earlier development of atherosclerotic plaque rather than in reducing events in patients with existing plaque.

It is unclear whether increased triglyceride can directly induce apoptosis or is affected by dietary taurine. In this study, we showed that plasma triglyceride is not affected by dietary taurine and that the prevention of endothelial apoptosis can occur regardless of the triglyceride level. This finding is supported by in vitro experiments, whereby Nyblom et al[51] showed reduced β-cell apoptosis, although the triglyceride level did not change. Taken together, these results suggest that triglyceride might not be an important determinant of cellular apoptosis, at least in endothelial or β cells.

Taurine supplementation did not significantly affect the endothelial level of proteins associated with the NOS, RAS, or oxidative stress systems. For this discussion, please see the data supplement.

In conclusion, we show that the addition of 2.5% taurine to an atherogenic diet reduces left main coronary artery wall pathology on a background of a worse lipid profile. As well, taurine also significantly reduces endothelial ER stress, hyperhomocysteinemia, and hypermethioninemia and impairs left main coronary artery endothelial cell apoptosis without detectable effects on the NOS, RAS, or oxidative stress systems.

Perspectives

Atherogenesis is clearly related to factors other than purely the lipid profile. It is possible that dietary taurine might be used independently to not only impair coronary artery disease but also to reduce the burden of hyperhomocysteinemia caused by excess dietary intake of processed foods high in methionine. As well, therapeutic intervention aimed at reduc-

tion in ER stress could be a novel avenue for drug development for the further prevention of cardiovascular disease.

Acknowledgment

We acknowledge the expert technical support of Angela Kourakis, who aided in the processing of the left main coronary artery for paraffin and immunohistochemistry.

Sources of Funding

This work was supported by the National Health Medical Research Council and National Heart Foundation of Australia.

Disclosures

None.

References

1. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I–general considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation*. 2001;104:2746–2753.
2. World Health Organization. *Global Burden of Disease: 2004 Update*. 2008. Geneva, Switzerland: WHO Press; 2008.
3. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, Orazem J, Magorien RD, O'Shaughnessy C, Ganz P. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med*. 2005;352:29–38.
4. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA*. 2006;296:1885–1899.
5. Nittynen L, Nurminen ML, Korpela R, Vapaatalo H. Role of arginine, taurine and homocysteine in cardiovascular diseases. *Ann Med*. 1999;31: 318–326.
6. Yamori Y, Nara Y, Ikeda K, Mizushima S. Is taurine a preventive nutritional factor of cardiovascular diseases or just a biological marker of nutrition? *Adv Exp Med Biol*. 1996;403:623–629.
7. Nandhini AT, Thirunavukkarasu V, Ravichandran MK, Anuradha CV. Effect of taurine on biomarkers of oxidative stress in tissues of fructose-fed insulin-resistant rats. *Singapore Med J*. 2005;46:82–87.
8. Tsuboyama-Kasaoka N, Shozawa C, Sano K, Kamei Y, Kasaoka S, Hosokawa Y, Ezaki O. Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. *Endocrinology*. 2006;147: 3276–3284.
9. Zulli A, Hare DL, Buxton BF, Black MJ. High dietary methionine plus cholesterol exacerbates atherosclerosis formation in the left main coronary artery of rabbits. *Atherosclerosis*. 2004;176:83–89.
10. Zulli A, Widdop RE, Hare DL, Buxton BF, Black MJ. High methionine and cholesterol diet abolishes endothelial relaxation. *Arterioscler Thromb Vasc Biol*. 2003;23:1358–1363.
11. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, McQueen MJ, Probstfield J, Fodor G, Held C, Genest J Jr. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med*. 2006;354:1567–1577.
12. Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurol*. 2007;6:830–838.
13. Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y, Sun N, Liu L, Xu X. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet*. 2007;369:1876–1882.
14. Martin-Venegas R, Rodriguez-Lagunas MJ, Mercier Y, Geraert PA, Ferrer R. Effect of pH on L- and D-methionine uptake across the apical membrane of Caco-2 cells. *Am J Physiol Cell Physiol*. 2009;296: C632–C638.
15. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;360:7–22.
16. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med*. 2000;342:145–153.
17. Hazell LJ, Stocker R. Oxidation of low-density lipoprotein with hypochlorite causes transformation of the lipoprotein into a high-uptake form for macrophages. *Biochem J*. 1993;290:165–172.
18. Panzenboeck U, Raitmayer S, Reicher H, Lindner H, Glatter O, Malle E, Sattler W. Effects of reagent and enzymatically generated hypochlorite on physicochemical and metabolic properties of high density lipoproteins. *J Biol Chem*. 1997;272:29711–29720.
19. Marsche G, Hammer A, Oskolkova O, Kozarsky KF, Sattler W, Malle E. Hypochlorite-modified high density lipoprotein, a high affinity ligand to scavenger receptor class B, type I, impairs high density lipoprotein-dependent selective lipid uptake and reverse cholesterol transport. *J Biol Chem*. 2002;277:32172–32179.
20. Abebe W. Effects of taurine on the reactivity of aortas from diabetic rats. *Life Sci*. 2008;82:279–289.
21. Tan B, Jiang DJ, Huang H, Jia SJ, Jiang JL, Hu CP, Li YJ. Taurine protects against low-density lipoprotein-induced endothelial dysfunction by the DDAH/ADMA pathway. *Vascul Pharmacol*. 2007;46:338–345.
22. Abebe W, Mozaffari MS. Taurine depletion alters vascular reactivity in rats. *Can J Physiol Pharmacol*. 2003;81:903–909.
23. Abebe W, Mozaffari MS. Effects of chronic taurine treatment on reactivity of the rat aorta. *Amino Acids*. 2000;19:615–623.
24. Schaffer SW, Lombardini JB, Azuma J. Interaction between the actions of taurine and angiotensin II. *Amino Acids*. 2000;18:305–318.
25. Azuma M, Takahashi K, Fukuda T, Ohyabu Y, Yamamoto I, Kim S, Iwao H, Schaffer SW, Azuma J. Taurine attenuates hypertrophy induced by angiotensin II in cultured neonatal rat cardiac myocytes. *Eur J Pharmacol*. 2000;403:181–188.
26. Li C, Cao L, Zeng Q, Liu X, Zhang Y, Dai T, Hu D, Huang K, Wang Y, Wang X, Li D, Chen Z, Zhang J, Li Y, Sharma R. Taurine may prevent diabetic rats from developing cardiomyopathy also by downregulating angiotensin II type2 receptor expression. *Cardiovasc Drugs Ther*. 2005; 19:105–112.
27. Wang J, Peng YJ, Zhu DN. Amino acids modulate the hypotensive effect of angiotensin-(1–7) at the caudal ventrolateral medulla in rats. *Regul Pept*. 2005;129:1–7.
28. Olszanecki R, Marcinkiewicz J. Taurine chloramine and taurine bromamine induce heme oxygenase-1 in resting and LPS-stimulated J774.2 macrophages. *Amino Acids*. 2004;27:29–35.
29. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation*. 2001;104:365–372.
30. Farb A, Burke AP, Tang AL, Liang TY, Mannan P, Smialek J, Virmani R. Coronary plaque erosion without rupture into a lipid core: A frequent cause of coronary thrombosis in sudden coronary death. *Circulation*. 1996;93:1354–1363.
31. Arbustini E, Dal Bello B, Morbini P, Burke AP, Bocciarelli M, Specchia G, Virmani R. Plaque erosion is a major substrate for coronary thrombosis in acute myocardial infarction. *Heart*. 1999;82:269–272.
32. Sugiyama S, Okada Y, Sukhova GK, Virmani R, Heinecke JW, Libby P. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. *Am J Pathol*. 2001;158:879–891.
33. Vissers MC, Pullar JM, Hampton MB. Hypochlorous acid causes caspase activation and apoptosis or growth arrest in human endothelial cells. *Biochem J*. 1999;344:443–449.
34. Cave AC, Brewer AC, Narayanapanicker A, Ray R, Grieve DJ, Walker S, Shah AM. NADPH oxidases in cardiovascular health and disease. *Antioxid Redox Signal*. 2006;8:691–728.
35. Zou MH, Shi C, Cohen RA. High glucose via peroxynitrite causes tyrosine nitration and inactivation of prostacyclin synthase that is associated with thromboxane/prostaglandin H(2) receptor-mediated apoptosis and adhesion molecule expression in cultured human aortic endothelial cells. *Diabetes*. 2002;51:198–203.
36. Casey RG, Gang C, Joyce M, Bouchier-Hayes DJ. Taurine attenuates acute hyperglycaemia-induced endothelial cell apoptosis, leucocyte-endothelial cell interactions and cardiac dysfunction. *J Vasc Res*. 2007;44: 31–39.
37. Wu QD, Wang JH, Fennessy F, Redmond HP, Bouchier-Hayes D. Taurine prevents high-glucose-induced human vascular endothelial cell apoptosis. *Am J Physiol*. 1999;277:C1229–C1238.
38. Ulrich-Merzenich G, Zeitler H, Vetter H, Bhonde RR. Protective effects of taurine on endothelial cells impaired by high glucose and oxidized low density lipoproteins. *Eur J Nutr*. 2007;46:431–438.
39. Nonaka H, Tsujino T, Watari Y, Emoto N, Yokoyama M. Taurine prevents the decrease in expression and secretion of extracellular superoxide dismutase induced by homocysteine: amelioration of homocysteine-induced endoplasmic reticulum stress by taurine. *Circulation*. 2001;104:1165–1170.
40. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS. Chemical chaperones reduce ER stress and

restore glucose homeostasis in a mouse model of type 2 diabetes. *Science*. 2006;313:1137–1140.
41. Schuller-Levis GB, Park E. Taurine and its chloramine: modulators of immunity. *Neurochem Res*. 2004;29:117–126.
42. Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM, Jacobs DR Jr. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr*. 2006; 83:1369–1379.
43. Outinen PA, Sood SK, Pfeifer SI, Pamidi S, Podor TJ, Li J, Weitz JI, Austin RC. Homocysteine-induced endoplasmic reticulum stress and growth arrest leads to specific changes in gene expression in human vascular endothelial cells. *Blood*. 1999;94:959–967.
44. Chang PY, Lu SC, Lee CM, Chen YJ, Dugan TA, Huang WH, Chang SF, Liao WS, Chen CH, Lee YT. Homocysteine inhibits arterial endothelial cell growth through transcriptional downregulation of fibroblast growth factor-2 involving G protein and DNA methylation. *Circ Res*. 2008;102: 933–941.
45. Lee SJ, Kim KM, Namkoong S, Kim CK, Kang YC, Lee H, Ha KS, Han JA, Chung HT, Kwon YG, Kim YM. Nitric oxide inhibition of homocysteine-induced human endothelial cell apoptosis by down-regulation of p53-dependent Noxa expression through the formation of S-nitrosohomocysteine. *J Biol Chem*. 2005;280:5781–5788.
46. Ferretti G, Bacchetti T, Marotti E, Curatola G. Effect of homocysteinylation on human high-density lipoproteins: a correlation with paraoxonase activity. *Metab Clin Exp*. 2003;52:146–151.
47. Barbato JC, Catanescu O, Murray K, DiBello PM, Jacobsen DW. Targeting of metallothionein by L-homocysteine: a novel mechanism for disruption of zinc and redox homeostasis. *Arterioscler Thromb Vasc Biol*. 2007;27:49–54.
48. Colgan SM, Austin RC. Homocysteinylation of metallothionein impairs intracellular redox homeostasis: the enemy within! *Arterioscler Thromb Vasc Biol*. 2007;27:8–11.
49. Zhou J, Werstuck GH, Lhotak S, Shi YY, Tedesco V, Trigatti B, Dickhout J, Majors AK, DiBello PM, Jacobsen DW, Austin RC. Hyperhomocysteinemia induced by methionine supplementation does not independently cause atherosclerosis in C57BL/6J mice. *FASEB J*. 2008;22: 2569–2578.
50. Zhou J, Werstuck GH, Lhotak S, de Koning AB, Sood SK, Hossain GS, Moller J, Ritskes-Hoitinga M, Falk E, Dayal S, Lentz SR, Austin RC. Association of multiple cellular stress pathways with accelerated atherosclerosis in hyperhomocysteinemic apolipoprotein E-deficient mice. *Circulation*. 2004;110:207–213.
51. Nyblom HK, Sargsyan E, Bergsten P. AMP-activated protein kinase agonist dose dependently improves function and reduces apoptosis in glucotoxic beta-cells without changing triglyceride levels. *J Mol Endocrinol*. 2008;41:187–194.

ONLINE SUPPLEMENT

HIGH DIETARY TAURINE REDUCES APOPTOSIS AND ATHEROSCLEROSIS IN THE LEFT MAIN CORONARY ARTERY: ASSOCIATION WITH REDUCED CHOP AND TOTAL PLASMA HOMOCYSTEINE BUT NOT LIPIDEMIA.

Anthony Zulli[a,b*], Eza Lau[a], Bagus PP Wijaya[a], Xin Jin[a], Komang Sutarga[a], Grace D Schwartz[a], Jonathon Learmont[a], Peter J Wookey[a,b], Angelo Zinello[c], Ciriaco Carru[c], David L Hare[a,b]

Departments of Cardiology[a], and Medicine[b], University of Melbourne, Austin Health, Australia Department of Biomedical Sciences[c] - University of Sassari Viale S.Pietro 43/b - 07100 Sassari- Italy

METHODS SUPPLEMENT

Isometric tension in the abdominal aorta

Abdominal aortae were dissected into 3 x 3mm rings and sequentially mounted between two metal hooks in organ baths attached to force displacement transducers (Grass FT03) coupled to a data acquisition system (MacLab). The baths will be filled with Krebs solution and kept at a constant temperature of 37 °C and continuously bubbled with 95% O_2/5% CO_2. After 1hour, vessels were gently stretched to a resting tension of 2.5g. After 30 minutes, the vessels were gently re-stretched to a resting tension of 2.5g. After the vessels reached plateau tension, maximum constriction was determined by a high potassium krebs solution (KPSS, 124mM K^+). After plateau (6 minutes), vessels were rinsed with krebs solution. After 45 minutes, the vessel rings were subjected to a phenylephrine concentration curve (10^{-8} – 10^{-5} M, half log units). After the final concentration of phenylephrine was added and the constriction reached plateau, the rings were subjected to an acetylcholine dose response curve (10^{-8} – 10^{-6} M, half log units).

Left main coronary artery analysis

The left main coronary artery was excised from the heart and processed for paraffin. Groups were staggered into 2 batches and the LMCA from each rabbit from each batch were mounted in the same paraffin block. Sections were cut at 5 micron until the bifurcation. Approximately 30 sections were studied. Sections were randomly selected, dewaxed, rehydrated and placed in 10mM TrisCl, pH 7.4. Sections were then preincubated with 1% goat serum in 10mM TrisCl (pH7.4) for twenty minutes before incubating with the primary antibody diluted in 1% goat serum in 10mM TrisCl (pH7.4). Mouse monoclonal IgG, ACE2 (Cat# ALX 804-715, Alexis Biochemicals, diluted 1:150), AT2R (Cat# MAB3659, R&D Systems, Minneapolis, diluted 1:150), AT1R (Cat# sc-57036, Santa Cruz Biotechnology, diluted 1:50), ACE (Cat# MAB3502, Millipore, diluted 1:50), eNOS, (Cat#610296, BD Australia, diluted 1:100), phosphor-eNOS 1177 (Cat#612392, BD Australia, diluted 1:100), phosphor-eNOS 495 (Cat#612706, BD Australia, diluted 1:100), caveolin-1 (Cat# 036000, Zymed Laboratories, diluted 1:50), HSP70 (Cat#MAB3516, Millipore, diluted 1:50), nitrotyrosine (Cat#MAB5404, Millipore, diluted 1:100), HO-1 (Cat#Ab13248, Abcam, diluted 1:100), myeloperoxidase (Cat#sc-59600, Santa Cruz Biotechnology, diluted 1:50) , CHOP (Cat#MA1-250, ABR, USA) were incubated overnight and immunohistochemistry was performed as previously described [1-3]. Antigenic sites were developed with DAB, counterstained with hematoxylin, dehydrated and mounted with DPX mounting media.

Apoptosis detection by ssDNA

Apoptotic endothelial cells were detected by a monoclonal antibody to single stranded DNA (ssDNA) Sections were cut from paraffin blocks and attached to superfrost plus slides. Slides were placed in oven at 60°C for 1 hour, deparaffinised and hydrated. Slides were then incubated with saponin (0.1mg/ml in PBS) at room temperature for 20 minutes. Sections were washed in 1xPBS and incubated with Proteinase K (20ug/ml in 1xPBS) for 20 minutes at room temperature. Slides were then washed in three changes of 1xPBS and subsequently transferred into a coplin jar containing 50ml of 50% formamide (v/v dH_2O) preheated in water bath to 56°C and incubated for 20 minutes. Importantly, the temperature of formamide solution inside the jar was kept at 56°C. The slides were transferred into a container of ice-cold 1xPBS for 5 min. Endogenous peroxidase was quenched in 3% hydrogen peroxide for 5 minutes and then rinsed in dH_2O and then treated with 3% nonfat dry milk + 1% goat serum diluted in 1xPBS for 15 minutes to block non-specific antibody binding. Sections were then rinsed in 1xPBS and incubated with primary antibody to ssDNA (Cat# MAB3034, 1:100 dilution in 1%goat serum in 1xPBS) and left overnight. Sections were rinsed in 1xPBS for 5 minutes, and then incubated with the 'Envision' molecule (Cat# K4001, Dakocytomation) for 1 hour at room temperature. Slides were rinsed again in 1xPBS for 5 minutes, incubated with DAB chromagen for 1 minute, rinsed in dH_2O for 1 min, counterstained with haematoxylin for 1 minute, rinsed in dH_2O, blued in Scotts tapwater, dehydrated and then mounted in DPX media mount.

Wall pathology

Wall pathology was quantified by image analysis software (MCID Elite 6.0). Briefly, digital images of the LMCA (4 images, top, bottom, left and right) were obtained using a Leica DC480. The area of the intima and media was obtained by digital trace. The areas of plaques were determined separately. The intima:media ratio or plaque:media ratio was then obtained. To determine the intensity and proportional area of the endothelium that was immunostained, each trace was repeated three times, each time re-selecting the hue, saturation and intensity to obtain the most accurate representation of colour. The ribbon tool (MCID software) was selected, and the endothelial layer was traced, including other binding cells. The data from all four images were averaged to obtain one result. This was done for three traces. All three traces were then averaged to obtain one result from each LMCA. This value was then used as n=1. All experimental groups were normalised to a percentage increase over control[4, 5].

Western blot analysis

Plasma samples (0.5µL) were added to 9.5µL sample buffer, heated to 100°C for 5minutes, immediately placed on ice, and loaded into PAGE gels (5% stacking, 8% resolving). Initial voltage was 100V until samples entered resolving gel, and then 180V for 2 hours. Proteins were transferred onto PVDF membrane using a semidry transfer cell (Biorad) using standard transfer buffer. Membranes were washed with 25 ml TBS for 1 min at room temperature and non-specific binding was blocked by incubating membrane with 5% skim milk for 45min. Then, membranes were washed 5 times for 3 minutes each with 50 ml TBS-Tween, incubated with HOCl-LDL antibody (3µL of Cat#MAB 3232, in 10 ml) for 1hour. Serum HOCl-LDL levels were expressed as HOCl-LDL/LDL ratios. For comparison between MC and MCT, these ratios were normalized to MCT increases equivalent to 0%. Membranes were wash 5 times for 3 minutes each with 50 ml TBS-Tween and then incubated with 'Envision' molecule (25µL of Cat# K4003, in 10mL, Dakocytomation). Membranes were then washed 5 times for 3 min each with 50 ml TBS-Tween, and exposed to ECL.

Then, membranes were stripped with Stripping Buffer (Pierce, Cat #46430), and then washed with 25 ml TBS for 1 min at room temperature and non-specific binding was blocked by incubating membrane with 5% skim milk for 45min. Then, membranes were washed 5 times

for 3 minutes each with 50 ml TBS-Tween, incubated with a monoclonal antibody to ApoB (3μL of Cat# MAB4124, R&D systems, in 10 ml) for 1hour. Membranes were wash 5 times for 3 minutes each with 50 ml TBS-Tween and then incubated with goat anti mouse IgG conjugated to peroxidise (1:20,000 dilution, Sigma Cat# AO168). Membranes were then washed 5 times for 3 min each with 50 ml TBS-Tween, and exposed to ECL and detected on a Fuji Film LAS3000 and western blot bands were then analysed using FujiFilm MultiGauge Software.

Amino acid profile

Methionine was measured by capillary electrophoresis (CE) UV detection as previously described with some modifications [6]. In brief, 400 μL of obtained plasma was filtered in Vivaspin 500 microconcentrators by centrifugation at 3000*g* for 20min to remove proteins. Filtered samples were directly injected into CE. An MDQ capillary electrophoresis system equipped with a diode array detector was used (Beckman Instruments, Fullerton, CA, USA). Analysis was performed in an uncoated fused-silica capillary (75 μm I.D. and 60.2 cm length), injecting 39 nL of sample. Separation was carried out in a 125 mmol/L Tris buffer titrated with 1mol/L phosphoric acid to pH 2.3, 15 °C, and 15 kV .

Taurine was measured by capillary electrophoresis laser induced fluorescence detection as previously described[7]. Briefly, 50 μL of plasma was mixed with 50 μL of internal standard homocysteic acid (200 μmol/L) and 100 μL of trichloroacetic acid (10%) was then added to precipitate the proteins. After centrifugation at 3,000g for 5 min, 10 μL of clear supernatant was mixed with 90 μL of 100 mmol/L Na_2HPO_4 of pH 9.5 and 11 μL of 15 mmol/L FITC (fluorescein isothiocyanate). After 20 min incubation time at 100°C, the samples were diluted 100-fold and injected in CE. Analysis of taurine was performed by a CE system (P/ACE 5510) equipped with a laser-induced fluorescence (LIF) detector (Beckman, Palo Alto, CA, USA). Analysis was performed in an uncoated fused silica capillary, 75 μm I.D. and 47 cm length, injecting 18 nL of sample. Separation was carried out in a 20 mmol/L tribasic sodium phosphate buffer, pH 11.8, 23°C at normal polarity 22 kV.

Plasma thiols (Cys, GSH, Glu-Cys and Cys-Gly) were measured by capillary electrophoresis laser induced fluorescence detection as previously described [8]. In brief, 100 μL of plasma sample were mixed with 10 μL of tri-*n*-butylphosphine (10%) were mixed, vortexed for 30 s and subsequently incubated at 4°C for 10 min. At the end of incubation 100 μL of 10% trichloroacetic acid were added, vortexed for 10 s and then centrifuged for 10 min at 3,000*g*. 100 μL of supernatant were mixed with 100 μL of 300 mmol/L Na_3PO_4 at pH 12.5 and with 25 μL of 5-iodoacetamidofluorescein (4.1 mmol/L), and subsequently incubated at room temperature for 10 min. The mix was diluted 1/100 before injection on CE-LIF. Thiols analysis was carried out on a P/ACE 5510 system. The dimension of the uncoated fusedsilica capillary was 75 μm I.D. and 57 cm length. Analysis was performed applying 14 nL of sample under nitrogen pressure and using 5 mmol/L sodium phosphate/ 4 mmol/L boric acid as electrolyte solution with 75 mmol/L *N*-methyl-D-glucamine at pH 11. The separating conditions (28 kV, 70 μA, normal polarity, 40°C) were reached in 30 s and held at a constant voltage for 5 min.

RESULTS SUPPLEMENT

Analysis of plasma cysteinylglycine (Figure S1– A) , glutathionine (Figure S1 – B) and cysteine (Figure S1 – C) showed no changes between groups.

Analysis of the expression of endothelial proteins of the NOS system showed a trend towards increased total eNOS and peNOS-S1177 in the MC group, however the addition of taurine to the diet increased endothelial caveolin-1 protein by 29% vs control ($p=0.059$) and endothelial peNOS-T495 by 34% ($p=0.095$). This was not associated with a concurrent increase in eNOS or peNOS-S1177.

Analysis of the expression of endothelial proteins of the oxidative, nitrative and endoplasmic reticulum stress system shows that the MC group exhibited elevated oxidative stress, as detected by an increase in HSP 70 by 33% vs control ($p<0.05$), and taurine treatment further increased endothelial HSP70 ($p<0.02$). Interestingly, endothelial heme-oxygenase-1 (HO-1), myeloperoxidase (MPO), nitrotyrosine (NT), and iNOS appeared to decrease in the MC group and the addition of taurine only resulted in a trend towards increased levels above control (p=ns). Analysis of the expression of endothelial proteins of the RAS system in the MC group showed a significant increase of ACE2 by 19% ($p<0.01$) and ACE by 11 % ($p=0.01$) vs control. In addition, endothelial AT1R appeared to increase but this did not reach significance. The addition of taurine to the MC diet appeared to reduce the expression of endothelial ACE2, AT2R, ACE and AT1R, but this was not significant from MC. There was no significant change in endothelial AT2R.

Analysis of serum hypochlorous LDL/LDL ratio by western blot (Figure S2-A) showed a trend to increase hypochlorous LDL in the MC group, but this normalized at week 4 (Figure S2-C). Plaque hypochlorous LDL appeared to be increased in the MCT group, but this failed to reach significance (Figure S2-B). As taurine in the MCT group completely inhibited the increase in tHcy observed in the MC group, a very strong trend towards a positive correlation ($r^2=0.6986$) was observed ($p=0.0401$, Figure S2-D) between plasma tHcy and the plasma hypochlorous LDL/LDL ratio in the MC group (normalized to MCT).

DISCUSSION SUPPLEMENT

The failure of the following relationships to reach significance could possibly be a type 2 error due to an insufficient number of animals studied in these exploratory studies and have thus not been included in main text

Taurine resulted in a significant reduction in plasma tHcy, this being strongly associated with reduction in serum hypochlorous LDL. Thus, it is possible that a potentially atherogenic property of homocysteine might include the formation of hypochlorous LDL, although independent yet still closely correlated, effects of taurine could be implicated. Certainly one can say that this current study suggests a role for high dietary taurine in the prevention of dietary induced hyperhomocysteinemia. It is possible that this could be a better method of lowering tHcy than the methods used in previous clinical studies, although this is obviously speculative at this stage.

Moreover, the increase in endothelial HSP70 in both the atherogenic group and the taurine treated group indicates a clear role for HSP70 in coronary artery atherosclerosis. HSP70 is a cytoprotective protein and acts as a molecular chaperone to restore normal protein function[9]. The further increase in HSP70 observed by taurine treatment could be preventing the observed endothelial cell apoptosis in this model, as HSP70 is a regulator of apoptosis[10]. Furthermore, HSP70 has been shown to be an indicator for endothelial cell proliferation[11], indicating that the endothelial cell layer in the taurine treated group might be proliferating, and this theory is also supported by the lack of apoptotic endothelial cells in this group.

As oxidised LDL is a potent inducer of endothelial cell apoptosis[12], it is possible that taurine might inhibit apoptosis by impairing the formation of oxidized LDL. Potentially taurine could impair oxidised LDL by absorbing the hypochlorite anion ($HOCl/^{-}OCl$) produced by myeloperoxidase in macrophages, and thus reduce the formation of hypochlorous LDL[13] in serum. As circulating plasma hypochlorous LDL originates from mild oxidation in the arterial wall by myeoloperoxidase[12], we studied both plaque and serum hypochlorous LDL. We found that there was a trend towards reduced circulating hypochlorous LDL by the addition of taurine to the atherogenic diet over the four week period, compared to the atherogenic diet alone. This trend towards lower plasma hypochlorous LDL was associated with a trend towards increased plaque hypochlorous LDL in the taurine treated groups. If these trends are real, it could be possible that retaining hypochlorous LDL in plaque might result in less release of hypochlorous LDL into the circulation.

It is conceivable that dietary taurine, by impairing oxidation of LDL in plaques, might contribute to plaque stability and thus reduce the likelihood of acute coronary syndromes, histological examination of lethal plaques demonstrating more oxidised LDL and unstable angina having been previously correlated with circulating oxidised LDL[14]. In this regard, further studies into the mechanisms involved in inhibiting the release of hypochlorous LDL from plaque to the circulation are warranted, as this would decrease circulating oxidised LDL and possibly impair endothelial cell apoptosis and thus thrombosis.

Clinical data clearly established a strong association between higher cardiovascular events and a high total cholesterol/HDL ratio, and even more so for an apoB100/apoA1 ratio [15-18]. The current study highlights the possibility of discordance between a worsening lipid profile and a demonstrable improvement in coronary artery atherosclerosis. This possibility has been suggested in some clinical studies. For example, it is well known that there is no evidence of

premature coronary artery disease in human carriers of the ApoA1-Milano despite very low HDL levels[19-21]. Our results clearly show that a high LDL/low HDL lipid profile does not further induce atherosclerosis in the left main coronary artery if induced by high dietary taurine, clearly indicating other factors besides the lipid profile are involved in atherogenesis.

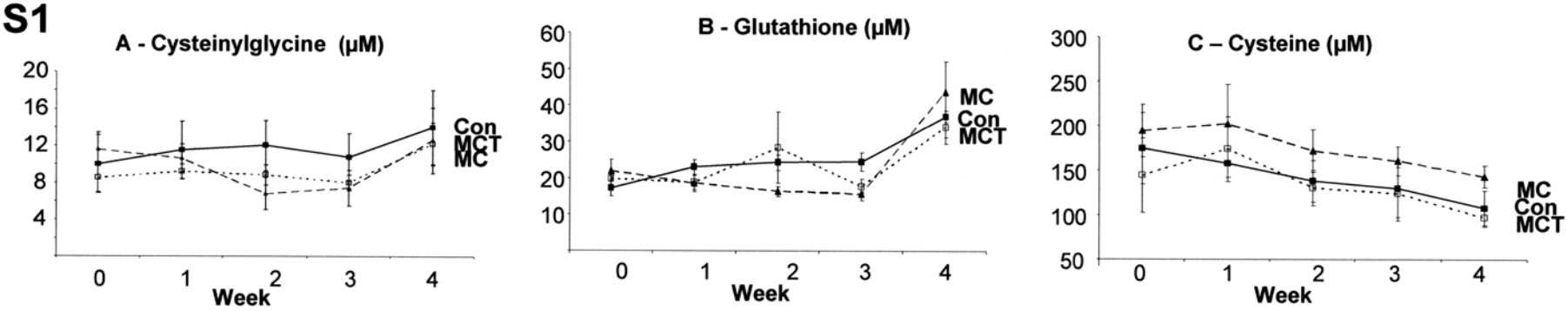
It is suggested that enhancing endothelial function via the restoration of NOS function or reduction in oxidative stress is a pre-requisite for the impairment of atherosclerosis[22]. In contrast, we show that atherosclerosis in the coronary artery is not related to an improvement in endothelial function in the abdominal aorta. To study endothelial function in the left main coronary artery, we investigated eNOS, RAS and oxidative activity in the endothelial layer. We found no evidence to suggest that eNOS activity was increased, as detected by eNOS activity markers phosporylated eNOS at serine 1177, dephosphorylated eNOS at threonine 495, and the eNOS inhibitor caveolin-1 in the endothelial layer left main coronary artery or that oxidative stress was decreased. However, we did find that ACE, ACE2, AT2R and AT1R were all slightly decreased, but these findings did not reach significance. Whether the activity of these enzymes are changed remain to be elucidated. Indeed, we did find that taurine treatment might decrease eNOS activity in the coronary artery, as suggested by a trend to increase in endothelial caveolin-1 ($p=0.059$) and phosphorylated eNOS at the threonine site ($p=0.095$). If these results hold true, taurine could be impairing the dysfunctional eNOS, thus reducing the amount of O_2^-. This view is supported by Ozaki and colleagues, who show that ApoE deficient mice overexpressing eNOS accelerated atherosclerosis [23]. As well, caveolin-1 has been shown to regulate apoptosis in cell lines[24] and vascular smooth muscle cells[25], raising the possibility that the increase in caveolin-1 might also impair the apoptosis observed in this study

REFERENCES

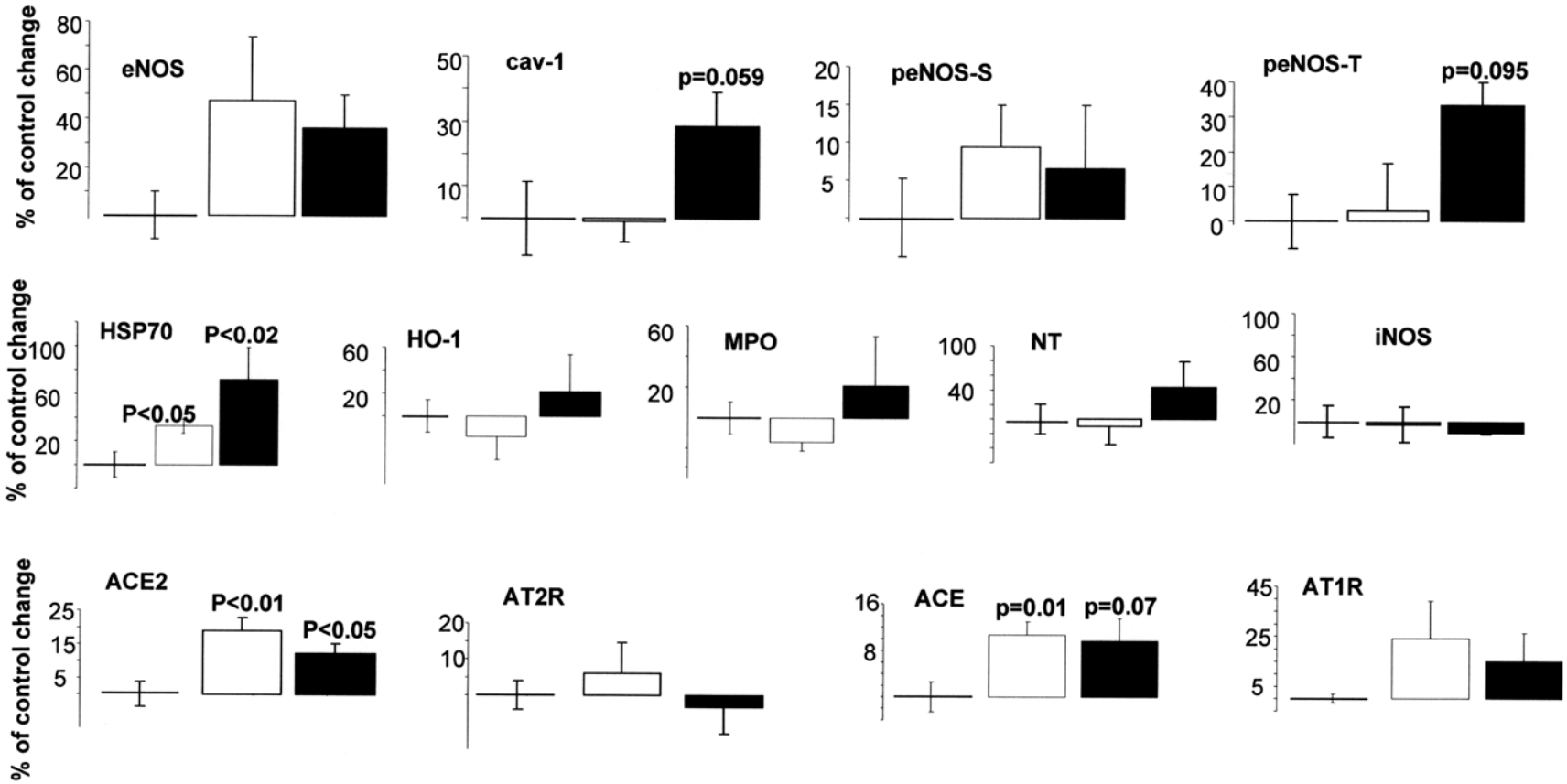
1. Zulli A, Burrell LM, Buxton BF, Hare DL. ACE2 and AT4R are present in diseased human blood vessels. *Eur J Histochem.* 2008;52:39-44.
2. Zulli A, Buxton BF, Merrilees M, Hare DL. Human diseased arteries contain cells expressing leukocytic and embryonic stem cell markers. *Hum Pathol.* 2008;39:657-665.
3. Zulli A, Burrell LM, Widdop RE, Black MJ, Buxton BF, Hare DL. Immunolocalization of ACE2 and AT2 receptors in rabbit atherosclerotic plaques. *J Histochem Cytochem.* 2006;54:147-150.
4. Zulli A, Buxton BF, Black MJ, Ming Z, Cameron A, Hare DL. The immunoquantification of caveolin-1 and eNOS in human and rabbit diseased blood vessels. *J Histochem Cytochem.* 2006;54:151-159.
5. Wookey PJ, Zulli A, Buxton BF, Hare DL. Calcitonin receptor immunoreactivity associated with specific cell types in diseased radial and internal mammary arteries. *Histopathology.* 2008;52:605-612.
6. Zinellu A, Sotgia S, Usai MF, Zinellu E, Posadino AM, Gaspa L, Chessa R, Pinna A, Carta F, Deiana L, Carru C. Plasma methionine determination by capillary electrophoresis-UV assay: application on patients affected by retinal venous occlusive disease. *Anal Biochem.* 2007;363:91-96.
7. Zinellu A, Sotgia S, Bastianina S, Chessa R, Gaspa L, Franconi F, Deiana L, Carru C. Taurine determination by capillary electrophoresis with laser-induced fluorescence detection: from clinical field to quality food applications. *Amino acids.* 2009;36:35-41.
8. Zinellu A, Carru C, Galistu F, Usai MF, Pes GM, Baggio G, Federici G, Deiana L. N-methyl-D-glucamine improves the laser-induced fluorescence capillary electrophoresis performance in the total plasma thiols measurement. *Electrophoresis.* 2003;24:2796-2804.
9. Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci.* 2005;62:670-684.
10. Giffard RG, Han RQ, Emery JF, Duan M, Pittet JF. Regulation of apoptotic and inflammatory cell signaling in cerebral ischemia: the complex roles of heat shock protein 70. *Anesthesiology.* 2008;109:339-348.
11. Zhu W, Roma P, Pirillo A, Pellegatta F, Catapano AL. Human endothelial cells exposed to oxidized LDL express hsp70 only when proliferating. *Arteriosclerosis, thrombosis, and vascular biology.* 1996;16:1104-1111.
12. Mertens A, Holvoet P. Oxidized LDL and HDL: antagonists in atherothrombosis. *Faseb J.* 2001;15:2073-2084.
13. Hazell LJ, Stocker R. Oxidation of low-density lipoprotein with hypochlorite causes transformation of the lipoprotein into a high-uptake form for macrophages. *The Biochemical journal.* 1993;290:165-172.
14. Faviou E, Vourli G, Nounopoulos C, Zachari A, Dionyssiou-Asteriou A. Circulating oxidized low density lipoprotein, autoantibodies against them and homocysteine serum levels in diagnosis and estimation of severity of coronary artery disease. *Free Radic Res.* 2005;39:419-429.
15. Sniderman AD, Jungner I, Holme I, Aastveit A, Walldius G. Errors that result from using the TC/HDL C ratio rather than the apoB/apoA-I ratio to identify the lipoprotein-related risk of vascular disease. *J Intern Med.* 2006;259:455-461.
16. Amarenco P, Steg PG. The paradox of cholesterol and stroke. *Lancet.* 2007;370:1803-1804.

17. Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy--a review of the evidence. *J Intern Med.* 2006;259:493-519.
18. Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet.* 2007;370:1829-1839.
19. Parolini C, Chiesa G, Gong E, Caligari S, Cortese MM, Koga T, Forte TM, Rubin EM. Apolipoprotein A-I and the molecular variant apoA-I(Milano): evaluation of the antiatherogenic effects in knock-in mouse model. *Atherosclerosis.* 2005;183:222-229.
20. Wang L, Sharifi BG, Pan T, Song L, Yukht A, Shah PK. Bone marrow transplantation shows superior atheroprotective effects of gene therapy with apolipoprotein A-I Milano compared with wild-type apolipoprotein A-I in hyperlipidemic mice. *Journal of the American College of Cardiology.* 2006;48:1459-1468.
21. Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, Eaton GM, Lauer MA, Sheldon WS, Grines CL, Halpern S, Crowe T, Blankenship JC, Kerensky R. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA.* 2003;290:2292-2300.
22. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation.* 2004;109:III27-32.
23. Ozaki M, Kawashima S, Yamashita T, Hirase T, Namiki M, Inoue N, Hirata K, Yasui H, Sakurai H, Yoshida Y, Masada M, Yokoyama M. Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *The Journal of clinical investigation.* 2002;110:331-340.
24. Zhao X, Liu Y, Ma Q, Wang X, Jin H, Mehrpour M, Chen Q. Caveolin-1 negatively regulates TRAIL-induced apoptosis in human hepatocarcinoma cells. *Biochemical and biophysical research communications.* 2009;378:21-26.
25. Ingueneau C, Huynh-Do U, Marcheix B, Athias A, Gambert P, Negre-Salvayre A, Salvayre R, Vindis C. TRPC1 is regulated by caveolin-1 and is involved in oxidized LDL-induced apoptosis of vascular smooth muscle cells. *Journal of cellular and molecular medicine.* 2008. *In press.*

S1



ENDOTHELIAL IMMUNOQUANTIFICATION



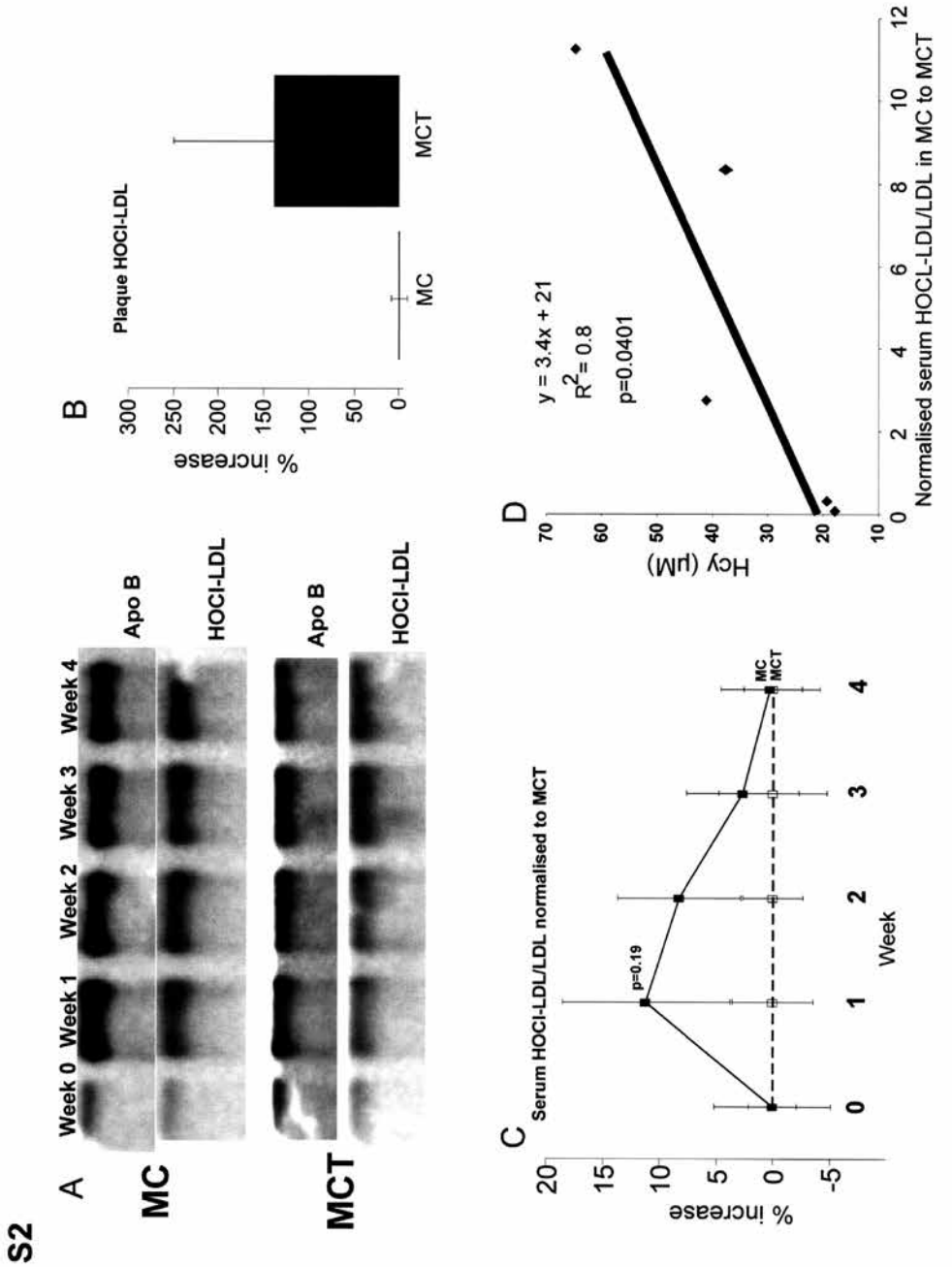


Figure S1
Plasma cysteineglycine (A), glutathionine (B) and cysteine (C) were not increased in any diet. Immunohistochemical quantification of factors involved in atherogensis in the LMCA endothelial layer. The addition of taurine to the MC diet failed to significantly affect the NOS system, oxidative stress system or renin-angiotensin system. For all endothelial immunoquantification bar graphs, the first column is from the Con group, the second (white box) is from the MC group, and the third is from the MCT group (black box).

Figure S2
Western blot analysis of serum hypochlorous LDL/LDL (A,C) as well as immunoquantification of plaque hypochlorous LDL (B). Taurine appears to increase plaque hypochlorous LDL (B) and reduce serum hypochlorous LDL/LDL (C). Interestingly, a positive association between plasma tHcy in the MC group and serum hypochlorous LDL was observed ($p<0.05$).

Atherosclerosis 208 (2010) 19–25

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Review

The potential protective effects of taurine on coronary heart disease☆

Oktawia P. Wójcik[a], Karen L. Koenig[a], Anne Zeleniuch-Jacquotte[a,b], Max Costa[a,b,c], Yu Chen[a,b,*]

[a] Department of Environmental Medicine, New York University School of Medicine, New York, NY 10016, USA
[b] New York University Cancer Institute, New York University School of Medicine, New York, NY 10016, USA
[c] Department of Pharmacology, New York University School of Medicine, New York, NY 10016, USA

ARTICLE INFO

Article history:
Received 17 October 2008
Received in revised form 29 May 2009
Accepted 2 June 2009
Available online 11 June 2009

Keywords:
Taurine
Coronary heart disease
Cholesterol
Blood pressure
Antioxidant

ABSTRACT

In humans, taurine (2-aminoethanesulfonic acid) is mainly obtained from diet. Despite the fact that the health effects of taurine are largely unknown, taurine has become a popular supplement and ingredient in energy drinks in recent years. Evidence from mechanistic and animal studies has shown that the main biological actions of taurine include its ability to conjugate bile acids, regulate blood pressure (BP), and act as a potent antioxidant and anti-inflammatory agent. These actions suggest that high levels of taurine may be protective against coronary heart disease (CHD). However, data from epidemiologic and intervention studies in humans are limited. We review what is known about taurine's metabolism, its transportation in the body, its food sources, and evidence of its effect on cardiovascular health from *in vitro*, animal, and epidemiologic studies. We also discuss shortcomings of the human studies that need to be addressed in the future. The identification of taurine as a preventive factor for CHD may be of great public health importance.

Contents

1. Introduction

Coronary heart disease (CHD) has decreased in the US as a result of preventive measures such as smoking cessation and treatment of hypertension, dyslipidemia, diabetes mellitus, and obesity. However, CHD remains the single largest killer of American men and women, with an estimate of 8.7 million US men and 7.3 million US women affected by CHD in 2005 [1]. Identification of new factors

☆ This research was supported by U.S. grants: NIH grants ES000260, CA16087, CA098661 and American Heart Association grant 0835569D.

* Corresponding author at: New York University School of Medicine, 650 First Avenue, 5th Floor, Room 510, New York, NY 10016-3240, USA. Tel.: +1 212 263 4839; fax: +1 212 263 8570.
E-mail address: yu.chen@nyumc.org (Y. Chen).

0021-9150/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2009.06.002

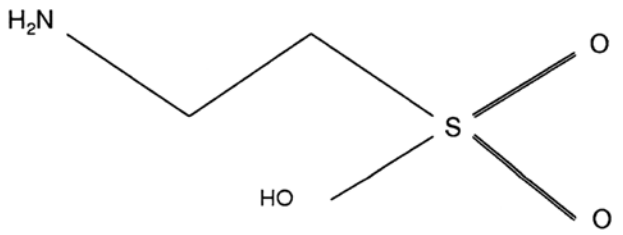


Fig. 1. Taurine structure.

that may help reduce incidence of CHD could have an important public health impact.

Diet can influence heart health, and recent evidence strongly supports the idea that beneficial dietary factors such as fruits, vegetables, legumes, whole grains, and vegetable oils should be consumed adequately. In humans, diet is the main source of taurine (2-aminoethanesulfonic acid), a sulfur-containing molecule (Fig. 1). Smaller amounts of taurine are also synthesized endogenously in the liver from methionine and cysteine. Taurine exists freely in cytosol and is most abundant in the heart, retina, developing brain and blood (Table 1). Today, taurine is a key ingredient in "energy" drinks such as Red Bull (1000 mg), Monster (2000 mg), and Rockstar (3000 mg), although there is no evidence of taurine's effects on physical activity. The high concentration of taurine in these popular drinks, however, underscores the importance of evaluating the potential health implications of taurine. We review the taurine content of different foods and the metabolism and transport of taurine in the body. We examine evidence from *in vitro*, animal and human studies on the potential of taurine in protecting against CHD. We also discuss the shortcomings of previous human studies and suggest future directions.

2. Taurine metabolism and transport in humans

Synthesis of taurine begins in the liver with a magnesium-catalyzed methylation of methionine to form homocysteine, a process which can be reversed by the vitamin B12 and folate-dependent enzyme methionine synthetase (Fig. 2 adapted from [2]). Next, homocysteine donates its sulfur group to form cystathionine and under the influence of pyridoxal-5′-phosphate (P5P) cystathionine is broken down to cysteine. Cysteine, catalyzed by

Table 1
Taurine concentrations in various tissues.

Tissue type	Taurine concentration[a]
Human	
Brain	
Developing	4–20 μmol/g [40]
Adult	1–9 μmol/g [40]
Heart	6 μmol/g [41]; 15–25 μmol/g [42]
Liver	2 μmol/g [41]
Skeletal muscles	5 μmol/g [41]
Retina	30–40 μmol/g [41]
Plasma	50–80 μmol/L [41,42]; 100 μmol/L [43]
Leukocytes and platelets	13–17 μmol/L [44]; 10–50 μmol/L [43]
Rat	
Brain	3 μmol/g [41]; 5 μmol/g [45,46]
Heart	20 [46]; 30 μmol/g [47]
Liver	3 μmol/g [41]; 4 μmol/g [45,46]
Skeletal muscles	7 μmol/g [41]; 16 μmol/g [45]
Retina	27 μmol/g [41]; 50 μmol/g [47]
Plasma	360 μmol/L [45]; 450 μmol/L [41]
Kidney	7 μmol/g [46]; 9 μmol/g [45]

[a] Units for some values have been changed from those originally published for uniformity.

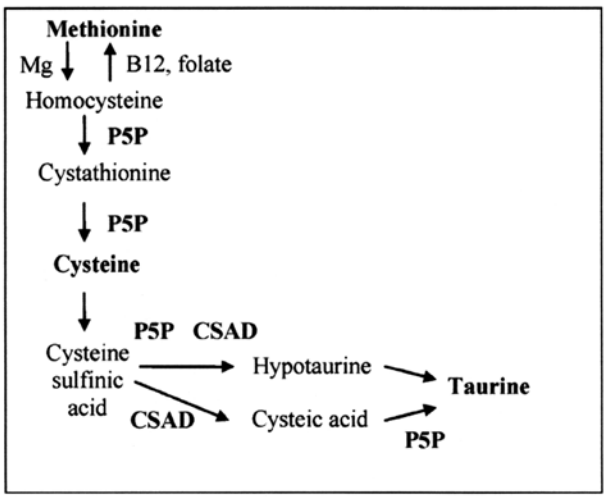


Fig. 2. Synthesis of taurine (adapted from [2]).

cysteine deoxygenase, combines with dioxygen to become cysteine sulfinic acid, which is then decarboxylated by cysteine sulfinic acid decarboxylase (CSAD) and P5P to hypotaurine. Hypotaurine is oxidized to taurine by hypotaurine dehydrogenase. Alternatively, taurine is formed following the oxidation of cysteine sulfinic acid to cysteic acid and the decarboxylation of cysteic acid by P5P [3].

Humans have a low level of CSAD, and, therefore, obtain most of their taurine from foods [4]. Taurine obtained from food is absorbed by the small intestine. After absorption, carrier-mediated active transport in the brush border membrane moves taurine to enterocytes, which deliver it to the portal vein [5]. Taurine is then transported to the liver and released into circulation and can then enter cells via the taurine transporter (TauT), which in turn responds to the concentration of taurine in cells [6]. A high concentration of taurine downregulates *TauT*, and taurine is excreted from the body in urine. Conversely, when the taurine concentration is low, *TauT* is upregulated and taurine is reabsorbed into circulation through the renal tubules in the kidney.

3. Taurine level in foods

The mean content of taurine in selected foods is shown in Table 2. Overall, low amounts of taurine are found in dairy, such as ice cream and cow's milk. The highest amounts of taurine can be found in shellfish, especially scallops, mussels, and clams. High amounts of taurine can also be found in the dark meat of turkey and chicken, and turkey bologna. Cooking has been shown to have no adverse effect on taurine levels [7], and taurine values from the same food sources are fairly consistent across different studies. The mean daily taurine intake for adult human non-vegetarians has been estimated between 40 and 400 mg [8], typically falling closer to the lower end of the range. However, the amount of taurine bioavailable in humans after consuming foods containing taurine is not known. In human trauma patients, a dose–response was found between taurine given intravenously at 0–50 mg/kg and the amount of taurine in serum [9].

4. Mechanisms of taurine protection against CHD

We review data from *in vitro*, animal, and limited human studies of the ability of taurine to conjugate bile acids, regulate blood pressure (BP), and reduce oxidative stress and inflammation.

Table 2
Taurine amounts in foods.

Food	Method of preparation	Mean taurine content mg/100 g[a] (SEM)[b]
Beef	Raw	43.1 (7.6) [48]; 46.3 (4.6) [49]
	Broiled	38.4 (9.7) [48]
Chicken dark meat	Raw	82.6 (4.6) [49]; 169.6 (37.4) [48]
	Broiled	199.1(27.4) [48]
Chicken light meat	Raw	17.5 (0.4) [49]; 17.8 (3.3) [48]
	Broiled	14.5 (3.9) [48]
Turkey dark meat	Raw	306 (69) [48]
	Roasted	299.6 (52.2) [48]
Turkey light meat	Raw	29.5 (6.9) [48]
	Roasted	11.1 (1.1) [48]
Veal	Raw	39.8 (12.5) [48]
	Broiled	46.7 (10.3) [48]
Pork, loin	Raw	50.1 (3.8) [49]; 61.2 (10.6) [48]
	Roasted	56.8 (11.5) [48]
Lamb dark meat	Raw	43.8 (4.1) [49]; 47 [50]
Ham, picnic	Baked	49.8 (5.8) [48]
Salami, cotto beef	Cured	59.2 (7.8) [48]
Bologna, pork/beef	Cured	31.4 (4) [48]
Bologna, turkey	Cured	122.7 (5.3) [48]
Tuna, albacore	Canned	41.5 (12.8) [48]
Tuna, chunk light		39 (11.8) [48]
White fish	Raw	113.9 (13) [49]; 151.2 (22.9) [48]
	Cooked	172.1 (53.6) [48]
Shrimp, small	Cooked	10.5 (1.4) [48]
Shrimp, medium	Raw	39.4 (12.8) [48]; 155.2 (3.8) [49]
Mussels	Raw	655.4 (72) [48]
Oysters	Fresh	70 [50]; 396.7 (29) [48]
Cod	Frozen	31 [50]
Clams	Raw	240 [50]; 513.1 (50.1) [49]; 520.7 (97.4) [48]
	Canned	152 [50]
Octopus	Raw	388 (12.5) [49]
Scallop	Raw	827.7 (15.4) [48]
Squid	Raw	356.7 (95) [48]
Cow's milk	Unprocessed	<0.5 (7)
3.5% fat, whole		2.4 (0.3) [48]
2.0% fat, low fat		2.3 (0.2) [48]
0.5%, non-fat		2.5 (0.3) [48]
Non-fat, dried		7.0 [48]
Yogurt, low-fat plain		3.3 (0) [48]
Yogurt, low-fat peach		7.8 (0.9) [48]
Ice cream/vanilla		1.9 [48]
Pasteurized milk		6 [50]

[a] Units for some values in Table 2 have been adapted from those previously published for uniformity
[b] SEM: standard error of the mean

4.1. Lipid detoxification

4.1.1. In vitro and animal studies

Taurine's main function in the body is the conjugation of cholesterol into bile acids, changing cholesterol's solubility and enabling its excretion. This process can be accelerated through the upregulation of 7-alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in the production of bile acids [10]. Taurine has been shown to have time- and dose-response effects on CYP7A1 mRNA levels in Hep G2 cells (human hepatoblastoma cells used to study cholesterol function). In these cells, the level of CYP7A1 mRNA increased with increasing concentrations of taurine (2, 10 and 20 mmol/L) both in the presence and absence of 0.2 mmol/L cholesterol. Furthermore, the expression of CYP7A1 was significantly greater 4 h after taurine treatment than in cells without taurine treatment, and continued to significantly increase at 24 and 48 h [11], suggesting that the effect of taurine may be sustained.

The cholesterol profiles of rats, mice, hamsters, guinea pigs, and rabbits have all been shown to be affected by taurine. For example, taurine supplementation of 0.25–50 g/kg for two weeks led to significant dose-dependent attenuation in the increase of serum cholesterol in Wistar rats fed a diet high in cholesterol compared to a group fed a high cholesterol diet without supplementation. This effect has been attributed to an increased level of CYP7A1 mRNA in the liver observed in the taurine supplemented group [12].

Taurine may also decrease cholesterol levels through an upregulation of the hepatic low-density lipoprotein receptor (LDLR) and/or through an improvement in the binding of LDL to LDLR. Golden Syrian hamsters fed a high fat diet supplemented with 1% taurine for two weeks compared to unsupplemented hamsters had significantly reduced serum total cholesterol (317 mg/dL versus 543 mg/dL) and LDL+VLDL cholesterol (213 mg/dL versus 460 mg/dL). Radiolabelled LDL tracers in the body revealed that taurine upregulated the activity of LDLR, increased LDL uptake by the liver, and increased LDL turnover in blood [13]. C57BL/6 mice fed a high fat diet supplemented with 1% taurine for one month showed a significant decrease, compared to control mice fed a high fat diet without taurine, in total serum cholesterol (126 mg/dL versus 181 mg/dL, respectively) and LDL+VLDL cholesterol (70 mg/dL versus 120 mg/dL, respectively). However, liver LDLR protein levels measured by Western blot showed no difference between the two groups [14]. Additional studies are needed to clarify taurine's role in regulating LDL.

4.1.2. Human studies

The effects of taurine on lipid levels were examined in several small randomized trials (Table 3). A single-blind study of 22 healthy male Japanese volunteers between the ages of 18–29 [15] examined the effects of 6 g/day of taurine supplementation versus placebo on participants' lipid profiles. Volunteers were placed on a three-week diet designed to increase their cholesterol levels. The control group had a statistically significant increase in total cholesterol, LDL cholesterol, and LDL, while the corresponding increases of the taurine supplemented group were smaller and not statistically significant. However, it is unknown whether the beneficial effects of taurine would only be seen among individuals with high-fat diet.

In a double-blind randomized study of 30 overweight or obese college students (body mass index [BMI] $\geq$ 25), who received either 3 g/day of taurine supplementation or placebo for seven weeks, average changes in lipid levels over time in the treatment group were compared with those in the placebo group. At baseline, there were no differences in any parameters between the two groups. After seven weeks of supplementation, plasma triglycerides decreased by 8 mg/dL in the taurine supplemented group, and increased by 3 mg/dL in the placebo group. These changes were statistically significantly different between the two groups ($p = 0.04$). Additionally, the atherogenic index [(total cholesterol – HDL cholesterol)/HDL cholesterol] was reduced in the taurine supplemented group (2.75–2.30) after seven weeks, and this reduction was statistically significantly different from the changes in the placebo group (2.91–2.99). Changes in other measures such as total cholesterol and HDL-cholesterol were not statistically different between the two groups [16]. These findings suggest that taurine may reduce triglyceride levels; however, the study's limitations, including small sample size, with only 15 participants in each arm, short length of supplementation, and baseline health status of the participants, call for future large studies to confirm the results.

4.2. Effects on BP

4.2.1. In vitro and animal studies

The main mechanism through which taurine may decrease BP is thought to be the attenuation of angiotensin II signaling, which

Table 3
Human studies assessing the association of taurine with heart disease and CHD risk factors.

Citation	Country	Study design	Dosage	Sample size and characteristics	Age (years)	Endpoint	Findings
Mizushima et al. [26]	Japan and Brazil	Cross-sectional	N/A	433 Japanese in Japan 269 Japanese in Brazil	45–59	Hypercholesterolemia Hypertension	Hypercholesterolemia prevalence[a]: *Men*: 5.8% in Japan vs. 28.3% in Brazil[b] *Women*: 19.0% in Japan vs. 22.1% in Brazil[c] Hypertension prevalence[a]: *Men*: 20.0% in Japan vs. 26.7% in Brazil[c] *Women*: 14.0% in Japan vs. 32.0% in Brazil[b]
Liu et al. [25]	China	Cross-sectional	N/A	775 Han 510 Uygur 204 Kazak 125 Tibetan	49–54	Partial correlation coefficient of taurine excretion with BP	*Han*: SBP $r=-0.06$[c]; DBP $r=-0.12$[d] *Uygur*: SBP $r=-0.01$[c]; DBP $r=-0.09$[c] *Kazak*: SBP $r=-0.09$[c]; DBP $r=-0.04$[c] *Tibetan*: SBP r and DBP $r=-0.25$[d]
Yamori et al. [36]	16 countries	Ecologic	N/A	1352 males 1382 females	48–56	Urinary taurine excretion vs. age-adjusted IHD mortality rates	Men, $\beta=-0.38$ per 100,000/μmol/day[e] Women, $\beta=-0.15$ per 100,000/μmol/day[e]
Yamori et al. [37]	16 countries	Ecologic	N/A	2462 males	45–74	Urinary taurine excretion vs. age-adjusted IHD mortality rates	Men, $\beta=-0.3$ per 100,000/μmol/day[e]
Fujita et al. [27]	Japan	RCT	6 g taurine or placebo/day for 1 week	19 borderline hypertensives	20–25	ΔSBP ΔDBP ΔEpinephrine	ΔSBP = −9.0 mmHg vs. −2.7 mmHg[f] ΔDBP = −4.1 mmHg vs. −1.2 mmHg[f] ΔEpinephrine = −14.6 pg/mL vs. −1.9 pg/mL[d,f]
Milei et al. [35]	Argentina	RCT	5 g taurine or placebo 1–3 h before CABG	12 patients with stable angina	30–60	ΔOxidative stress	Ratio of reperfusion and preischemic sample means = 1.12 vs. 2.45[b,f]
Mizushima et al. [15]	Japan	RCT	High cholesterol diet with 6 g taurine or placebo/day for 3 weeks	22 male volunteers	18–29	ΔTotal cholesterol ΔLDL-cholesterol ΔLDL Δ Norepinephrine	ΔTotal cholesterol = 22.1 mg/dL vs. 25.4 mg/dL[c,f] ΔLDL-C = 6.7 mg/dL vs. 17.1 mg/dL[c,f] ΔLDL = 28.1 mg/dL vs. 43.9 mg/dL[c,f] ΔNorepinephrine = 7 μg/day vs. 35 μg/day[d,f]
Zhang et al. [16]	China	RCT	3 g taurine or placebo/day for 7 weeks	30 overweight or obese college students	18–23	ΔTriglyceride ΔTotal cholesterol ΔHDL-C ΔAtherogenic index[h]	ΔTriglyceride = −8.12 mg/dL vs. +3.09 mg/dL[d,f,g] ΔTotal cholesterol = −9.67 mg/dL vs. 0 mg/dL[c,f,g] ΔHDL-C = +3.09 mg/dL vs. −0.39 mg/dL[c,f,g] ΔAtherogenic index = −0.45 vs. −0.08[e,f]

[a] Hypercholesterolemia and hypertension prevalences are weighted averages calculated from original article.
[b] $p<0.001$.
[c] Not significant.
[d] $p<0.05$.
[e] $p<0.01$.
[f] Taurine vs. placebo.
[g] Converted from mmol/L to mg/dL.
[h] Atherogenic index = [(TC − HDL-C)/HDL-C].

causes vasoconstriction and consequently increases BP [17]. Taurine may also reduce BP through enhancement of the kinin–kallikrein system in the kidney that causes vasodilation [18]. Taurine may also lower BP by decreasing levels of epinephrine (which increases heart rate) and norepinephrine (which causes vasoconstriction). In hypertensive rats supplemented with 1.5% taurine in drinking water for eight weeks, the mean plasma norepinephrine level in the taurine supplemented rats was 383 pg/mL, significantly lower than in the control rats (615 pg/mL). There was also a significant difference between the mean epinephrine levels in the taurine supplemented rats (232 pg/mL) compared to the control group (892 pg/mL) [19].

Taurine supplementation effectively controlled high BP in the most common animal models of hypertension including: spontaneously hypertensive rats (SHR) [19], deoxycorticosterone acetate-salt rats (DOCA-salt/Sprangue–Dawley) [20], salt-sensitive Dahl-S rats [21], renovascular hypertensive rats [22], and hyperinsulinemic rats (Wistar) [23]. For example, hypertension in SHR and SHR stroke-prone (SHR-SP) rats was significantly attenuated by the addition of 3% taurine to the drinking water. After 72 days of the experiment, the difference in BP between the control group and the SHR-SP group was 30 mmHg [24].

4.2.2. Human studies

Analyses from the WHO Cardiovascular Diseases and Alimentary Comparison (WHO-CARDIAC), a multi-center cross-sectional study, have suggested an inverse correlation between urinary excretion of taurine and BP (Table 3, [25]). After adjusting for age, sex and potassium levels, a study of different ethnic Chinese populations found a significant inverse correlation between 24-h taurine excretion and diastolic BP in 755 Han participants and a significant inverse correlation between 24-h taurine excretion and both diastolic and systolic BP in 125 Tibetan participants. The Uygur or the Kazak populations showed small, non-significant negative correlations between 24-h taurine excretion and both systolic and diastolic BP [25]. One major limitation of the study is its cross-sectional design in which taurine and BP status were measured at the same time, making it difficult to know which temporally preceded the other. Potential confounders that are related to both taurine level and BP were not considered in the study. In addition, population-specific factors that led to differences in the correlations among ethnic groups were not investigated.

An inverse correlation between BP and taurine excretion has also been seen in Japanese immigrants in Brazil [26]. In this cross-sectional study, a population-based sample of 433 middle-aged Japanese in Shimane and Okinawa, Japan, and 269 Japanese immigrates from Shimane and Okinawa to Brazil showed that native Japanese had a significantly greater urinary excretion of taurine compared to Japanese immigrants in Brazil. This observation was consistent with a gradient in the prevalence of hypertension and hypercholesterolemia with lower prevalence in the Japanese living in Japan compared to the Japanese immigrants living in Brazil [26], suggesting the environment and not genetics as the source of the prevalence gradients, including a possible role of taurine intake. Although the study acknowledged differences in diet, it did not discuss other lifestyle differences between the two groups. Since this study used prevalence rather than incidence data, the temporal sequence of events could not be established.

In a double-blind, placebo-controlled trial of 19 borderline hypertensive patients between the ages of 20 and 25 [27], 6 g of taurine supplementation/day significantly decreased systolic and diastolic BP over time, whereas in the placebo group BP did not change significantly. Furthermore, plasma epinephrine levels in the taurine treatment group decreased significantly but remained at a similar level in the placebo group. Norepinephrine levels decreased non-significantly in both the taurine supplemented and placebo groups [27]. Although the results suggest protective effects of taurine, statistical testing was not conducted to directly compare longitudinal changes in the treatment and placebo groups. Other limitations of the study are its small sample size, with only $\leq$10 participants in each of the study groups, short length of supplementation (7 days), and the fact that participants had preexisting borderline hypertension, limiting the generalizability of the results to healthy individuals. Nevertheless, the norepinephrine findings were consistent with the previously discussed study by Mizushima et al. [15], in which urinary norepinephrine levels increased significantly in individuals given a high cholesterol diet without taurine supplementation but did not change significantly in individuals fed a high cholesterol diet supplemented with taurine.

4.3. Antioxidation and anti-inflammation

4.3.1. In vitro and animal studies

Atherosclerosis is recognized as a chronic inflammatory process resulting from oxidation and oxygen radicals. Oxidant activity, measured by thiobarbituric acid reactive substances (TBARS), was significantly less in the plasma of male Wistar rats fed a high fat diet supplemented with 50 mg/kg/day taurine for six months (1.6 nmol/mL) compared to rats fed a high fat diet without taurine supplementation (2.4 nmol/mL) [28]. Serum TBARS were also significantly lower in apolipoprotein E-deficient mice after 2% taurine supplementation for 12 weeks (8.6 nmol/mL), compared to mice without taurine supplementation (11.1 nmol/mL) [29].

Taurine is also known to react with hypochlorous acid (HOCl), a powerful oxidant, to create a more stable taurine chloramine (TauCl) *in vivo* to block the production of pro-inflammatory cytokines. For example, 0.4 mM TauCl added to adherent leukocytes taken from healthy volunteers and activated with lipopolysacharides (LPS) significantly reduced the amount of interleukin-6 (IL-6) produced. In murine peritoneal neutrophils with acute inflammation activated by recombinant interferon-γ (INF-γ) and LPS, TauCl in concentrations ranging from 0.03 to 0.3 mmol/L significantly inhibited the production of IL-6 in a dose-dependent manner [30].

The adhesion of circulating leukocytes to endothelial cells and their transendothelial migration is an initiating step of atherosclerosis [31]. The expression of intracellular adhesion molecule-1 (ICAM-1), which mediates cell–cell adhesion, was decreased by taurine in Sprague–Dawley rats with impaired reactive oxygen species (ROS) scavenging capability. Taurine given intravenously at 200 mg/kg for 5 days before the induction of inflammation prevented a significant increase in the expression of ICAM-1 in the post-capillary venular (high endothelial cell region) [32].

The production of tumor necrosis factor-α (TNF-α), an important pro-inflammatory cytokine, has been shown to be downregulated by taurolidine, a derivative of taurine. Taurolidine blocked the production of TNF-α by 50–90% in human peripheral blood mononuclear cells from healthy donors stimulated by LPS and INF-γ [33]. Additionally, the amount of TNF-α released from mouse macrophage-like RAW 264.7 cells was reduced in a dose-dependent manner by TauCl given in concentrations ranging from 0.2 to 1 mmol/L [34].

4.3.2. Human studies

Taurine's antioxidant activity in humans has received little attention. In a placebo-controlled trial of 12 patients with stable angina [35], intravenous infusion of 5 g taurine one to three hours before coronary artery bypass surgery reduced the level of lipoperoxidation products, an indicator of ROS, during reperfusion (restoration of blood flow). The mean oxidative stress ratio comparing reperfusion to pre-operative biopsy samples was 1.12 in the taurine pretreated group versus 2.45 in the placebo group [35].

Larger studies are needed to evaluate the effect of taurine in healthy individuals.

5. Human studies of taurine and heart disease

Table 3 includes two other human studies of the association between taurine and heart disease. The WHO-CARDIAC study [36,37], which recruited random samples of men and women 48–56 years of age from 24 study centers in 16 countries, investigated the ecological correlation between dietary factors and ischemic heart disease (IHD). As expected, urinary taurine levels were highest in Japanese men (2180.6 μmol/day) and women (1590.0 μmol/day), who had the greatest seafood consumption, and lowest in Canadian men (191.6 μmol/day) and Russian women (127.5 μmol/day). A significant inverse correlation was found between the group-level median value of urinary taurine excretion and age-adjusted IHD mortality rates in the study areas, both in men and women. The associations remained significant after adjustment for serum total cholesterol, BMI and urinary sodium to potassium excretion ratios [36]. A separate analysis of the male participants in the 16 countries found age-adjusted IHD mortality rates in the area populations to be significantly negatively associated with the average urinary taurine excretion after adjusting for group means of BMI, total cholesterol, urinary sodium/potassium ratio, polyunsaturated fatty acids, and polyunsaturated fatty acids/saturated fatty acids ratio [37]. However, the findings of this study are subject to ecologic fallacy because it is unknown whether the individuals who died of IHD actually had low levels of urinary taurine excretion, and adjustment of group means of potential confounders may not address the confounding effects at the individual level. Also, other potential confounders related to both CHD and taurine intake such as smoking status, physical activity, and socioeconomic status were not considered. Additionally, urinary taurine levels are unstable and may be highly dependent on daily food consumption which may be influenced by seasonal changes in food availability.

6. Discussion

In summary, animal and *in vitro* studies have provided insights into the mechanisms by which taurine can improve lipid profile, lower BP, and act as an antioxidant and anti-inflammation agent, suggesting great potential of taurine in improving the profile of cardiovascular risk factors and reducing occurrences of cardiovascular disease. A few small clinical trials and observational studies in humans have also suggested short-term benefits of taurine supplementation on lipid and BP profiles.

The data from existing human studies indicate that taurine may confer substantial benefits in reducing the risk of CHD on the population level. For instance, based on a meta-analysis of individual data for one million adults in 61 prospective studies, a 2 mmHg lower usual systolic BP would decrease stroke mortality by 10% and IHD or other vascular cause mortality by 7% in the middle aged [38]. A clinical trial of taurine supplementation showed that taurine reduced blood pressure 6 mmHg more than placebo [27]. However, several limitations of the existing studies should be considered, including: (1) ecologic study design with analyses based on group-level data; (2) small sample sizes in the randomized clinical trials, with fewer than 30 participants in all of them; (3) characteristics of the study populations, including subjects with existing CHD, hypertension, or obesity; and (4) short-term duration of taurine supplementation in the randomized clinical trials (≤2 months); and (5) lack of information on potential confounders in observational studies. Future observational epidemiologic studies that address these limitations are needed to evaluate long-term human health effects of taurine on BP, cholesterol profile, and other risk factors for CHD.

Currently, no prospective epidemiologic studies have been conducted to investigate taurine's possible association with CHD incidence. One of the reasons for this could be the lack of a reliable measure of long-term taurine level. Use of questionnaires to estimate taurine dietary intake is difficult because the content of taurine differs appreciably by type of seafood and cut of meat, posing a challenge in calculating taurine intake from diet questionnaires. Biochemical measurements of taurine reflecting an "internal dose" would be more accurate. However, it is important to evaluate to what extent the level measured in urine or blood samples fluctuates over time before using these measurements in large epidemiologic studies. In addition, lifestyle or other nutritional factors that may be related to both taurine levels and cardiovascular outcomes are largely unknown. These data are needed to support the validity of findings in epidemiologic studies of taurine and CHD.

Although no minimum level of intake with adverse effect has been set for taurine, a recent risk assessment study designated the upper level of taurine supplementation at 3 g per day. This assessment was based on toxicological evidence from a review of all human clinical trials with taurine supplementation [39]. The only adverse effects noted after consuming a 3 g dose of taurine were gastrointestinal disturbances. It should be noted that the minimum dose used in the existing trials was 3 g/day, much greater than the usual intake of taurine from diet (<0.4 g/day). However, an inverse association between taurine and CHD-related outcomes has been reported in ecologic studies without taurine supplementation, suggesting that potential beneficial effects of taurine may exist at lower levels. Future studies are needed to evaluate the full dose–response relationship between taurine intake and CHD-related outcomes. Although some "energy drinks" contain high levels of taurine (>1 g/serving), they also contain high amounts of caffeine and other ingredients; therefore, health effects relating to their use should be evaluated separately.

The relationship between dietary sources of taurine and the biochemical availability of taurine in the human body await research investigation. For example, knowledge of the specific equation relating food intake to serum level of taurine would be useful if taurine has preventive effects. In addition to taurine, fish and shellfish may contain other nutrients or environmental contaminants that may influence heart health, including cholesterol, omega-3 fatty acids, mercury, PCBs (polychlorinated biphenyls), and dioxins. Understanding taurine's role in CHD etiology may help improve current dietary guidelines for CHD. Further research will be needed to evaluate whether taurine is beneficial for subgroups in the population with high risk of CHD, or those who do not or cannot regularly consume meat or seafood.

In conclusion, considering the *in vitro*, animal, and human studies reviewed, there are several plausible mechanisms through which taurine may decrease the risk of CHD. However, the evidence from epidemiologic studies is limited due to the shortcomings in study design, sample size, and the characteristics of study populations. Nutritional studies of dietary sources of taurine and the biochemical availability of taurine, as well as epidemiologic studies using CHD or CHD risk factors as endpoints are needed to provide more definitive answers about the influence of long-term taurine levels on preclinical and clinical CHD outcomes.

Conflict of interest

The authors had no conflict of interest.

Acknowledgments

Contributors: The contributions of the authors were as follows—OPW, KLK, AZJ, MC, and YC contributed to the conception, revision and editing of the manuscript.

References

[1] AHA. Heart disease and stroke statistics—2008 update at a glance. Dallas, Texas: American Heart Association; 2008. p. 1–43.
[2] Birdsall TC. Therapeutic applications of taurine. Alt Med Rev 1998;3:128–36.
[3] Huxtable RJ. Expanding the circle 1975–1999: sulfur biochemistry and insights on the biological functions of taurine. Adv Exp Med Biol 2000;483:1–25.
[4] Schuller-Levis G, Park E. Is taurine a biomarker? Adv Clin Chem 2006;41:1–21.
[5] O'Flaherty L, Stapleton PP, Redmond HP, Bouchier-Hayes DJ. Intestinal taurine transport: a review. Eur J Clin Invest 1997;27:873–80.
[6] Tappaz ML. Taurine biosynthetic enzymes and taurine transporter: molecular identification and regulations. Neurochem Res 2004;29:83–96.
[7] Stapleton PP, Charles RP, Redmond HP, Bouchier-Hayes DJ. Taurine and human nutrition. Clin Nutr 1997;26:103–8.
[8] Finnegan D. The health effects of stimulant drinks. Br Nutr Foundation Nutr Bull 2003;28:147–55.
[9] Paauw JD, Davis AT. Taurine supplementation at three different dosages and its effects on trauma patients. Am J Clin Nutr 1994;60:203–6.
[10] Yamori Y, Murakami S, Ikeda K, Nara Y. Fish and lifestyle-related disease prevention: experimental and epidemiological evidence for anti-atherogenic potential of taurine. Clin Exp Pharmacol Physiol Suppl 2004;31:S20–3.
[11] Lam NV, Chen W, Suruga K, Nishimura N, Goda T, Yokogoshi H. Enhancing effect of taurine on CYP7A1 mRNA expression in Hep G2 cells. Amino Acids 2006;30:43–8.
[12] Yokogoshi H, Mochizuki H, Nanami K, Hida Y, Miyachi F, Oda H. Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. J Nutr 1999;129:1705–12.
[13] Murakami S, Kondo Y, Toda Y, et al. Effect of taurine on cholesterol metabolism in hamsters: up-regulation of low density lipoprotein (LDL) receptor by taurine. Life Sci 2002;70:2355–66.
[14] Chen W, Matuda K, Nishimura N, Yokogoshi H. The effect of taurine on cholesterol degradation in mice fed a high-cholesterol diet. Life Sci 2004;74:1889–98.
[15] Mizushima S, Nara Y, Sawamura M, Yamori Y. Effects of oral taurine supplementation on lipids and sympathetic nerve tone. Adv Exp Med Biol 1996;403:615–22.
[16] Zhang M, Bi L, Fang JH, et al. Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. Amino Acids 2004;26:267–71.
[17] Schaffer SW, Lombardini JB, Azuma J. Interaction between the actions of taurine and angiotensin II. Amino Acids 2000;18:305–18.
[18] Kohashi N, Okabayashi Y, Hama J, Katori R. Decreased urinary taurine in essential hypertension. Prog Clin Biol Res 1983;125:73–87.
[19] Yamamoto J, Akabane S, Yoshimi H, Nakai M, Ikeda M. Effects of taurine on stress-evoked hemodynamic and plasma catecholamine changes in spontaneously hypertensive rats. Hypertension 1985;7:913–22.
[20] Fujita T, Sato Y. Hypotensive effect of taurine. Possible involvement of the sympathetic nervous system and endogenous opiates. J Clin Invest 1988;82:993–7.
[21] Ideishi M, Miura S, Sakai T, Sasaguri M, Misumi Y, Arakawa K. Taurine amplifies renal kallikrein and prevents salt-induced hypertension in Dahl rats. J Hypertens 1994;12:653–61.
[22] Militante JD, Lombardini JB. Treatment of hypertension with oral taurine: experimental and clinical studies. Amino Acids 2002;23:381–93.
[23] Anuradha CV, Balakrishnan SD. Taurine attenuates hypertension and improves insulin sensitivity in the fructose-fed rat, an animal model of insulin resistance. Can J Physiol Pharmacol 1999;77:749–54.
[24] Nara Y, Yamori Y, Lovenberg W. Effect of dietary taurine on blood pressure in spontaneously hypertensive rats. Biochem Pharmacol 1978;27:2689–92.
[25] Liu L, Liu L, Ding J, et al. Ethnic and environmental differences in various markers of dietary intake and blood pressure among Chinese Han and three other minority peoples of China: results from the WHO cardiovascular diseases and alimentary comparison (CARDIAC) study. Hepertens Res 2001;24:315–22.
[26] Mizushima S, Moriguchi EH, Ishikawa P, et al. Fish intake and cardiovascular risk among middle-aged Japanese in Japan and Brazil. J Cardiovasc Risk 1997;4:191–9.
[27] Fujita T, Katsuyuki A, Noda H, Yasushi I, Sato Y. Effects of increased adrenomedullary activity and taurine in young patients with borderline hypertension. Circulation 1987;75:525–32.
[28] Sethupathy S, Elanchezhiyan C, Vasudevan K, Rajagopal G. Antiatherogenic effect of taurine in high fat diet fed rats. Indian J Exp Biol 2002;40:1169–72.
[29] Kondo Y, Toda Y, Kitajima H, et al. Taurine inhibits development of atherosclerotic lesions in apolipoprotein E-deficient mice. Clin Exp Pharmacol Physiol 2001;28:809–15.
[30] Marcinkiewicz J, Grabowska A, Bereta J, Bryniarski K, Nowak B. Taurine chloramine down-regulates the generation of murine neutrophil inflammatory mediators. Immunopharmacology 1998;40:27–38.
[31] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;326:801–9.
[32] Casey RG, Gang C, Joyce M, Bouchier-Hayes DJ. Taurine attenuates acute hyperglycaemia-induced endothelial cell apoptosis, leucocyte–endothelial cell interactions and cardiac dysfunction. J Vasc Res 2007;44:31–9.
[33] Bedrosian I, Duane Sofia R, Wolff SM, Dinarello CA. Tauroline, an analogue of the amino acid taurine, suppresses interleukin 1 and tumor necrosis factor synthesis in human peripheral blood mononuclear cells. Cytokine 1991;3:568–75.
[34] Park E, Quinn MR, Wright CE, Schuller-Levis G. Taurine chloramine inhibits the synthesis of nitric oxide and the release of tumor necrosis factor in activated RAW 264.7 cells. J Leukoc Biol 1993;54:119–24.
[35] Milei J, Ferreira R, Llesuy S, Forcada P, Covarrubias J, Boveris A. Reduction of reperfusion injury with preoperative rapid intravenous infusion of taurine during myocardial revascularization. Am Heart J 1992;123:339–45.
[36] Yamori Y, Liu L, Ikeda K, et al. Distribution of twenty-four hour urinary taurine excretion and association with ischemic heart disease mortality in 24 populations of 16 countries: results from the WHO-CARDIAC Study. Hypertens Res 2001;24:453–7.
[37] Yamori Y, Liu L, Mizushima S, Ikeda K, Nara Y. Male cardiovascular mortality and dietary markers in 25 population samples of 16 countries. J Hypertens 2006;24:1499–505.
[38] Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002;360:1903–13.
[39] Shao A, Hathcock JN. Risk assessment for the amino acids taurine, L-glutamine and L-arginine. Regul Toxicol Pharmacol 2008;50:376–99.
[40] Sturman JA, Rassin DK, Gaull G. Taurine in the development of the central nervous system. In: Babeau A, Huxtable RJ, editors. Taurine and neurological disorders. New York: Raven; 1978. p. 47–91.
[41] Hayes KC, Sturman JA. Taurine in metabolism. Annu Rev 1981;1:401–25.
[42] Sole MJ, Jeejeebhoy KN. Conditioned nutritional requirements and the pathogenesis and treatment of myocardial failure. Curr Opin Clin Nutr Metab Care 2000;3:417–24.
[43] Hansen SH. The role of taurine in diabetes and the development of diabetic complications. Diabetes Metab Res Rev 2001;17:330–46.
[44] Learn DB, Fried VA, Thomas EL. Taurine and hypotaurine content of human leukocytes. J Leukoc Biol 1990;48:174–82.
[45] Brosnan JT, Brosnan ME. The sulfur-containing amino acids: an overview. J Nutr 2006;136. p. S1636–S1640.
[46] Spaeth DG, Schneider DL, Sarett HP. Taurine synthesis, concentration, and bile salt conjugation in rat, guinea pig, and rabbit. Proc Soc Exp Biol Med 1974;147:855–8.
[47] Huxtable RJ. Physiological actions of taurine. Physiol Rev 1992;72:101–63.
[48] Laidlaw SA, Grosvenor M, Kopple JD. The taurine content of common foodstuffs. J Parenter Enteral Nutr 1990;14:183–8.
[49] Pasantes-Morales H, Quesada O, Alcocer L, Sánchez Olea R. Taurine content in foods. Nutr Rep Int 1989;40:793–801.
[50] Lourenço R, Camilo ME. Taurine: a conditionally essential amino acid in humans? An overview in health and disease. Nutr Hosp 2002;17:262–70.

Diabetes and Vascular Disease Research
http://dvr.sagepub.com/

Two weeks taurine supplementation reverses endothelial dysfunction in young male type 1 diabetics

Michael A Moloney, Rowan G Casey, David H O'Donnell, Patricia Fitzgerald, Chris Thompson and David J Bouchier-Hayes

Diabetes and Vascular Disease Research 2010 7: 300 originally published online 28 July 2010
DOI: 10.1177/1479164110375971

The online version of this article can be found at:
http://dvr.sagepub.com/content/7/4/300

Published by:
SAGE
http://www.sagepublications.com

Diabetes & Vascular Disease Research
7(4) 300–310

DOI: 10.1177/1479164110375971
dvr.sagepub.com
SAGE

Two weeks taurine supplementation reverses endothelial dysfunction in young male type 1 diabetics

Michael A Moloney[1], Ronan G Casey[1], David H O' Donnell[1], Patricia Fitzgerald[2], Chris Thompson[3] and David J Bouchier-Hayes[1]

Abstract

Type 1 diabetics have a well-recognised risk of accelerated cardiovascular disease. Even in the absence of clinical signs there are detectable abnormalities of conduit vessel function. Our group has previously reported reversal of endothelial dysfunction in diabetics with pravastatin. In young asymptomatic smokers, taurine supplementation has a beneficial impact on macrovascular function, assessed by FMD, and shows an up-regulation of nitric oxide from monocyte–endothelial cell interactions. We hypothesise that taurine supplementation reverses early endothelial abnormalities in young male type 1 diabetics, as assessed by applanation tonometry, brachial artery ultrasound and laser Doppler fluximetry. Asymptomatic, male diabetics (n=9) were scanned prior to treatment and then randomised in a double-blind cross-over fashion to receive either 2 weeks placebo or taurine. Control patients (n=10) underwent a baseline scan. Assessed diabetics had detectable, statistically significant abnormalities when compared with controls, in both arterial stiffness (augmentation index) and brachial artery reactivity (FMD). Both of these parameters were returned to control levels with 2 weeks taurine supplementation. In conclusion, 2 weeks taurine supplementation reverses early, detectable conduit vessel abnormalities in young male diabetics. This may have important implications in the long-term treatment of diabetic patients and their subsequent progression towards atherosclerotic disease.

Key words

Applanation tonometry, diabetes mellitus, flow-mediated dilatation, laser Doppler fluximetry, taurine

Introduction

In patients with type 1 diabetes, cardiac autonomic neuropathy may be present at a very early age, even in an asymptomatic population.[1] This is associated with a significant increase in mortality.[2] Several clinical trials have generated important results that validate effective strategies for modifying cardiovascular risk in diabetics.[3] Even in the clinically asymptomatic type 1 diabetic patient there are statistical differences in blood pressure.[4] These individuals have statistically higher end systolic blood pressure, even in the setting of clinically asymptomatic disease, when compared with controls. Diabetic retinopathy is considered an early sign of widespread overall vascular damage.[5] Interestingly, even in the absence of retinopathy, nephropathy and neuropathy, young assumed-asymptomatic diabetics have clinically detectable abnormalities, predictive of the development of future cardiovascular complications.[6-11] There is even evidence of cardiovascular abnormalities in diabetic children as young as 11 years.[9] Clinical assessment for nephropathy, neuropathy and retinopathy is commonplace, each reflecting both control and stage of disease. Non-invasive assessment of endothelial function in diabetics is both reproducible and reliable. Pulse wave velocity and pulse wave assessment can be determined from measurements of pulse waveform. Increased pulse wave velocity has been associated with increasing age, arterial blood pressure, diabetes, smoking and end-stage renal disease.[12] Arterial and endothelial dysfunction is different in high and low-risk subjects,[13] with arterial elasticity assessment by radial pulse waveform analysis

[1]Department of Vascular Surgery, Beaumont Hospital, Dublin, Ireland
[2]Department of Non-Invasive Vascular Imaging, Beaumont Hospital, Dublin, Ireland
[3]Department of Endocrinology, Beaumont Hospital, Dublin, Ireland

Corresponding author:
Dr Tony Moloney,
Royal College of Surgeons in Ireland, Department of Vascular Surgery, Beaumont Hospital, Beaumont Road, Dublin 9, Ireland.
Email: tmoloney@rcsi.ie

correlating with FMD in young healthy subjects and older type-2 diabetics.

HMG CoA reductase inhibitors are known to reverse endothelial dysfunction in young, normoalbuminuraemic, male diabetics.[4,14] Experimental evidence suggests that HMG CoA reductase inhibitors increase both expression and activity of endothelial nitric oxide synthase,[15,16] and therefore up-regulate nitric oxide and reverse endothelial dysfunction. Lifelong statin treatment presents economical, ethical and psychological dilemmas, with a recognised incidence of adverse side effects such as muscle and liver toxicity. The incidence of transaminase increases greater than threefold is approximately 1% for all statins, and is dose related.[17] Myopathy occurs in 1 in 1000 patients.[18] Also, the risk of rhabdomyolysis and other adverse effects with statin use can be exacerbated by several factors, including diabetes.[18]

β-aminoethane sulphonic acid, or taurine, is a conditionally essential amino acid, found abundantly in tissues that are excitable and rich in membranes that generate oxidants. It is the most prevalent of all amino acids in skeletal tissue, cardiac muscles and the brain, and is essential in the functioning of the brain, heart, lungs, blood, liver, pancreas, gall bladder and the kidney. Food sources include dairy products, oatmeal and seafood. It is now well accepted that taurine has an important role to play in prevention of hypertension and stroke.[19] In diabetic patients a hypoglycaemic effect has previously been reported.[20] Several papers have reported that taurine potentiates the effect of insulin,[21] and possibly the insulin receptor.[22] In type 1 diabetics both plasma and platelet taurine levels are reduced,[23] with oral supplementation returning them to above control levels.[24,25] Long-term treatment with taurine in streptozotocin-induced diabetic rats reduced mortality.[26] Taurine has also been shown to inhibit ischaemia-induced apoptosis in cardiac myocytes[27] and endothelial cells.[28] It protects against myocardial injury in hyperhomocysteinaemia in rats,[29] it reduces iron-mediated myocardial oxidative stress and preserves cardiovascular function in a murine model,[30] and its therapeutic role in the reduction following ischaemia/reperfusion injury is well documented.[31]

We therefore hypothesised that taurine supplementation in normoalbuminuraemic type 1 diabetics reverses early, detectable, endothelial abnormalities assessed by applanation tonometry, brachial artery ultrasound and laser Doppler fluximetry.

Methods

Patient population

Young male patients with type 1 diabetes mellitus were recruited from the diabetic day care centre at Beaumont Hospital, Dublin, Ireland, under the supervision of a Consultant Endocrinologist. All patients were male and under 30 years of age. They were all type 1 diabetics, with no evidence of macrovascular or microvascular disease and on no other medication apart from the appropriate insulin dose. All patients had a 24-h urine collection and urinary albumin excretion status to exclude microalbuminuria (Cobasmiras, Roche).

Exclusion criteria:

- Other risk factors for the development of cardiovascular disease, including smoking, hyperlipidaemia, hypertension, family history of premature vascular disease
- Female sex
- BMI>30

Control subjects underwent the same exclusion criteria. All patients were age, sex and weight matched.

Treatment protocol

All patients were provided with a thorough explanation of the study. Subjects and General Practitioners were given an information leaflet, and subjects gave informed consent to the study. The Beaumont Hospital Ethics Committee approved this study, and the Irish Medicines Board approved the use of placebo and taurine. Diabetics were supplemented with 1.5 g/day taurine (500 mg three times daily) (Twinlab) for 14 days. Placebo was an identical tablet administered three times daily for 14 days also.

Controls and diabetics were assessed at baseline. Diabetics were then randomised in a double-blind, cross-over fashion to either placebo or control and treated for 2 weeks, reassessed and treated for a further 2 weeks with the other medication and then reassessed again (Figure 1). Randomisation was achieved by placing of the tablets, placebo or taurine, in identical envelopes by an independent, non-medical member of staff. The envelopes were then labelled with numbers only, thereby not giving away identify of the treatment limb but allowing for identification of the various limbs at the end of the study. Diabetics were assessed at three separate time points, 2 weeks apart. Controls were assessed at baseline.

Haemodynamic studies

All studies were performed in the non-invasive vascular laboratory at Beaumont Hospital, Dublin, Ireland, under the supervision of a consultant in vascular imaging. All non-invasive assessments took place in a quiet, temperature-controlled room (20°C) with the subject lying comfortably in a resting supine state for 15 min. Subjects were asked to avoid caffeine and exercise for the preceeding 12 h. Blood pressure was then assessed on three occasions and the mean value, along with patient demographics, height and weight were recorded.

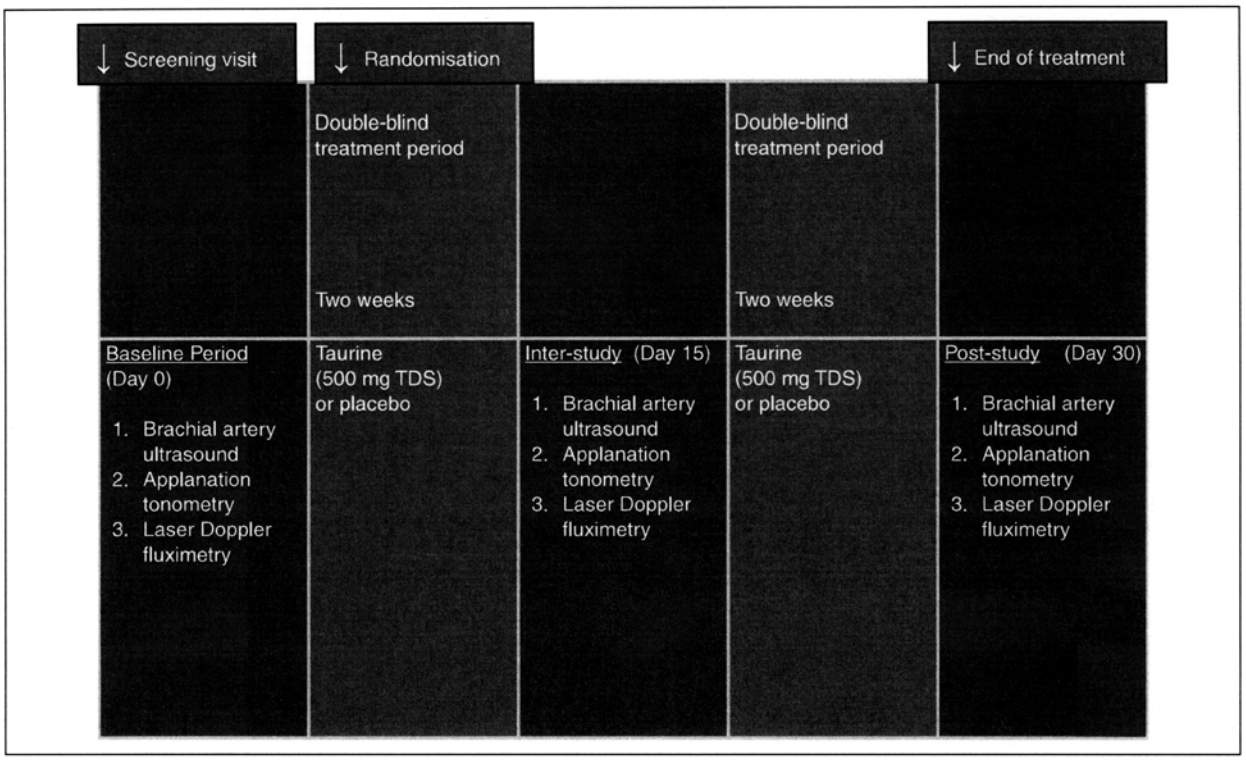


Figure 1. Study protocol for the trial.

Brachial artery ultrasound and flow-mediated dilatation

Flow-mediated changes in conduit artery diameter are caused by shear stress-induced generation of endothelial derived vasoactive mediators,[32] reflected in FMD. This technique involves measurement of the brachial artery,[33] assessed by high-resolution external vascular ultrasound in response to an increase in blood flow (causing shear stress) during reactive hyperaemia (inflation of a distal cuff placed around the wrist). This leads to endothelium-dependent dilatation; the response is then contrasted with that to sublingual GTN, an endothelium-independent dilator. The artery is therefore scanned at three time points: at baseline, immediately following reactive hyperaemia and finally after administration of GTN. A cuff inflation time of 270 s is now widely accepted to produce adequate hyperaemia for assessment of FMD.[34] This technique for assessment of endothelial function has been extensively described and is accurate and reproducible in vivo.[32,33,35-38] The brachial artery is imaged using a 13 MHz linear array transducer ultrasound system (Acuson 128XP/10 system, Acuson, California). The subject's right arm is comfortably immobilised in the extended position to allow constant imaging of the brachial artery. The artery is scanned in longitudinal section with a pulsed Doppler signal at a 70° angle to the vessel and at a location in the antecubital fossa where the clearest anterior posterior M lines are visible. Images are recorded on videotape for subsequent off-line analysis using ultrasound calipers. Baseline readings are taken for 2 min. A blood pressure cuff applied to the distal right forearm is then inflated to 240 mmHg for 4.5 min and subsequently deflated. The endothelial-dependent vasomotor responses to reactive hyperaemia are recorded 45–60 s after cuff deflation. GTN is then administered sublingually and endothelial-independent dilatation is assessed as above. At later off-line analysis the baseline brachial artery diameter, FMD in response to reactive hyperaemia and endothelial-independent dilatation are calculated. Diameter changes are expressed as the percentage change relative to the mean baseline scan (100%). Using ultrasonic calipers, measurements are taken from the anterior to the posterior M line co-incident with the end of diastole (on the ECG tracing) using the 'm' mode. The mean diameter is calculated from four cardiac cycles incident with the 'R' wave on the ECG.

Applanation tonometry – assessment of central and peripheral haemodynamics

With each beat of the left ventricle a new pulse wave is formed, each reflective and predictive of cardiac function.

Arterial tonometry permits accurate representation of the pressure waveform at sites where the artery can be compressed against underlying bone. Ideal sites are radial and carotid arteries.[39] Radial tonometry is more reproducible; it gives an accurate pressure wave contour and is a fair representation of pulse pressure. The technique is relatively simple and involves placement of a flat sensor which flattens the arterial wall, eliminating tangential pressures and exposing the artery to pressure within the artery and therefore accurately recording it. Synthesised waveforms correspond well to those recorded invasively in the ascending aorta, or to surrogate waveforms measured non-invasively.[40] The waveform is then transferred into the corresponding central arterial waveform, using a generalised transfer factor based on data from invasive recordings.[41] Therefore pulse waveform analysis can be used to record the peripheral waveform and to generate a corresponding central waveform. Both waveforms can then be analysed to give information on the augmentation index and central pressures. The augmentation index was initially described by Kelly et al.[42,43] as the ratio of augmentation index pressure (differences in pressure between early and late systolic shoulders of the pulse waveform) and pulse pressure expressed as a percentage. This is now a well-accepted technique for the assessment of arterial stiffness.[41,44-47] In this pilot clinical trial the right radial pulse was used for assessment of the pulse waveform. Peripheral and central parameters were measured using the applanation tonometry probe.

Pulse pressure waveform and amplitude are obtained with a probe that incorporates a high fidelity strain transducer (Model TCB-500, Millar Instruments). Pulse waves are obtained by placing the pencil-like probe perpendicularly over the point of strongest pulsation of the radial artery. The sensor is used to flatten the arterial wall against a bony surface, tangential pressures are eliminated, and the sensor is exposed to the true intra-arterial pressure. The wrist is held dorsiflexed using a support and the probe is applied with steady pressure until a good, recurring waveform signal appears within the display. A good waveform is defined as one that is consistent, large (at least 3 cm on screen) and in a steady position. Twenty consecutive pressure waveforms are recorded, averaged, and peripheral parameters such as dP/dt, the differential of the pressure wave, are obtained.[43] When 20 waveforms are analysed the PHV is calculated. This is a percentage of mean pulse height (difference between maximum and minimum of each pulse). The PHV parameter gives a numerical value and will increase with beat-to-beat pulse height variations, typically as a result of poor quality signals. Diastolic variability is the averaged variability of the diastolic points (minimums) from the mean diastolic as a percentage of the pulse height. It increases with beat-to-beat variations of the minimums and is a measure of how steadily the tonometer is held during the measurements (if the tonometer is held perfectly steady then the PHV% and diastolic variability is zero). Values greater than 4% will not be recorded, and the software programme expects repeat measurements to be made until they fall within 0–4% variability. With the use of the constant transfer function from ascending aorta to the radial artery the aortic pressure waveform and left ventricular function can be measured. The differential pressure wave (dP/dt) that is measured from the radial artery can be used to measure the amplitude of reflected peripheral waves from the vascular tree. When compared with the central cardiac impulse this gives the augmentation index. Left ventricular properties, such as ejection duration and heart rate, can be derived from the aortic pressure wave. This aortic wave can be subdivided into systolic and diastolic components, and allows ventriculo–vascular interactions at baseline and following stressors to be calculated.

Laser Doppler fluximetry

A variety of methods have been used to assess skin viability and tissue micro-perfusion.[48] The principle of laser Doppler flowmetry utilises the fact that a laser light beam incident on tissue is scattered both in static and moving structures (red cells). Light beams scattered in moving red cells undergo a frequency shift according to the Doppler effect, while beams scattered in static tissue alone remain unshifted. A portion of the backscattered light is brought to impinge on the surface of a photodetector where beat notes produced by mixing of waves scattered in different structures are formed. When assessing skin blood flow, laser Doppler flowmetry is found to be more specific and sensitive to changes in blood flow than xenon-washout techniques. This concept has been previously used to predict patency of skin flaps,[49] to visualise the nature of rhythmical variations in healthy human skin,[50] to assess skeletal muscle blood flow,[51] and to assess renal[52] and testicular blood flow.[53] It has also recently been documented that type 1 diabetics have impaired pressure-induced vasodilatation, assessed by laser Doppler flowmeter.[54] The venoarteriolar response (postural vasoconstriction) assessed by laser Doppler flowmetry has also been found to be both reliable and reproducible.[55,56]

Laser Doppler fluximetry is a well-established technique employed in the measurement of the microcirculation following various stimuli, including acute hyperglycaemia and cigarette smoke.[57-60] A laser signal is emitted from a probe. The depth of penetration is determined by the wavelength of emitted light, and the shape is determined by the probe configuration. Therefore, this system measures red blood cell movement in a fixed volume of tissue, which is then an indirect measure of red blood cell flow or 'flux'. There is a small zone of injury around the tip of the probe, but the penetration of the signal is greater and therefore is measuring flux in normal tissues. Measurement of red

blood cell motion is recorded continuously in the outer layer of the tissue under study, with little or no influence on physiological blood flow. This output value constitutes the flux of red cells, defined as the number of red cells times their velocity, and is reported as microcirculatory perfusion units. No direct information concerning oxygen, nutrient or waste metabolite exchange in the surrounding tissue is obtained with this technique. The relationship between the flowmeter output signal and the flux of red blood cells is linear. The beam can penetrate unbroken, non-pigmented tissue to a depth of 1–2 mm. In this study we chose to use one endothelial-dependent and one endothelial-independent stimulus. A postural change from lying to sitting/standing causes a precapillary arteriolar vasoconstriction known as the venoarteriolar reflex, which is thought to protect capillaries by preventing rises in capillary hydrostatic pressure and ultimately transudation and tissue oedema.[61]

Laser Doppler fluximetry results in arbitrary values of flux units. As such there are no absolute measurements of blood flow, and comparisons between baseline and following a stimulus must be made while the Doppler probe is positioned against the skin at the same sitting under the same environmental and study conditions. For this reason it is very difficult to compare baseline flow among groups of subjects, and results are generally given as percent change from an arbitrary baseline.

Serum samples

Haemoglobin, packed cell volume, inflammatory indicators (white cell count, erythrocyte sedimentation rate and C-reactive protein), urea and electrolytes, liver function tests, cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, glucose, glycosylated haemoglobin (HA-8140, Menarini) and frustosamine were assessed at the various time points in both study groups.

Statistical analysis

Statistical analysis was performed with SPSS version 12.0 statistics software. Descriptive statistics as mean ± SEM. ANOVA, with post hoc Tukey-Kramer multiple comparisons testing. The $p<0.05$ level was set as significant. All patients were entered in the study on an intention-to-treat basis and no subjects dropped out or were removed from the study.

Results

Demographics, blood indices and glycaemic control (Table 1)

There were no statistical differences between controls and diabetics in age or BMI. 24-h urinary albumin in diabetics was within the normal reference range (Cobasmiras, Roche).

There were no statistical differences in haemoglobin, inflammatory indicators (white cell count, erythrocyte sedimentation rate and C-reactive protein), urea and electrolytes, liver function tests, cholesterol, low-density lipoprotein, high-density lipoprotein or triglycerides. Packed cell volume was statistically lower in diabetics (0.42 (0.01)) when compared with controls (0.45, $p=0.048$); this was unaltered with treatment. Serum glucose measurements were not significantly elevated in patients with diabetes at the various time points. Glycosylated haemoglobin (HA-8140, Menarini) was statistically higher in diabetics (7.8 (0.9)) compared with controls (4.3, $p=0.010$), This was unaltered throughout the study period. There was a statistical difference in fructosamine levels between controls (236) and diabetics (409) and throughout the study period ($p<0.050$) (see Table 1).

Applanation tonometry: myocardial workload parameters (Table 2)

End Systolic / Mean systolic / Mean diastolic blood pressures There were no statistical differences between controls and patients (baseline, placebo or taurine treated).

Augmentation index Diabetic patients at baseline (2.3 (4.8)) had a statistically higher augmentation index when compared with controls (-10.9 (1.9), $p=0.020$). This was returned to control levels with 2 weeks taurine supplementation (-15.3 (3.5), $p=0.001$) (see Figure 2).

Applanation tonometry: myocardial perfusion parameters (Table 2)

Heart rate Diabetic patients at baseline (69 (2.5) beats/min) had a statistically higher heart rate when compared with controls (58 (3.6) beats/min, $p=0.010$). This was unaltered by taurine or placebo supplementation.

Ejection duration Diabetic patients had a statistically lower ejection duration (298 (7) ms) when compared with controls (324 (9), $p=0.042$). This was unaltered by taurine or placebo supplementation.

Buckberg Index (SEVR) Diabetic patients at baseline (171 (5)) had a statistically lower SEVR when compared with controls (208 (15), $p=0.041$), this was unaffected by either placebo or taurine supplementation.

Brachial artery reactivity (Table 2)

Baseline vessel diameter There were no statistical differences in brachial artery diameter between controls and diabetics and throughout the study period.

Table 1. Demographics, blood indices and glycaemic control.

	Control	DM-Baseline	DM-Taurine	DM-Placebo	p value
Age (Years)	29 (1.0)	28 (2.0)	28 (2.0)	28 (2.0)	NS
BMI (kg^2/m)	27.9 (1.2)	22.7 (0.9)	–	–	NS
Haemoglobin (g/dL)	15.5 (0.4)	14.9 (0.3)	14.9 (0.2)	14.7 (0.3)	NS
Haematocrit (PCV)	0.45 (0.009)	0.42 (0.01) $	0.41 (0.006)	0.41 (0.01)	<0.05
White cell count ($X10^9/L$)	5.8 (0.3)	5.8 (0.7)	6.0 (0.7)	5.3 (0.6)	NS
Erythrocyte sedimentation rate (mm/h)	3.9 (0.7)	4.8 (0.7)	3.2 (0.5)	4.2 (1.1)	NS
Total Cholesterol (mmol/L)	4.3 (0.2)	4.7 (0.5)	4.6 (0.4)	4.8 (0.5)	NS
Triglycerides (mmol/L)	1.14 (0.2)	1.2 (0.3)	1.2 (0.2)	1.6 (0.4)	NS
HDL cholesterol (mmol/L)	1.3 (0.06)	1.4 (0.2)	1.4 (0.1)	1.2 (0.1)	NS
LDL cholesterol (mmol/L)	2.5 (0.1)	2.7 (0.4)	2.7 (0.4)	2.9 (0.4)	NS
Serum von Willebrand factor (% control)	71 (4)	110 (10) $	111 (14)	93 (8)	<0.05
Serum glucose (mmol/L)	4.8 (0.2)	9 (2.8)	11 (3.8)	9 (3.4)	NS
Haemoglobin A_{1c}	4.3 (0.3)	7.8 (0.9) $	7.9 (0.9)	7.9 (1.0)	<0.05
Fructosamine	236 (17.5)	409 (70.6) $	422 (55.8)	401 (52.4)	<0.05

Key: DM = diabetes mellitus; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; $ - DM *vs.* Control $p<0.05$

Table 2. Subjects haemodynamic parameters assessed by applanation tonometry, brachial artery ultrasound and laser Doppler fluximetry.

	Control	DM-Baseline	DM-Taurine	DM-Placebo	P value
Myocardial Workload					
End systolic BP (mmHg)	93 (2)	93 (3)	91 (4)	94 (4)	NS
Mean systolic BP (mmHg)	99 (2)	97 (3)	98 (2)	98 (3)	NS
Mean diastolic BP (mmHg)	86 (8)	86 (3)	86 (2)	86 (3)	NS
Augmentation index	−10.9 (1.9)	2.3 (4.8) $	−15.3 (3.5)	0.3 (6.6)	0.001
Myocardial Perfusion Parameters					
Heart Rate (beats/min)	58 (3.6)	69 (2.5) £	71 (4.0)	69 (1.6)	<0.05
Ejection Duration (msec)	324 (9)	298 (7) £	289 (8)	293 (6)	<0.05
Buckberg index (SEVR)	208 (15)	171 (5) £	171 (10)	173 (8)	<0.05
Brachial Artery Reactivity					
Baseline vessel diameter	4.7 (0.2)	4.4 (0.1)	4.6 (0.04)	4.7 (0.3)	NS
Flow-mediated dilatation (%)	9.8 (1.1)	4.0 (0.6) $	9.0 (1.0)	4.3 (0.5)	0.004
Dilatation to GTN (%)	16.9 (1.9)	13.9 (2.1)	15.8 (1.4)	12.7 (1.4)	NS
Laser Doppler Fluximetry					
% Constriction	37 (4.3)	30 (9.3)	30 (12.5)	26 (5.1)	NS
% Dilatation	2113 (615)	794 (299) £	749 (170)	803 (198)	<0.05

Key: DM = diabetes mellitus; BP = blood pressure; SEVR = sub-endocardial viability ratio; GTN = glyceryl trinitrate; $ - DM-Baseline *vs.* Control & DM-Taurine $p<0.05$. £ - DM-Baseline *vs.* Control $p<0.05$.

Endothelial-dependent dilatation (Figure 3) Diabetics had a statistically lower FMD (4.0 (0.6)) when compared with controls (9.8 (1.1), $p=0.001$). This was returned to control levels with 2 weeks taurine supplementation (9.0 (1.0), $p=0.004$).

Endothelial-independent dilatation (smooth muscle dilatation) There were no statistical differences between any of the groups.

Laser Doppler fluximetry (Table 2)

Percentage constriction to leg dependency There was no significant difference between controls and diabetes groups (at baseline, taurine or placebo treated).

Percentage dilatation to heat Diabetics had a statistically lower percentage dilatation (794 (299)) when compared with controls (2113 (615), $p=0.047$), this was unaltered by treatment with taurine or placebo.

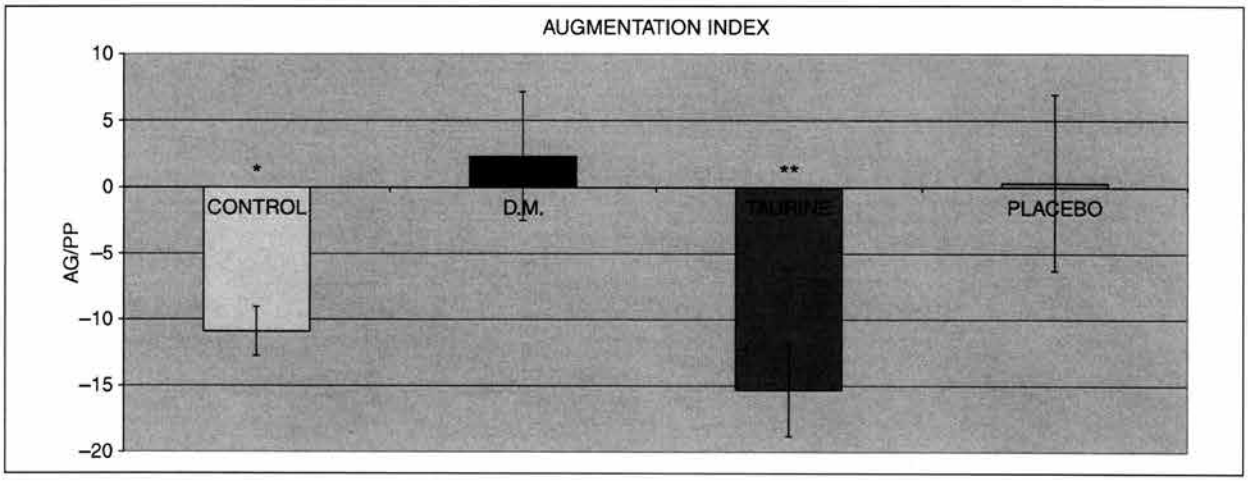


Figure 2. This graph demonstrates the Augmentation index (AI) in controls, diabetics at baseline (DM) and diabetics following supplementation with taurine and placebo for 2 weeks. Diabetics have a statistically higher AI compared with controls (* p=0.020). This is returned to control levels with 2 weeks taurine supplementation (** p=0.001).

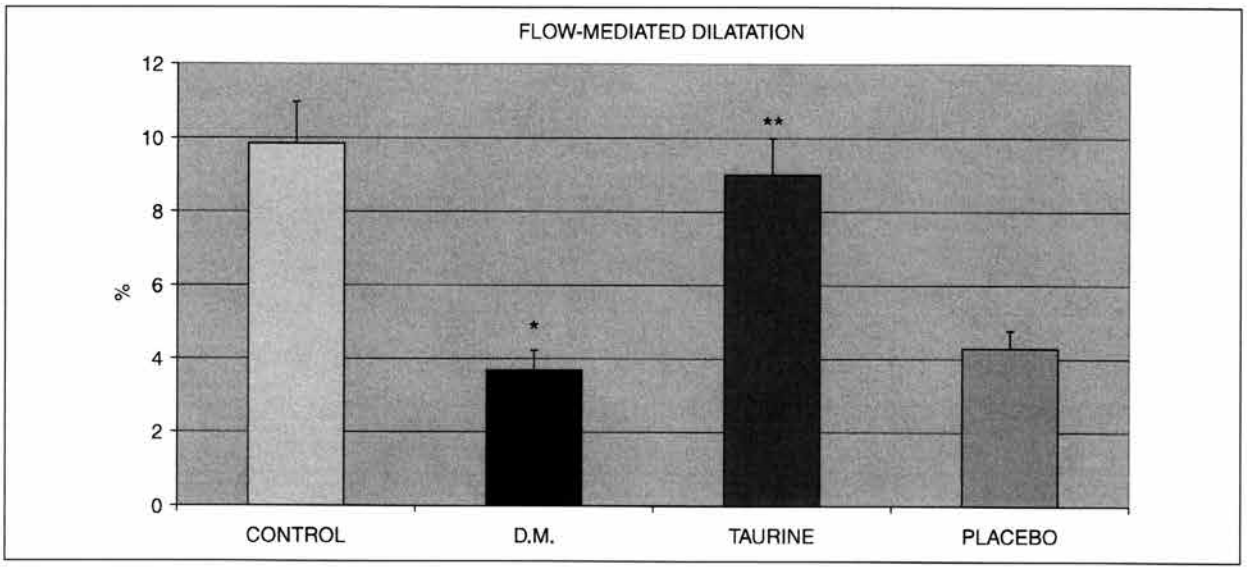


Figure 3. This graph demonstrates the flow-mediated dilatation (FMD) in controls, diabetics at baseline (DM) and diabetics following supplementation with taurine and Placebo for 2 weeks. Diabetics have a statistically lower FMD (* p=0.001) compared with controls. This returned to control levels with 2 weeks taurine supplementation (** p=0.004).

Discussion

This study confirms a multitude of detectable abnormalities in type 1 diabetics, all of which showed excellent glycaemic control. Also, we have for the first time demonstrated that 2 weeks taurine supplementation in young, normoalbuminuracmic, asymptomatic, type 1 diabetics reversed the augmentation index, a marker of arterial stiffness, and restored conduit vessel dysfunction, reflected in improvements in FMD.

Interestingly, in our study population of diabetics there were no statistical differences in glucose levels between controls and diabetics at the various time points. This is a reflection of the diabetic population's tight glycaemic control. Also, short-term glycaemic control, reflected in fructosamine levels, was unaltered in the diabetics across the various time points.

The incidence of many manifestations of coronary artery disease are increased in patients with type 1 diabetes.[62] Indeed, early markers of cardiovascular disease may be present in the absence of clinically detectable disease in diabetes, as reflected in this study in increased arterial stiffness and abnormal conduit vessel function, reflected in decreased FMD.

As with asymptomatic smokers,[63] our patient population of diabetics had a multitude of detectable, pre-clinical abnormalities. Augmentation index, assessed by non-invasive applanation tonometry, was statistically higher when compared with controls, even in the absence of any abnormal myocardial pressures parameters. Type 1 diabetics are reported to have stiffer large arteries in many,[64-66] although not all, studies.[67] Insulin therapy was found to decrease arterial stiffness in uncomplicated type 1 diabetes mellitus.[11] Aortic pulse wave velocity is a recognised independent predictor of mortality in both diabetics and glucose-tolerance-tested population samples. This is strongly correlated with a previous validated estimate of arterial stiffness.[47] Although we found no differences in central pressure parameters in the diabetic population assessed, coexistent raised arterial blood pressure in diabetes is associated with increased cardiovascular morbidity and mortality.[12] Our blood pressure findings are in contrast to previous reports of significant alterations in central pressure parameters in normoalbuminuraemic, type 1 diabetics.[4] This is possibly a reflection of our study population.

Diabetics assessed had multiple myocardial perfusion parameter abnormalities, which were unaltered by taurine. Both ejection duration and the SEVR were statistically lower in diabetics compared with controls. A decreased SEVR is a direct reflection of increased propensity to myocardial ischaemia, and failure to reverse this suggests that pulse rate is the dominant factor in the determination of the Buckberg index.

With regards to conduit vessel function, the diabetic population assessed had a statistically lower FMD when compared with controls. This is in keeping with previously published data.[5,7,9,14] Two weeks taurine supplementation reversed diabetic conduit vessel abnormalities to control values, as assessed by endothelial-dependent dilatation. This most likely resulted from improvements in bioavailability of nitric oxide due to increases in endothelial nitric oxide synthase.[68] Endothelial-independent dilatation, assessed by administration of sublingual GTN, was similar in all groups, thus confirming an endothelial-dependent abnormality as assessed by brachial artery reactivity.

Microcirculatory baseline flow was not statistically different in the diabetics compared with controls. Baseline flow was increased with taurine supplementation, but this was not statistically significant. With regards to the microcirculatory responses, there were no differences in percentage constriction between the groups, but diabetics had a statistically lower percentage dilatation response to heat. This was unaltered by taurine supplementation, possibly reflective of irreversible microcirculatory structural changes in our diabetic population. However, others have questioned both the reproducibility and reliability of the assessment of the skin microcirculatory bed, particularly in diabetics.

Our group, and others, have previously documented improvements in type 1 diabetic conduit vessel function with HMG CoA reductase inhibitors.[4,14] Lifelong incidence of adverse side effects from statin use is, as yet, unpublished. Also, the risk of rhabdomyolysis and other adverse effects with statin use can be exacerbated by several factors, including diabetes.[18]

Taurine, a semi-essential amino acid, therapeutic levels of which can be obtained by dietary manipulation, was found previously to have a hypoglycaemic effect in patients with diabetes mellitus.[20] Several papers have reported that taurine potentiates the effect of insulin,[21] and possibly the insulin receptor.[22] There have been several clinical trials on the assessment of taurine in type 1 diabetics.[23] One study demonstrated that both plasma and platelet taurine levels are reduced in these patients.[24] Oral supplementation returned them to above control levels.[24,25]

The pre-clinical, therapeutic potential of taurine in this 'at-risk' population of normoalbuminuraemic type 1 diabetics is clearly evident. The fact that therapeutic plasma concentrations of the amino acid can be achieved with dietary supplementation supports this strategy in the possible prevention of progression of type 1 normoalbuminuraemic diabetics to clinically overt vascular disease. Studies to evaluate the role of taurine in hyperglycaemic patients, type-2 diabetics and diabetics with microalbuminuria are essential, and assessment of taurine supplementation in these populations is mandatory, particularly in the setting of established cardiovascular disease.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Schnell, O., Muhr, D., Weiss, M., Desel, S., Haslbeck, M., Standl, E. (1996) Reduced myocardial ^{123}I-Metaiodobenzlguanidine uptake in newly diagnosed IDDM patients. *Diabetes* 45: 801–05.
2. Sampson, M.J., Wilson, S., Karagiannis, P., Edmonds, M., Watkins, P.J. (1990) Progression of diabetic autonomic neuropathy over a decade in insulin-dependent diabetics. *Q J Med* 278: 635–46.
3. Mazzone, T. (2004) Strategies in ongoing clinical trials to reduce cardiovascular disease in patients with diabetes mellitus and insulin resistance. *Am J Cardiol* 93(suppl): 27C–31C.
4. Casey, R., Joyce, M., Moore, K., Thompson, C., Fitzgerald, P., Bouchier-Hayes, D. (2007) Two-week treatment with pravastatin improves ventriculo-vascular haemodynamic interactions in young men with type 1 diabetes. *Diab Vasc Dis Res* 4(1): 53–61.
5. Sundell, J., Janatuinen, T., Ronnemaa, T., et al. (2004) Diabetic background retinopathy is associated with impaired

coronary vasoreactivity in people with Type 1 diabetes. *Diabetologica* 47: 725–31.
6. Ciarla, M.V., Bocciarelli, A., DiGregorio, S., et al. (2001) Autoantibodies and endothelial dysfunction in well-controlled uncomplicated insulin-dependent diabetes mellitus patients. *Atherosclerosis* 158: 241–46.
7. Dogra, G., Rich, L., Stanton, K., Watts, G.F. (2001) Endothelium-dependent and independent vasodilation studied at normoglycaemia in Type I diabetes mellitus with and without micoralbuminuria. *Diabetologica* 44: 593–601.
8. Group DER. (2003) Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med* 348: 2294–303.
9. Jarvisalo, M.J., Raitakari, M., Toikka, J.O., et al. (2004) Endothelial dysfunction and increased arterial intima-media thickness in children with type-1 diabetes. *Circulation* 109: 1750–55.
10. Schiekofer, S., Balletshofer, B., Andrassy, M., Bierhaus A., Nawroth, PP. (2000) Endothelial dysfunctin in diabetes mellitus. *Semin Thromb Hemost* 26: 503–11.
11. Westerbacka, J., Uosukainen, A., Makimattila, S., Schenzka, A., Yki-Jarvinen, H. (2000) Insulin-induced decrease in large artery stiffness is impaired in uncomplicated type 1 diabetes mellitus. *Hypertension* 35: 1043–48.
12. Lim, HS., Lip, G.Y.H. (2004) Arterial stiffness in diabetes and hypertension. *J Human Hypertens* 18: 467–68.
13. Wilson, A.M., O'Neal, D., Nelson, C.L., Prior, D.L., Best, J.D., Jenkins, A.J. (2004) Comparison of arterial assessments in low and high vascular disease risk groups. *Am J Hypertens* 17: 285–91.
14. Joyce, M., Moore, K., Thompson, C., Fennessy, F., Kelly, CJ., Bouchier-Hayes, D.J. (2004) Hydroxy-Methylglutaryl-Coenzyme A reducatse inhibition improves endothelial dysfunction in type-1 diabetes. *Eur J Vasc Endovasc Surg* 27: 432–37.
15. Joyce, M., Kelly, CJ., Chen, G., Bouchier-Hayes, D.J. (2001) Pravastatin attenuates lower torso ischaemia-reperfusion-induced lung injury by upregulating constitutive endothelial nitric oxide synthase. *Eur J Vasc Endovasc Surg* 21: 295–300.
16. Joyce, M., Kelly, CJ., Winter, D., Chen, G., Leahy, A., Bouchier-Hayes, D.J. (2001) Pravastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, attenuates renal injury in an experimental model of ischaemia-reperfusion. *J Surg Res* 101: 79–84.
17. Maron, D.J., Fazio, S., Linton, M.F. (2000) Current perspectives on statins. *Circulation* 101: 207–13.
18. Steinmetz, E.F., Buckley, C., Thompson, R.W. (2003) Prospects for the medical management of abdominal aortic aneurysms. *Vascular & Endovascular Surgery* 37(3): 151–63.
19. Yamori, Y., Nara, Y., Ikeda, K., Mizushima, S. (1996) Is taurine a preventive nutritional factor of cardiovascular diseases or just a biological marker of nutrition? *Adv Exp Med Biol* 403: 623–29.
20. Kulakowski, E., Maturo, J. (1984) Hypoglycaemic properties of taurine: not mediated by enhanced insulin release. *Biochem Pharmacol* 33: 2835–38.
21. Lampson, W.G., Kramer, J.H., Schaffer, S.W. (1983) Potentiation of the actions of insulin by taurine. *Can J Physiol Pharmacol* 61: 457–63.
22. Maturo, J., Kulakowski, E. (1988) Taurine binding to the purified insulin receptor. *Biochem Pharmacol* 37: 3755–60.
23. Hansen, S.H. (2001) The role of taurine in diabetes and the development of diabetic complications. *Diabetes Metab Res Rev* 17: 330–46.
24. Franconi, F., Bennardini, F., Mattana, A., et al. (1995) Plasma and platelet taurine are reduced in subjects with insulin-dependent diabetes mellitus. *Am J Clin Nutr* 61: 1115–1119.
25. Franconi, F., Miceli, M., Fazzini, A., et al. (1996) Taurine and diabetes - humans and experimental models. *Adv Exp Med Biol* 403: 579–82.
26. Di Leo, M.A.S., Santini, S.A., Silveri, N.G., Giardina, B., Franconi, F., Ghirlanda, G. (2004) Long-term taurine supplementation reduces mortality rate in streptozotocin-induced diabetic rats. *Amino Acids* 27: 187–91.
27. Takatani T., Takahashi K., Uozumi Y., et al. (2004) Taurine prevents the ischamia-induced apoptosis in cultured neonatal rat cardiomyocytes through Akt/caspase-9 pathway. *Biochem Biophys Res Comm* 216: 484–89.
28. Fennessy, F. *Endothelial physiology in young cigarette smokers: a multifunctional analysis of the effects of taurine supplementation*. Thesis. Dublin: RCSI, Trinity College Dublin, 1999.
29. Chang, L., Xu, J., Yu, F., Zhao, J., Tang, X., Tang, C. (2004) Taurine protected myocardial mitochondrial injury induced by hyperhomocysteinemia in rats. *Amino Acids* 27: 37–48.
30. Oudit, G.Y., Trivieri, M.G., Khaper, N., et al. (2004) Taurine supplementation reduces oxidative stress and improves cardiovascular function in an iron-overload murine model. *Circulation* 109: 1877–85.
31. Kingston, R., Kelly, CJ., Murray, P. (2004) The therapeutic role of taurine in ischaemia-reperfusion injury. *Curr Pharm Des* 10: 2401–10.
32. Raitakari, O.T., Celermajer, D.S. (2000) Flow-mediated dilatation. *Br J Clin Pharmacol* 50: 397–404.
33. Fathi, R., Marwick, T.H. (2001) Noninvasive tests of vascular function and structure: why and how to perform them. *Am Heart J* 141: 694–703.
34. Corretti, M.C., Plotnick, G.D., Vogel, R.A. (1995) Technical aspects of evaluating brachial artery vasodilatation using high-frequency ultrasound. *Am J Physiol* 268: 1397–404.
35. Agewall, S., Wright, S., Doughty, RN., et al. (2000) Does a glass of red wine improve endothelial function? *Eur Heart J* 21: 74–78.
36. Asmar, R.G., Topouchian, J.A., Benetos, A., Sayegh, F.A., Mourad, J-J., Safar, M.E. (1997) Non-invasive evaluation of

arterial abnormalities in hypertensive patients. *J Hypertens* 15(suppl 2): S99–S107.
37. Celermajer, D.S., Sorensen, K.E., Gooch, V.M., et al. (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340: 1111–15.
38. Hijmering, M.L., Stroes, E.S.G., Pasterkamp, G., Sierevogel, M., Banga, JD., Rabelink, T.J. (2001) Variability of flow-mediated dilation: consequences for clinical application. *Atherosclerosis* 157: 369–73.
39. O'Rourke, M.F. (1996) Arterial pressure: what should we be measuring? *Blood Press Monit* 1(suppl 2): S97–S103.
40. O'Rourke, M., Jiang, X.J. (1999) Use of radial artery applanation tonometry. *J Am Coll Cardiol* 34: 951–52.
41. Wilkinson, I.B., Cockcroft, J.R., Webb, D.J. (1998) Pulse wave analysis and arterial stiffness. *J Cardiovasc Pharmacol* 32(suppl 3): S33–S37.
42. Kelly, R., Hayward, C., Avolio, AP., O'Rourke, M. (1989) Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* 80:1652–59.
43. Kelly, R., Hayward, C., Ganis, J., Daley, J., Avolio, A.P., O'Rourke, M. (1989) Noninvasive registration of the arterial pressure pulse wavefrom using high fidelity applanation tonometry. *J Vasc Med Biol* 1: 142–49.
44. Cameron, J.D., McGrath, B.P., Dart, A.M. (1998) Use of radial artery applanantion tonometry and a generalised transfer function to determine aortic pressure augmentation in subjects with treated hypertension. *J Am Coll Cardiol* 32: 1214–20.
45. Roman, M.J., Ganau, A., Saba, P.S., Pini, R., Pickering, T.G., Devereux, R.B. (2000) Impact of arterial stiffening on left ventricular structure. *Hypertension* 36: 489–94.
46. Wilkinson, I.B., MacCallum, H., Flint, L., Cockcroft, J.R., Newby, D.E., Webb, D.J. (2000) The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol* 525: 262–70.
47. Yasmin, Brown, M.J. (1999) Similarities and differences between augmentation index and pulse wave velocity in the assessment of arterial stiffness. *QJM* 92: 595–600.
48. Oberg, P.A., Tenland, T., Nilsson, G.E. (1983) Laser-Doppler flowmetry – a non-invasive and continuous method for blood flow evaluation in microvascular studies. *Acta Med Scand* 687: 17–24.
49. Jones, B., Mayou, B. (1982) The laser Doppler flowmeter for macrovascular monitoring: a preliminary report. *Br J Plast Surg* 35: 147–49.
50. Salerud, E.G., Tenland, T., Nilsson, G.E., Oberg, P.A. (1982) Rhythmical variations in human skin blood flow. *Int J Microcirc Clin Exp* 2: 91–102.
51. Oberg, P.A., Nilsson, G.E., Tenland, T., Holmstrom, A., Lewis, D.H. (1979) Measurement of skeletal muscle blood flow in bullet wounding with a new laser Doppler flowmeter. *Microvasc Res* 18: 298.
52. Stern, M.D., Lappe, D.L., Bowen, P.D., et al. (1977) Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. *Am J Physiol* 232: H441–48.
53. Damber, J-E., Lindahl, O., Selstam, G., Tenland, T. (1982) Testicular blood flow measured with a laser Doppler flowmeter: acute effects of catecholamines. *Acta Physiol Scand* 115: 209–15.
54. Koitka, A., Abraham, P., Bouhanick, B., Sigaudo-Roussel, D., Demiot, C., Saumet, J.L. (2004) Impaired pressure-induced vasodilation at the foot in young adults with type-1 diabetes. *Diabetes* 53: 721–25.
55. Malanin, K. (1998) The venoarteriolar response of the skin in healthy legs measured at different depths. *Clin Physiol* 18: 441–44.
56. Malanin, K., Vilkko, P., Kolari, P.J. (1998) Blood flux and venoarteriolar response of the skin in legs with chronic venous insufficiency measures at two different depths by using a double-wavelength laser Doppler technique. *Angiology* 49: 441–46.
57. Asgeirdottir, L.P., Adnarsson, U., Johnnson, G.S. (2001) Lower extremity blood flow in healthy men: effect of smoking, cholesterol, and physical activity - a Doppler study. *Angiology* 52: 437–45.
58. Kilo, S., Berghoff, M., Hilz, M., Freeman, R. (2000) Neural and endothelial control of microcirculation in diabetic peripheral neuropathy. *Neurology* 54: 1246–52.
59. Pollock, F.E., Koman, L.A., Smith, B.P., Holden, M., Russell, G.B., Poehling, G.G. (1993) Measurement of hand microvascular blood flow with isolated cold stress and laser Doppler fluxmetry. *J Hand Surg* 18: 143–50.
60. Van den Brande, P., DeConnick, A., Lievens, P. (1997) Skin microcirculation responses to severe cooling. *Int J Microcirc Clin Exp* 17: 55–60.
61. Belcaro, G., Vasdekis, S., Rulo, A., Nicolaides, A.N. (1989) Evaluation of skin blood flow and venoarteriolar response in patients with diabetes and peripheral vascular disease by laser Doppler flowmetry. *Angiology* 40: 953–57.
62. Butler, R., MacDonald, T.M., Struthers, A.D., Morris, A.D. (1998) The clinical implications of diabetic heart disease. *Eur Heart J* 19: 1617–27.
63. Fennessy, F.M., Casey, R.G., Bouchier-Hayes, D.J. (2003) Peripheral and central arterial haemodynamic interactions are early abnormalities in young male cigarette smokers. *Eur J Vasc Endovasc Surg* 25: 152–58.
64. Berry, K.L., Skyrne-Jones, R.A., Cameron, J.D., O'Brien, R.C., Meredith, I.T. (1999) Systemic arterial compliance is reduced in young patients with IDDM. *Am J Physiol* 276: 1839–45.
65. Oxlund, H., Rasmussen, L.M., Andreassen, T.T., Heickendroff, L. (1989) Increased aortic stiffness in patients with type I diabetes. *Diabetologica* 32: 748–52.
66. Ryden-Ahlgren, A., Lanne, T., Wollmer, P., Sonesson, B., Hansen, F., Sundkvist, G. (1995) Increased in arterial

stiffness in women, not in men, with IDDM. *Diabetologica* 38: 1082–89.

67. Kool, M.J., Lambert ,J., Stehouwer, C.D., Hoeks, A.P.G., Van Bortel, L.M. (1995) Vessel wall properties of large arteries in uncomplicated IDDM. *Diabetes Care* 18: 618–24.
68. Fennessy, F.M., Moneley, D.S., Wang, J.H., Kelly, C.J., Bouchier-Hayes, D.J. (2003) Taurine and vitamin C modify monocyte and endothelial dysfunction in young smokers. *Circulation* 107: 410–15.

Therapeutic Effects of L-Carnitine and Propionyl-L-carnitine on Cardiovascular Diseases: A Review

ROBERTO FERRARI, E. MERLI, G. CICCHITELLI, D. MELE, A. FUCILI, AND C. CECONI

Department of Cardiology, University Hospital of Ferrara, Cardiovascular Research Center, Salvatore Maugeri Foundation, IRCCS, Gussago (Brescia), Italy

ABSTRACT: **Several experimental studies have shown that levocarnitine reduces myocardial injury after ischemia and reperfusion by counteracting the toxic effect of high levels of free fatty acids, which occur in ischemia, and by improving carbohydrate metabolism. In addition to increasing the rate of fatty acid transport into mitochondria, levocarnitine reduces the intramitochondrial ratio of acetyl-CoA to free CoA, thus stimulating the activity of pyruvate dehydrogenase and increasing the oxidation of pyruvate. Supplementation of the myocardium with levocarnitine results in an increased tissue carnitine content, a prevention of the loss of high-energy phosphate stores, ischemic injury, and improved heart recovery on reperfusion. Clinically, levocarnitine has been shown to have anti-ischemic properties. In small short-term studies, levocarnitine acts as an antianginal agent that reduces ST segment depression and left ventricular end-diastolic pressure. These short-term studies also show that levocarnitine releases the lactate of coronary artery disease patients subjected to either exercise testing or atrial pacing. These cardioprotective effects have been confirmed during aortocoronary bypass grafting and acute myocardial infarction. In a randomized multicenter trial performed on 472 patients, levocarnitine treatment (9 g/day by intravenous infusion for 5 initial days and 6 g/day orally for the next 12 months), when initiated early after acute myocardial infarction, attenuated left ventricular dilatation and prevented ventricular remodeling. In treated patients, there was a trend towards a reduction in the combined incidence of death and CHF after discharge. Levocarnitine could improve ischemia and reperfusion by (1) preventing the accumulation of long-chain acyl-CoA, which facilitates the production of free radicals by damaged mitochondria; (2) improving repair mechanisms for oxidative-induced damage to membrane phospholipids; (3) inhibiting malignancy arrhythmias because of accumulation within the myocardium of long-chain acyl-CoA; and (4) reducing the ischemia-induced apoptosis and the consequent remodeling of the left ventricle. Propionyl-L-carnitine is a carnitine derivative that has a high affinity for muscular carnitine transferase, and it increases cellular carnitine content, thereby allowing free fatty acid transport into the mitochondria. Moreover, propionyl-L-carnitine stimulates a better efficiency of the Krebs cycle during hypoxia by providing it with a very easily usable substrate, propionate, which is rapidly transformed into succinate without energy consumption (anaplerotic path-**

Address for correspondence: Prof. Roberto Ferrari, Chair of Cardiology, University Hospital of Ferrara, Corso Giovecca, 203, 44100 Ferrara, Italy. Voice: +0532/202143; fax: +0532/241885. fri@dns.unife.it

Ann. N.Y. Acad. Sci. 1033: 79–91 (2004).
doi: 10.1196/annals.1320.007

way). Alone, propionate cannot be administered to patients in view of its toxicity. The results of phase-2 studies in chronic heart failure patients showed that long-term oral treatment with propionyl-L-carnitine improves maximum exercise duration and maximum oxygen consumption over placebo and indicated a specific propionyl-L-carnitine effect on peripheral muscle metabolism. A multicenter trial on 537 patients showed that propionyl-L-carnitine improves exercise capacity in patients with heart failure, but preserved cardiac function.

KEYWORDS: **myocardial metabolism; myocardial ischemia; levocarnitine; propionyl-L-carnitine**

LEVOCARNITINE

Levocarnitine occurs naturally as an essential cofactor of fatty acid metabolism, which is synthesized endogenously or obtained from dietary sources. It is a cofactor of several enzymes (carnitine translocase, acylcarnitine transferases I and II). Its main role is to shuttle long-chain fatty acids and activated acetate across the inner mitochondrial membrane.[1] A specific translocase facilitates this exchange of long-chain acylcarnitine and acetylcarnitine. Levocarnitine acts as the shuttle for the end products of peroxisomal fatty acid oxidation and for the α-ketoacids derived from the branched-chain amino acids. The α-ketoacids are transferred into the mitochondrial matrix for completing oxidation. Furthermore, levocarnitine modulates the intramitochondrial acyl-CoA/CoA ratio. The reaction is freely reversible and is catalyzed by the mitochondrial enzyme, carnitine acetyltransferase. In the case of inadequate carnitine concentrations, the reaction would be pushed in the direction of acyl-CoA moiety, resulting in decreased free CoA concentrations within the mitochondrial matrix. This shift, in turn, interferes with all reactions that are dependent upon the availability of free CoA and downregulates intermediary metabolism within the mitochondrion.

Although the major role of levocarnitine is in free fatty acid metabolism, it also enhances carbohydrate utilization.[2] Several results obtained in intact animals support this view. Treatment with levocarnitine resulted in a significant improvement in several parameters of mechanical function of the heart during a period of mild ischemia.[3] These data were concomitant with a lower accumulation of acyl-CoA. No major changes of myocardial mechanical functions and systemic pressure were noted during the infusion of levocarnitine in the preischemic period. In addition, in these studies, the authors also investigated the effects of D-carnitine and DL-carnitine.[4] It is interesting to note that the D-isomer was biologically inert. In contrast, the results obtained in the DL-isomer-treated group were between L-isomer and control group results. These observations clearly demonstrate an isomer-specific effect, although the biochemical bases remain unknown.

Levocarnitine does not have hemodynamic effects in healthy volunteers or patients with CAD. However, an improvement of individual maximal aerobic power (VO_{2max}) is demonstrated in healthy subjects[5] and athletes[6] after chronic treatment with levocarnitine (4 g daily over a period of 2 weeks). Thus, levocarnitine has to affect heart metabolism. Its effects on myocardial metabolism at rest or during pacing-induced tachycardia have been studied in coronary-artery-diseased patients with normal left ventricular function.[7–9] At rest, levocarnitine (40 mg/kg administered intravenously as a single bolus over 5 to 45 min before the second pacing)

caused (a) a significant reduction in arterial concentration of free fatty acids owing to increased myocardial uptake; (b) a reduction in myocardial uptake of glucose; (c) no major changes in myocardial uptake of lactate; and (d) no significant increase in the overall oxygen consumption of the heart. During sinus pacing, levocarnitine caused (a) a decrease of myocardial lactate production, maintaining a positive extraction relative to that seen in the untreated or placebo-treated group; and (b) an increase in myocardial free fatty acid extraction.

There are widespread systemic metabolic effects of levocarnitine, including increased glucose utilization in patients with insulin-dependent diabetes[2] and reduction of blood lactate concentration.

There is a close relationship between tissue carnitine levels and liver glycogen content. Oral levocarnitine (3–4 g daily) normalizes plasma total cholesterol or triglyceride levels (or both) and increases high-density lipoprotein (HDL)–cholesterol in patients with type II and type IV hyperlipoproteinemia over a 2-month period.[10,11]

Placebo-controlled studies performed in patients with stable chronic effort angina suggest that levocarnitine given acutely (40 mg/kg iv) or chronically (1–3 g daily for a month) improves exercise capacity and the electrocardiographic manifestations of ischemia.[12–14]

Oral levocarnitine (4 g daily for 21 days) improves the maximal walking distance of patients with intermittent claudication caused by peripheral arterial disease.[15]

THERAPEUTIC USE OF LEVOCARNITINE

Interestingly and importantly, there is not any known disease or syndrome in which levocarnitine administration is contraindicated. Thus, levocarnitine is extremely safe.

There are several therapeutic uses of L-carnitine:

(1) Treatment of primary carnitine deficiency syndromes:
- *(i)* systemic carnitine deficiency;
- *(ii)* myopathic carnitine deficiency.

(2) Treatment of secondary carnitine deficiency/insufficiency states:
- *(i)* genetically determined metabolic errors (mainly organic acidurias);
- *(ii)* chronic intermittent hemodialysis in end-stage renal failure;
- *(iii)* valproate-induced hepatotoxicity;
- *(iv)* cardiac and/or skeletal muscle ischemia.

In this report, we concentrate only on secondary carnitine deficiency since primary has already been discussed elsewhere in this volume.

TREATMENT OF SECONDARY CARNITINE DEFICIENCY

Genetically Determined Metabolic Errors

The genetically determined conditions are recessive. No significant prevalence of these disorders is found between sexes or in different ethnic groups. The symptoms

appear in children from 0 to 13 years of age and affect fatty acid metabolism. The most common findings are weakness, coma, failure to thrive, hypoglycemia, metabolic acidosis, low serum carnitine (<20 μM), elevated esterified/free carnitine ratio (>0.40), and dicarboxylic aciduria.[1,16] Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency has emerged as the most distinctive organic aciduria associated with carnitine deficiency.[17] Some of the earliest examples of "primary systemic carnitine deficiency" have proved to be secondary to MCAD deficiency.[18–20] The metabolic errors are diverse. The specific defects identified are short-chain acyl-CoA dehydrogenase (SCAD) deficiency, MCAD deficiency, long-chain acyl-CoA dehydrogenase (LCAD) deficiency, multiple acyl-CoA dehydrogenase (MAD) deficiency, isovaleric acidemia, propionic acidemia, methylmalonic aciduria, hydroxymethylglutaryl-CoA lyase deficiency, glutaric aciduria type I, β-kethiolase deficiency, homocystinuria, 5-methylene tetrahydrofolate reductase efficiency, adenosine deaminase deficiency, ornithine transcarbamoylase heterozygote state, cystinosis, and cytochrome oxidase deficiency. These diverse conditions are associated with impaired esterification of carnitine and decreased serum or tissue carnitine concentrations.[18]

Administration of exogenous levocarnitine has positive metabolic effects, buffering the excess of acyl-CoAs in the mitochondria, and thus yielding formation of acylcarnitines. These are shuttled out of mitochondria and eliminated in the urine.[19] Improvement of the general conditions and muscle tone, and reduction of recurrent metabolic attacks, reflects the detoxification role of carnitine conjugation. The responder population is approximately 50% of the total.[20–22]

Carnitine Deficiency in Patients with End-Stage Renal Failure Undergoing Long-Term Intermittent Hemodialysis

Serum carnitine concentration was found to decline by ~75% during one hemodialysis session.[23] In patients undergoing hemodialysis, free carnitine concentration is subnormal and acylcarnitine is elevated, the latter probably being a result of abnormal fatty acid oxidation combined with diminished renal excretion. Often, the muscle concentration is also subnormal.[24] A variety of metabolic disorders and symptoms associated with the long-term intermittent hemodialysis are treated with levocarnitine. The doses range from 100 mg/kg when administered intravenously after each dialysis session, and from 1–2 g daily when administered orally. This treatment might reduce the increased concentration of triglycerides, which occurs in patients under dialysis,[25,26] but not univocally so.[27,28] Crossover, double-blind controlled trials demonstrated that levocarnitine treatment induces a reduction in the incidence of intradialytic hypotension and muscle cramps[24–29] and attenuates cardiac arrhythmias.[30] Similar observations have been reported using the oral or the intravenous administration. In addition, patients under dialysis receiving oral levocarnitine normally experience significant increases in hematocrit.[25,31,32]

Valproate-Induced Hepatotoxicity

Valproate has been in worldwide clinical use for the treatment of epilepsy. However, its chronic administration can cause hepatotoxicity and carnitine deficiency.[33,34] Treatment with carnitine was proposed to reduce valproate toxicity.

Studies demonstrated that oral levocarnitine administration (1–2 g daily) significantly restores the serum concentration of total carnitine and decreases the incidence of liver dysfunction.[35–37]

Cardiac and/or Skeletal Muscle Ischemia

The finding in experimental animals and in humans[38,39] that the ischemic and the failing myocardium has low content of free carnitine supports the concept that myocardial ischemia is often accompanied by relative carnitine insufficiency. Administration of carnitine (20–140 mg/kg iv) to CAD patients subjected to atrial pacing increases significantly pacing duration, maximal heart rate, and rate-pressure product. Concomitantly, it reduced left ventricular end-diastolic pressure and lactate production, which is converted into a net extraction.[7–9,40] A few placebo-controlled studies suggest that both the acute (40 mg/kg iv) and the prolonged oral administrations of levocarnitine (1–3 g daily) improve exercise capacity and the electrocardiographic manifestations of ischemia.[12–14] In no study was treatment with levocarnitine compared with standard antianginal therapy.

Tissue fatty acid accumulation during myocardial ischemia is a cause of ventricular arrhythmias. Because of this notion, a double-blind, parallel, placebo-controlled study was carried out in 56 patients suffering from acute myocardial infarction. After a clinical stratification, patients were randomly allocated to receive placebo or levocarnitine at a dose of 100 mg/kg every 12 h for 36 h. The end point of the study was the reduction in number of premature ventricular beats evaluated by Holter recording over a period of 48 h. Active treatment significantly decreased the ectopic ventricular beats, probably because of metabolic effects that avoid fatty acid myocardial accumulation. This hypothesis is suggested by the high concentration of long- and short-chain carnitine esters found in the urine of the treated patients at 48 h after drug infusion.[41]

All these data have prompted the conduction of a multicenter trial on 472 patients to evaluate the effects of levocarnitine administration on left ventricular remodeling after acute anterior infarction, called the Levocarnitine Ecocardiografia Digitalizzata Infarto Miocardico (CEDIM) trial.[42] This randomized, double-blind, placebo-controlled, multicenter study was performed to evaluate the effects of levocarnitine administration on long-term left ventricular dilatation in patients with acute myocardial infarction. Placebo or levocarnitine (9 g iv daily for the first 5 days and then 6 g orally daily) was administered for 12 months. The primary end points of the trial were left ventricular volumes and ejection fraction, at 12 months after the emergent event, assessed by two-dimensional echocardiography.

Treatment with the active compound resulted in a significant reduction of left ventricular dilatation. The percentages of both end-diastolic and end-systolic volumes were reduced significantly in the levocarnitine-treated group. No modification of left ejection fraction was observed. The incidences of death, congestive heart failure, and/or ischemic events were less in the carnitine-treated groups.

These encouraging data on survival have prompted another multicenter trial on over 2000 patients aimed to evaluate the short-term effects (6 months) of early levocarnitine administration on clinical end points (the CEDIM II trial) in patients with acute myocardial infarction. The study has been completed and the results will be available soon.

The effects of levocarnitine on peripheral muscle have also been studied.[43] The first double-blind, crossover study was designed to evaluate the effects of levocarnitine in 20 patients randomly assigned to receive placebo or levocarnitine (2 g twice daily, orally) for a period of 3 weeks. The end point was the difference of absolute walking distance at the end of each treatment, which was significantly increased by active treatment.

These data were concomitant with a reduction of popliteal venous lactate concentration and no important modification of blood flow. Biopsy of the ischemic muscle, performed before and after levocarnitine treatment, showed an increase of total carnitine level. The authors concluded that levocarnitine treatment improves the exercise duration of patients with peripheral vascular disease, probably through a metabolic mechanism.[43] To further prove this hypothesis, another study was carried out in which blood flow was measured in the affected limb by impedance plethysmography under resting conditions at 2-min intervals for 10 min after a 5-min period of ischemia in the same limb. After a washout period of 2 weeks, patients were randomly assigned to receive placebo or levocarnitine, 3 g iv as a bolus, followed by continuous intravenous infusion of 2 mg/kg/min for 30 min. At the end of perfusion, ischemia was induced by abolishing the blood flow.

Levocarnitine treatment did not modify blood flow under resting conditions; however, it significantly increased postischemic blood flow, suggesting an improvement in functional circulatory reserve in patients with peripheral vascular disease.

PROPIONYL-L-CARNITINE (PLC)

PLC is formed via carnitine acetyltransferase from propionyl-CoA, a product of methionine, threonine, valine, and isoleucine, as well as of odd-chain fatty acids. Pharmacokinetic studies demonstrated that, in humans, plasma concentration of PLC increases following intravenous administration and then decreases to baseline values within 6 to 24 h.[44] This life span varies with dosage. PLC increases plasma and cellular carnitine content, thus enhancing free fatty acid (FFA) oxidation in carnitine-deficient states, as well as increasing glucose oxidation rates.[45]

PLC is highly specific for skeletal and cardiac muscle;[46] it carries the propionyl group and enhances the uptake of this agent by myocardial cells.[47] This is particularly important because propionate can be used by mitochondria as an anaplerotic substrate, thus providing energy in the absence of oxygen consumption.[48] Note that propionate alone cannot be administered because of its toxicity.[49] Finally, because of the particular structure of the molecule with a long lateral tail, PLC has a specific pharmacologic action that is independent of its effect on muscle metabolism; this results in peripheral dilatation and positive inotropic effects.[50,51]

Because of PLC's characteristics, it was hypothesized that it could provide adjuvant benefit over standard therapy by specifically improving impaired metabolism of skeletal and heart muscle in patients with CHF. When administered acutely to an isolated and perfused heart preparation, PLC does not modify left ventricular pressure.[52] When administered intravenously through *in vivo* preparation, PLC causes a dose-dependent, short-lasting enhancement of cardiac output in dogs studied under open and closed chest conditions.[44,45] These responses are not modified by α- or β-adrenergic blockade or by administration of calcium antagonists. In

addition, PLC causes coronary vasodilatation with reduced oxygen extraction; these effects are not seen with levocarnitine alone. Thus, all of the cardiovascular actions of PLC can be attributed to its pharmacologic properties rather than to its role as a metabolic intermediate.

PLC hemodynamic effect was evaluated in 10 patients with coronary artery disease with normal LV function.[46,47] The drug was intravenously administered at 15 mg/kg. PLC improved the stroke volume and reduced the ejection impedance as a result of decreased systemic and pulmonary resistances and increased arterial compliance. Total external heart power improved with a proportionally smaller increase in the energy requirement; this suggested that PLC has a positive inotropic property.

PLC increased the performance of the aerobic myocardium independently from changes of peripheral hemodynamics or coronary flow when administered chronically to the animals several days before the isolation of the heart.[48–52] To investigate whether the chronic effect is specific for PLC or is due to levocarnitine or propionic acid, we designed experiments in which rabbits were treated for 10 days with saline, levocarnitine, propionic acid, or PLC (all at 1 mmol/kg). Propionic acid resulted in 98% mortality after 10 days. No deaths occurred after PLC or levocarnitine treatment. Treatment with levocarnitine failed to modify the volume-pressure curves of the isolated heart. Conversely, treatment with PLC prevented the decrease of the optimum developed pressure and the rise in end-diastolic pressure, which remained constant even after overstretching. It has been postulated that the rise in end-diastolic pressure enhanced the mechanical performance of the heart by improving its metabolism.[53] It is known that pyruvate increases heart contractility, which allows a more efficient energy use.[49] Administration of pyruvate leads to a higher cytosolic phosphorylation potential, which in conjunction with a reduced inorganic phosphate (Pi) concentration translates into an increased contraction. We investigated whether a similar mechanism is the basis for the PLC effects. Energy metabolism does not seem to be involved because high-energy phosphates, Pi, and mitochondrial function remain unchanged after chronic PLC administration. These findings, however, led to some important implications. Usually, typical inotropic agents, such as digitalis, calcium, and adrenergic compounds, stimulate contractility by increasing myofibrillar energy use at the expense of energy supply. Consequently, these agents cause a decline in the phosphocreatinine (PCr)/Pi ratio; this suggested that they place the heart in a supply/demand imbalance.[49] This was not the case for PLC.

Energy metabolism remained unchanged despite the increase in myocardial performance. During the repolarization phase, which is modified by PLC, important events occurred that influenced contractility.[54] It is appealing to correlate the effect on papillary muscle action potential duration with that on cardiac mechanical performance because PLC, but not levocarnitine, affects both of them.

Broderick *et al.*[55] conducted research with ischemic myocardium on isolated rat hearts and global no-flow ischemia that showed that, during the reperfusion of previously ischemic hearts, PLC stimulated glucose oxidation and significantly improved the functional recovery as measured by heart rate and peak systolic pressure. This supported the theory that carnitine's beneficial effects on ischemic myocardium are the result of its ability to overcome the inhibition of glucose oxidation that is induced by increased levels of fatty acids. Another study suggested an intracellular mechanism of action and implied that better protection is provided if the agent is administered before ischemic insult.[56]

Paulson *et al.*[57] studied isolated rat hearts that were subjected to global low-flow ischemia. During reperfusion, the group that was treated with PLC exhibited significantly greater recovery of all hemodynamic variables. In a similar preparation, 1 mmol PLC had no protective effect, whereas 5.5 and 11 mmol improved the recovery of cardiac output. The beneficial effect is greater than that of L-acetylcarnitine or levocarnitine on a molar basis. PLC was also found to directly improve postischemic stunning.[57] Specific experimental studies were conducted on the efficacy of this agent with respect to CHF.[57,58] In particular, treatment with PLC (50 mg/kg, intra-arterially) for 4 days significantly improved the hemodynamics of pressure overloaded (by constriction of the abdominal aorta) in conscious rats.[58] In another study, papillary muscles were isolated from rats that had been treated with 180 mg/kg PLC for 8 weeks, starting from weaning.[59] Aortic constriction was performed at 8 weeks of age and lasted for 4 weeks. The papillary muscles of untreated animals showed increased time-to-peak tension and a reduced peak rate of tension rise and delay. PLC normalized all of these parameters.[59]

In an infarct model of CHF, chronic administration of PLC (60 mg/kg orally given for 5 months) positively influenced ventricular remodeling; it was equally as effective as the ACE inhibitor, enalapril (1 mg/kg orally), in limiting the magnitude of LV dilatation estimated by pressure-volume curves. PLC limited the alterations in ventricular chamber stiffness that were induced by infarction at low and high filling pressures.[60] In isolated myocytes obtained from infarcted rats, PLC increased peak systolic calcium, peak shortening, and velocity of cell shortening to a greater extent than in normal cells.[61] Pasini *et al.*[62] investigated the effects of PLC (250 mg/kg by intraperitoneal injection for 2 months) on the isolated and perfused heart from rabbits with streptozotocin-induced diabetes.[62] Cardiac performance was determined under basal conditions and during a stepwise increase in volume of a saline-filled balloon that was inserted into the LV. PLC prevented the decrease in developed pressure and the increase in diastolic pressure because of the progressive filling of the LV balloon. The same treatment also prevented the depression in the function of the sarcoplasmic reticulum that was observed in untreated rats. Calcium-stimulated ATPase activity, calcium uptake, and magnesium ATPase activity were similar to those of the nondiabetic heart. On the contrary, treatment with PLC failed to rescue the diabetes-induced changes in the sarcolemmal calcium ATPase.[63]

The effects of PLC in a number of models of CHF are particularly evident under conditions of high-energy demand that is induced by increases in workload. Therefore, it seems likely that PLC is able to correct some metabolic steps of the process that leads to heart failure.

Besides its effect on the heart, PLC could be helpful in CHF for a specific action on peripheral heart muscle. In CHF, exertional fatigue is not simply the result of skeletal muscle underperfusion.[64–67] In most patients, there is a decrease in flow responses to exercise as a result of an abnormality of arterial vasodilatation, evidenced by a failure of leg vascular resistances to decrease during exercise.[65,68] Alterations in mitochondrial population or substrate use also may be responsible for the depressed exercise performance as well as metabolic deconditioning. Interestingly, at rest, the peripheral muscle of patients with CHF does not extract FFA, but only glucose.[69,70] This suggests that impairment of FFA oxidation might be due to a lack of carnitine; or conversely, glucose uptake is enhanced. During moderate exercise, there is an increase of glucose uptake with excessive production of lactate, but no

recruitment of FFA.[37,38] All of these observations, and the positive data obtained by the use of PLC to improve the walking capacities of patients with peripheral arterial disease,[71–75] suggested that PLC could specifically improve metabolism and function of skeletal muscle in patients with CHF.

THERAPEUTIC USE OF PROPIONYL-L-CARNITINE

PLC is used for treatment of cardiovascular diseases. Anand *et al.*[76] studied the effects of acute and chronic administration of PLC (1.5 g/day) on hemodynamics, hormonal levels, exercise capacity, and oxygen consumption in 30 patients with CHF New York Heart Association (NYHA) classes II and III, and LV ejection fraction (EF) less than 40%. There were no changes in the hemodynamics or neurohormonal levels after acute or chronic administration, except for a reduction in pulmonary artery pressure. After 1 month of treatment, however, a significant increase in exercise capacity and peak VO_2 was observed; this suggested a possible improvement of peripheral muscle metabolism.

Another study examined the effects of PLC (15 g/day for 1 month) on limb metabolism, at rest and during exercise.[70] Skeletal muscle metabolism was assessed as femoral arterial venous (A–V) difference for lactate, pyruvate, and FFA. At rest, PLC caused a reduction of arterial and venous blood levels of FFA, but did not change the overall muscle extraction of FFA, lactate, or pyruvate. After maximal exercise, PLC decreased the negative A–V difference for lactate, restored a positive A–V difference for pyruvate, and did not change that for FFA. The investigators concluded that PLC improved skeletal muscle metabolism in patients with idiopathic dilated cardiomyopathy by increasing pyruvate flux into the Krebs cycle and decreasing lactate production. This effect, which occurs in the absence of major hemodynamic and neuroendocrine changes, may underlie the ability of PLC to increase exercise performance in patients with CHF.

Caponnetto *et al.*[77] reported the effects of PLC on 50 patients with mild CHF (NYHA class II) who were symptomatic despite therapy with digitalis and diuretics, with an EF less than 45%. The patients were randomized to receive 1.5 g/day of PLC or placebo orally for 6 months. Maximal exercise time in the treated group was significantly increased (1 min longer vs. placebo), whereas lactate production was significantly reduced. LV shortening fraction and left ventricular ejection fraction (LVEF) showed a significant increase in the group that was given PLC, and systemic vascular resistances lowered. The greatest changes occurred after the first month of treatment and persisted throughout the entire period of treatment. Other authors confirmed similar data.[78] Bachetti *et al.*[79] reported that, when PLC was given to patients with severe heart failure (NYHA IV), it was able to reduce the increase in tumor necrosis factor-α (TNF-α) and, in particular, its soluble receptor that is elevated in CHF,[80–82] and that it is responsible for intracellular signaling of the effects of TNFα. An increased TNF was implicated in the skeletal muscle changes of patients with CHF.[83]

These data have encouraged a multicenter, international study on the effects of PLC on exercise duration.[84] A total of 574 patients under stable mandatory therapy with angiotensin-converting enzyme inhibitors, diuretics, and digitalis were studied. The primary efficacy variable was the maximum exercise test duration on a bicycle; rates of negative outcomes and quality of life were the main secondary variables. A

slight (nonsignificant) difference of 15 s in favor of PLC was noted in the adjusted 6-month means of maximum exercise duration in the completer/compiler population (353 patients: 188 in the PLC group and 165 in the placebo group). In a subgroup of patients with EF between 30% and 40% and baseline exercise duration within 480 s, there was a 57.7-s increase in exercise test duration after PLC administration; this suggested that patients with some degree of deconditioning and relatively preserved myocardial function are likely to benefit from treatment. There are several studies on the effects of PLC in peripheral artery disease, but this is beyond the scope of this review; this topic will be addressed in detail by William Hiatt in this volume.

ACKNOWLEDGMENTS

This work was supported by a grant from the Fondazione Cassa di Risparmio di Ferrara.

REFERENCES

1. De Vivo, D.C. & I. Tein. 1990. Primary and secondary disorders of carnitine metabolism. Int. Pediatr. **5:** 134–141.
2. Lopaschuk, G.D. 1991. Current Concepts in Carnitine Research, pp. 1–5. Atlanta.
3. Liedtke, A.J., H. Nellis & L.F. Whitesell. 1981. Effects of carnitine isomers on fatty acid metabolism in ischemic swine hearts. Circ. Res. **48:** 859–866.
4. Liedtke, A.J., H. Nellis, L.F. Whitesell & C.Q. Mahar. 1982. Metabolic and mechanical effects using L- and D-carnitine in working swine hearts. Am. J. Physiol. **243:** H691–H697.
5. Angelini, C., L. Vergani, L. Costa *et al.* 1986. Clinical study of efficacy of L-carnitine and metabolic observations in exercise physiology. *In* Clinical Aspects of Human Carnitine Deficiency, p. 38. Pergamon. New York.
6. Marconi, C., G. Sassi, A. Carpinelli & P. Cerretelli. 1985. Effects of L-carnitine loading on the aerobic and anaerobic performance of endurance athletes. Eur. J. Appl. Physiol. **54:** 131–135.
7. Thomsen, J.H., A.L. Shug, V.U. Yap *et al.* 1979. Improved pacing tolerance of the ischemic human myocardium after administration of carnitine. Am. J. Cardiol. **43:** 300–306.
8. Ferrari, R., F. Cucchini & O. Visioli. 1984. The metabolic effects of L-carnitine in angina pectoris. Int. J. Cardiol. **5:** 213–216.
9. Reforzo, G., P.L. De Andreis Bessone, F. Rebaudo & M. Tibaldi. 1986. Effects of high doses of L-carnitine on myocardial lactate balance during pacing-induced ischemia in aging subjects. Curr. Ther. Res. **40:** 374–383.
10. Rossi, C.S. & N. Siliprandi. 1982. Effect of carnitine on serum HDL-cholesterol: report of two cases. Johns Hopkins Med. J. **150:** 51–54.
11. Pola, P., P. Tondi, A. Dal Lago *et al.* 1983. Statistical evaluation of long-term L-carnitine therapy in hyperlipoproteinaemias. Drugs Exp. Clin. Res. **12:** 925–935.
12. Kamikawa, T., Y. Suzuki, A. Kobayashi *et al.* 1984. Effects of L-carnitine on exercise tolerance in patients with stable angina pectoris. Jpn. Heart J. **25:** 587–597.
13. Kololcharoen, P., J. Nappi, P. Peduzzi *et al.* 1981. Improved exercise tolerance after administration of carnitine. Curr. Ther. Res. **30:** 753–764.
14. Canale, C., V. Terrachini, A. Biagini *et al.* 1988. Bicycle ergometer and echocardiographic study in healthy subjects and patients with angina pectoris after administration of L-carnitine: semiautomatic computerized analysis of M-mode tracings. Int. J. Clin. Pharmacol. Ther. Toxicol. **26:** 221–224.
15. Brevetti, G., M. Chiariello, G. Ferulano *et al.* 1988. Increases in walking distance in patients with peripheral vascular disease treated with L-carnitine: a double-blind, cross-over study. Circulation **77:** 767–773.

16. FDA. 1989. The pink sheet. FDA Reports **51:** 7–9.
17. STANLEY, C.A., D.E. HALE & P.M. COATES. 1983. Medium-chain acyl-CoA dehydrogenase deficiency in children with non-ketotic hypoglycemia and low carnitine levels. Pediatr. Res. **17:** 877–884.
18. ROE, C.R., D.S. MILLINGTON, D.A. MAITBY *et al.* 1984. L-Carnitine enhances excretion of propionyl coenzyme A as propionylcarnitine in propionic acidemia. J. Clin. Invest. **73:** 1785–1788.
19. CHALMEAR, R.A., R.C. ROE, C.E. STACEY & C.L. HOPPEL. 1984. Urinary excretion of L-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence for secondary insufficiency of L-carnitine. Pediatr. Res. **18:** 1325–1328.
20. WINTER, S.C., M.Z. ELINOR, H. VANCE *et al.* 1991. The clinical use of carnitine for the treatment of organic acidurias. Int. Pediatr. **6:** 62–65.
21. DURAN, M., D. KETTING, J.E. BECKERING *et al.* 1986. Direct identification of propionyl-carnitine in propionic acidemia: biochemical and clinical results of oral carnitine supplementation. J. Inherited Metab. Dis. **9:** 202–207.
22. SECCOMBE, D.W., F. SNYDER & H.G. PARSONS. 1982. L-Carnitine for methylmalonic aciduria. Lancet **1982:** 1401.
23. LESCHKE, M., K.W. RUMPF, T. EISENHAUER *et al.* 1983. Quantitative assessment of carnitine loss during hemodialysis and hemofibrillation. Kidney Int. **24:** 143–146.
24. BELLINGHIERI, G., V. SAVICA, A. MALLAMA *et al.* 1983. Correlation between increased serum and tissue L-carnitine levels and improved muscle symptoms in hemodialyzed patients. Am. J. Clin. Nutr. **38:** 523–531.
25. ALBERTAZZI, A., P. CAPELLI, B. DI PAOLO *et al.* 1982. Endocrine metabolic effects of L-carnitine in patients on regular dialysis treatment. Proc. EDTA **19:** 302–307.
26. DI MARCO, V., V. GINARDI, E. DELL'SIRA *et al.* 1982. La carnicina nel trattamento dei crampi muscolari nei pazienti in emodialisi periodica. Policlinico, Sezione Medica **89:** 104–110.
27. AUBIA, J., J. MASRAMON, J. LHOVERAS *et al.* 1980. Carnitine in haemodialysis patients. Lancet **1980:** 1028.
28. CASCIANI, C.V., U. CARUSO, E. CRAVOTTO *et al.* 1982. Beneficial effects of L-carnitine in post-dialysis syndrome. Curr. Ther. Res. **32:** 116–126.
29. AHMAD, S., H.T. ROBERTSON, T.A. GOLPER *et al.* 1990. Multicenter trial of L-carnitine in maintenance hemodialysis patients. II. Clinical and biochemical effects. Kidney Int. **38:** 912–918.
30. SUZUKI, Y., M. NARITA & N. YAMAKAZI. 1982. Effects of L-carnitine on arrhythmias during hemodialysis. Jpn. Heart J. **23:** 349–358.
31. TROVATO, G.M., V. GINARDI, V. DI MARCO *et al.* 1987. Long-term carnitine treatment of chronic anemia of patients with end-stage renal failure. Curr. Ther. Res. **31:** 1042–1049.
32. VACHA, G.M., G. GIOCELLI, N. SILIPRANDI & M. CORSI. 1983. Favorable effects of L-carnitine treatment on hypertriglyceridemia in hemodialysis patients: decisive role of low levels of high-density lipoprotein-cholesterol. Am. J. Clin. Nutr. **38:** 532–540.
33. OHTANI, Y., F. ENDO & I. MATSUDA. 1980. Carnitine deficiency and hyperammonemia associated with valproic acid therapy. J. Pediatr. **101:** 782–785.
34. DREIFFUS, F.E., N. SANTILLI, D.H. LANGER *et al.* 1987. Valproic acid hepatic fatalities: a retrospective review. Neurology **37:** 379–385.
35. BRATTON, S.L., A.L. GARDEN, T.P. BOHAN *et al.* 1992. A child with valproic acid–associated carnitine deficiency and carnitine-responsive cardiac dysfunction. J. Child Neurol. **7:** 413–416.
36. CASTRO-CAGO, M., I. NOVO & S. RODRIGUEZ-SEGADE. 1990. Effects of valproic acid on the urea cycle and carnitine metabolsim. Int. Pediatr. **5:** 54–57.
37. SUGIMOTO, T., N. NISHIDA, K. MURAKAMI *et al.* 1990. Valproate-induced hepatotoxicity: protective effect of L-carnitine supplementation. Jpn. J. Psychiatry Neurol. **44:** 387–388.
38. SPAGNOLI, L.G., M. CORSI, S. VILLASCHI *et al.* 1982. Myocardial carnitine deficiency in acute myocardial infarction. Lancet **1982:** 1419–1420.
39. SUZUKI, Y. 1982. Myocardial carnitine deficiency in chronic heart failure. Lancet **1982:** 116.
40. PEPINE, C.J. 1992. The therapeutic potential of carnitine in cardiovascular disorders. Clin. Ther. **13:** 3–22.

41. RIZZON, P., C. BIASCO & F. BOSCIA. 1989. High doses of L-carnitine in acute myocardial infarction: metabolic and antiarrhythmic effects. Eur. Heart J. **10:** 502–508.
42. ILICETO, S., D. SCRUTINIO, P. BRUZZI *et al.* 1995. Effects of L-carnitine administration on left ventricular remodelling after acute anterior myocardial infarction (the CEDIM trial). J. Am. Coll. Cardiol. **26:** 380–387.
43. BREVETTI, G., M. CHIARIELLO, G. FERULANO *et al.* 1988. Increases in walking distance in patients with peripheral vascular disease treated with L-carnitine: a double blind, cross-over study. Circulation **77:** 767–773
44. CEVESE, A., F. SCHENA & G. CEROTTI. 1989. Short-term hemodynamic effects of intravenous propionyl-L-carnitine in anesthetized dogs. Cardiologia **34:** 95–101.
45. CEVESE, A., F. SCHENA & G. CERUTTI. 1991. Short-term hemodynamic effects of intravenous propionyl-L-carnitine in anesthetized dogs. Cardiovasc. Drugs Ther. **5**(suppl. II)**:** 45–56.
46. CHIDDO, A., S. MUSCI, A. BORTOME *et al.* 1989. Effetti emodinamici e sul circolo coronarico della propionyl-L-carnitina. Cardiologia **54**(suppl. 1)**:** 111–117.
47. CHIDDO, A., A. GAGLIONE, S. MUSCI *et al.* 1991. Hemodynamic study of intravenous propionyl-L-carnitine in patients with ischemic heart disease and normal left ventricular function. Cardiovasc. Drugs Ther. **5**(suppl. I)**:** 107–112.
48. TASSANI, V., L. CATTAPAN, L. MAGNANIMI *et al.* 1994. Anaplerotic effect of propionyl-L-carnitine in rat heart mitochondria. Biochem. Biophys. Res. Commun. **199:** 949–953.
49. ZWEIER, J.L. & E. JACOBUS. 1987. Substrate-induced alteration of high energy phosphate metabolism and contractile function in the perfused heart. J. Biol. Chem. **262:** 8015–8021.
50. FERRARI, R., F. DI LISA, J.W. DE JONG *et al.* 1992. Prolonged propionyl-L-carnitine pretreatment of rabbit: biochemical, hemodynamic, and electrophysiological effects on myocardium. J. Mol. Cell. Cardiol. **24:** 219–232.
51. BARBIERI, M., P.U. CARBONIN, E. CERBAI *et al.* 1991. Lack of correlation between the antiarrhythmic effect of L-propionylcarnitine on reoxygenation-induced arrhythmias and its electrophysiological properties. Br. J. Pharmacol. **102:** 73–78.
52. FERRARI, R., E. PASINI, E. CONDORELLI *et al.* 1991. Effect of propionyl-L-carnitine on mechanical function of isolated rabbit heart. Cardiovasc. Drugs Ther. **5:** 17–24.
53. FERRARI, R., A. CARGNONI, C. CECONI *et al.* 1990. Effect of L-carnitine derivatives on lipid peroxidation of cardiac mitochondria. Soc. Ital. Biochim. **2:** 200.
54. NOBLE, D. 1984. The surprising heart: a review of recent progress in cardiac electrophysiology. J. Physiol. **353:** 1–50.
55. BRODERICK, T.L., H.A. QUINNEY, C.C. BARKER *et al.* 1993. Beneficial effect of carnitine on mechanical recovery of rat hearts reperfused after a transient period of global ischemia is accompanied by a stimulation of glucose oxidation. Circulation **87:** 972–981.
56. LEIPALA, J.A., R. BHATNAGAR, E. PINEDA *et al.* 1991. Protection of the reperfused heart by L-propionylcarnitine. J. Appl. Physiol. **71:** 1518–1522.
57. PAULSON, D.J., J. TRAXLER, M. SCHMIDT *et al.* 1986. Protection of the ischaemic myocardium by L-propionylcarnitine: effects on the recovery of cardiac output after ischaemia and reperfusion, carnitine transport, and fatty acid oxidation. Cardiovasc. Res. **20:** 536–541.
58. YANG, X.P., M. SAMAJA, E. ENGLISH *et al.* 1992. Haemodynamic and metabolic activities of propionyl-L-carnitine in rats with pressure overload cardiac hypertrophy. J. Cardiovasc. Pharmacol. **20:** 88–98.
59. MICHELETTI, R., G. GIACALONE, M. CANEPARI *et al.* 1994. Propionyl-L-carnitine prevents myocardial mechanical alterations due to pressure overload in rats. Am. J. Physiol. **266:** H2190–H2197.
60. MICHELETTI, R., A. SCHIAVONE, E. ENGLISH *et al.* 1993. Propionyl-L-carnitine limits chronic ventricular dilatation after myocardial infarction in rats. Am. J. Physiol. **264:** H1111– H1117.
61. LI, P., R. MICHELETTI, C. PARK *et al.* 1994. Propionyl-L-carnitine ameliorates myocyte performance after infarction [abstract]. Can. J. Cardiol. **10:** 85.
62. PASINI, E., L. COMINI, R. FERRARI *et al.* 1992. Effect of propionyl-L-carnitine on experimental induced cardiomyopathy in rats. Am. J. Cardiovasc. Pathol. **4:** 216–222.

63. LOPASCHUCK, G.D., A.G. TAHILIANI, R.V.S.U. VADLAMUDI *et al.* 1983. Cardiac sarcoplasmic reticulum in insulin- or carnitine-treated diabetic rats. Am. J. Physiol. **245:** H969–H976.
64. WILSON, J.R., J.L. MARTIN, D. SCHWARTZ *et al.* 1984. Exercise intolerance in patients with chronic heart failure: role of impaired skeletal muscle nutritive flow. Circulation **69:** 1079–1087.
65. LIPKIN, D.P. & F.A. POOLE-WILSON. 1986. Symptoms limiting exercise in chronic heart failure. BMJ **292:** 1030–1031.
66. WILSON, J.R. & M.D. MANCINI. 1993. Factors contributing to exercise limitation of heart failure. J. Am. Coll. Cardiol. **22:** 93A–98A.
67. CLARK, A.L., F.A. POOLE-WILSON & A.J.S. COATS. 1996. Exercise limitation in chronic heart failure: central role of the periphery. J. Am. Coll. Cardiol. **28:** 1092–1102.
68. SULLIVAN, M.J., J.D. KNIGHT, M.B. HIGGINBOTHAM *et al.* 1989. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Circulation **80:** 769–781.
69. FERRARI, R., E. PASINI, F. DE GIULI *et al.* 1994. Limb uptake of substrate in patients with congestive heart failure (CHF) [abstract]. Can. J. Cardiol. **10**(suppl. A)**:** 73A.
70. OPASICH, C., E. PASINI, R. AQUILANI *et al.* 1997. Skeletal muscle function at low work level as a model for daily activities in patients with chronic heart failure. Eur. Heart J. **18:** 1626–1631.
71. BREVETTI, G., M. CHIARIELLO, G. FERULANO *et al.* 1988. Increases in walking distance in patients with peripheral vascular disease treated with L-carnitine: a double blind, cross-over study. Circulation **77:** 767–783.
72. TAYLOR, D.J., A. AMATO, U. HANDS *et al.* 1996. Changes in energy metabolism of calf muscle in patients with intermittent claudication assessed by 31 pMRS: a phase II open study. Vasc. Med. **1:** 241–245.
73. BREVETTI, G., S. PERNA, C. SABBÀ *et al.* 1995. Propionyl-L-carnitine in intermittent claudication: double-blind, placebo-controlled, dose-titration, multicenter study. J. Am. Coll. Cardiol. **26:** 1411–1416.
74. HIATT, W.R., J.G. REGENSTEINER, M.A. CREAGER *et al.* 2001. Propionyl-L-carnitine improves exercise performance and functional status in patients with claudication. Am. J. Med. **110:** 616–622.
75. BREVETTI, G., C. DIEHM & D. LAMBERT. 1999. European multicenter study on propionyl-L-carnitine in intermittent claudication. J. Am. Coll. Cardiol. **34:** 1618–1624.
76. ANAND, I., Y. CHANDRASHEKAR, F. DE GIULI *et al.* 1998. Acute and chronic effects of propionyl-L-carnitine on the hemodynamics, exercise capacity, and hormones in patients with congestive heart failure. Cardiovasc. Drugs Ther. **12:** 291–299.
77. CAPONNETTO, S., C. CANALE, M.A. MASPERONE *et al.* 1994. Efficacy of L-propionyl-carnitine treatment in patients with left ventricular dysfunction. Eur. Heart J. **15:** 1167–1173.
78. MANCINI, M., S. RENGO, M. LINGETTI *et al.* 1992. Control study on the therapeutic efficacy of propionyl-L-carnitine in patients with congestive heart failure. Arzneim. Forsch. **42:** 1101–1104.
79. BACHETTI, T., A. CORTI, G. CASSANI *et al.* 1994. Cytokines in end stage congestive heart failure: effect of propionyl-L-carnitine. Eur. Heart J. **15:** 1267–1273.
80. FERRARI, R., T. BACHETTI, R. CONFORTINI *et al.* 1995. Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure. Circulation **92:** 1479–1486.
81. FERRARI, R. 1998. Tumor necrosis factor in CHF: a double facet cytokine. Cardiovasc. Res. **37:** 554–559.
82. AGNOLETTI, L., S. CURELLO, T. BACHETTI *et al.* 1999. Serum from patients with severe heart failure downregulates enol and is proapoptotic—role of tumor necrosis factor-α. Circulation **100:** 1983–1991.
83. LEVINE, B., J. KALMAN, L. MAYER *et al.* 1991. Elevated circulating level of tumor necrosis factor in severe chronic heart failure. N. Engl. J. Med. **323:** 236–241.
84. THE INVESTIGATORS OF THE STUDY ON PROPIONYL-L-CARNITINE IN CHRONIC HEART FAILURE. 1999. Study on propionyl-L-carnitine in chronic heart failure. Eur. Heart J. **20:** 70–76.

Oxford Journals Medicine Cardiovascular Research Volume 51, Issue 1 Pp. 21-29.

Cardiovascular Research
cardiovascres.oxfordjournals.org

Cardiovasc Res (2001) 51 (1): 21-29. doi: 10.1016/S0008-6363(01)00313-3

Influence of L-carnitine and its derivatives on myocardial metabolism and function in ischemic heart disease and during cardiopulmonary bypass

Abstract

Carnitine and its derivatives have recently been shown to protect cardiac metabolism and function in ischemic heart disease and other clinical conditions of myocardial ischemia. Potential mechanisms of this effect include an increase in glucose metabolism, a reduction of toxic effects of long-chain acyl-CoA and acyl-carnitine in myocytes, an increase in coronary blood flow and anti-arrhythmic effect. It has also been shown that propionyl-L-carnitine which penetrates faster than carnitine into myocytes is effective in inhibiting production of free radicals. Beneficial effects of carnitine supplementation have been demonstrated under a variety of clinical conditions such as acute cardiac ischemia, during extracorporeal circulation, in carnitine-dependent cardiomyopathy as well as in patients with chronic circulatory failure and in cardiogenic shock. However, further studies are required before carnitine administration could be recommended as a routine procedure in ischemic heart disease or before cardiopulmonary bypass.

Key words Coronary disease Energy metabolism Heart failure Ischemia Myocytes Ventricular function

Time for primary review 42 days.

1. Introduction

L-Carnitine (β-hydroxy-γ-trimethyl-amino-butyric acid) is a crucial component of activated fatty acids transport mechanism across the mitochondrial membrane [1]. Carnitine facilitates oxidation of long-chain fatty acids, modulates the ratio of CoA to CoA-SH, and is involved in trapping acyl residues from peroxisomes and mitochondria. Carnitine also participates in metabolism of branched chain amino acids and stabilizes cellular membranes. It is also a free radical scavenger and is likely to take part in control of nuclear transcription [1,2]. The primary sites for carnitine synthesis from 6-*N*-trimethylolysine are the liver and the kidneys, although the brain does have a small potential as well. Substrates that are indispensable for carnitine synthesis and are present in the diet are lysine and methionine, although the presence of vitamins C and B_6, and iron [3] is equally important. However, if the diet is deficient in carnitine, a considerable drop soon develops in its plasma concentration [4]. Minimum supplementation of carnitine in the diet to maintain its body stores constant ranges from 8 to 11 mg per day [4]. The richest source of carnitine is red meat, which contains about 5.5 μmol/g of tissue, and among plant-origin products — beans (0.72 μmol/g) and avocado.

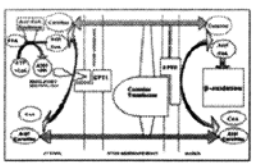
The total content of carnitine in the human body is about 100 mmol (16 g) but it depends on the diet, muscle mass and the age [1]. Muscles contain 98% of that total amount, while 1.5 and 0.5% of carnitine are found in the liver and other tissues, respectively [5]. The total carnitine concentration in

plasma is usually in the range of 42–85 μmol/l, and that of free carnitine in the range of 35–70 μmol/l [6]. Carnitine concentration in the heart is about 4.2 μmol/g of tissue, which is over three times higher, than that in the striated muscles (1.26 μmol/g), four times higher than that in the liver (0.94 μmol/g), and eight times higher than that in the kidney (0.52 μmol/g) [7]. The skeletal muscles and the heart lack potential for synthesizing carnitine.

Accumulation of carnitine in the heart is facilitated by active extraction of carnitine from plasma against a 60-fold concentration gradient [8]. Unlike in the liver, carnitine concentration in the myocardium and skeletal muscle is relatively independent of its temporary supply [9].

Over 95% of ingested carnitine is excreted in urine in humans [10]. The clearance of free carnitine is about four times lower than of acyl-carnitine (1.1 ml/min and 4.8 ml, respectively). There is evidence that a Na^+ dependent system exists driven by energy from the ATP hydrolysis. It is responsible for carnitine transport through the muscle cell membrane [11]. Carnitine transport to the mitochondria is carried out by a specific protein carrier [12]. Long-chain fatty acids constitute a basic substrate for oxidative energy metabolism in the myocardium. Following transport through the cellular membrane, they undergo activation to acyl-CoA in the cytoplasm or on the external mitochondrial membrane. Though some of activated fatty acids undergo esterification to triglycerides, majority becomes a substrate for β-oxidation in mitochondria. Carnitine has a basic role in transporting activated fatty acids from the cytoplasm into mitochondria, where β-oxidation occurs. Short- and medium-chain fatty acids are transported into the mitochondrial matrix without any carnitine assistance in the process. Long-chain fatty acid acyl groups are transported exclusively as carnitine esters by a carnitine carrier the translocase, which constitutes a trans-membraneous protein in the inner mitochondrial membrane [13,14].

The 'carnitine system' consists mainly of carnitine, carnitine acyl-transferases, translocase, and transporting proteins located in plasma membranes. Carnitine palmitoyl transferase 1 (CPT 1) which is located on the internal side of the external mitochondrial membrane transfers activated long-chain acyl residues from acyl-CoA into carnitine. Carnitine translocase exchanges acyl-carnitine for carnitine from the matrix via the internal mitochondrial membrane. On the internal side of the inner mitochondrial membrane, carnitine palmitoyl transferase 2 (CPT 2) catalysis acyl-CoA synthesis from acyl-carnitine and matrix pool of CoA-SH (Fig. 1). Finally acyl-CoA undergoes mitochondrial β-oxidation with a release of energy in the ATP form. Carnitine lowers the ratio of intramitochondrial acyl-CoA to CoA and causes detoxification of accumulated acyl-CoA esters in patients with defective metabolism of glucose or acyl-oxidation. The above mechanism is impaired when mitochondrial β-oxidation disturbances develop in carnitine deficient patients [15].



View larger version:
In this page In a new window
Download as PowerPoint Slide

Fig. 1
Carnitine dependent transport of long-chain acyl groups through the mitochondrial membrane.

Systemic carnitine deficiency manifests mainly as a dysfunction of skeletal muscles and myocardium, where fatty acids constitute a basic energy substrate. Other symptoms of carnitine deficiency also include hypoglycemia due to exhaustion of glucose reserves, which is an alternative substrate to fatty acids, hyperammonemia, hypoketonemia, coma, seizures, and developmental retardation, as well as the presence of lipid deposits in the histopathologic picture of the liver and muscles. Primary carnitine deficiencies result from inborn defects in specific proteins or in carnitine transferases. Secondary causes of L-carnitine

deficiency include metabolic defects in fatty acid oxidation, mitochondrial myopathy, prematurity, L-carnitine deficiency in the diet, dialysis therapy, diabetes, and inadequate absorption from the gastrointestinal tract. Studies on carnitine concentration can indicate either an absolute deficit of carnitine when its level drops below 20 μmol/l, or its relative deficit when the ratio of carnitine esters to free carnitine is above 0.4 [11]. Absolute or relative carnitine deficits develop in chronic congestive heart failure, acute myocardial ischemia, diseases of peripheral blood vessels, diabetes, and disturbances of lipid metabolism. The clinical importance of carnitine in the treatment of circulatory disorders was first demonstrated in 1973 when its deficit was discovered in patients with lipid cardiomyopathy and reduced fatty acid oxidation [16]. Additionally, carnitine administration has been proven clinically beneficial in other diseases characterized by carnitine deficit that include, among others, ischemic cardiomyopathy and peripheral atherosclerosis [2].

Positive clinical effects of carnitine administration were also observed in nervous system degenerative diseases, brain ischemia, chronic fatigue syndrome, Alzheimer's disease, and AIDS [17–19]. L-Carnitine in a long-term supplementation has been shown to be beneficial to the function of erythrocytes in hemodialysed patients [20]. Positive results of carnitine therapy are due to, among others, oxidation of fatty acids, the process which is linked with saving muscle glycogen reserves. A number of therapeutic effects possibly come from the interaction of carnitine and its derivatives with the elements of cellular membranes [21].

Some improvement in the muscle blood supply after carnitine supplementation has been related to vasodilatation. In diabetes, L-carnitine supplementation causes a decrease in triglyceride synthesis, a drop in the cellular free fatty acids uptake, and the removal from organism of excessive long-chain carnitine esters, as well as increase in glycolysis, oxidation of pyruvate, and improvement in neuronal transmission [22].

2. Disturbances of myocardial function and carnitine metabolism — experimental data

Carnitine is released from ischemic myocardium, and its concentration in the coronary sinus is proportional to the concentration of lactate [23,24]. These changes are reflected by a change in the ratio of free carnitine to carnitine esters in the heart. Anoxia caused by myocardial ischemia has been experimentally proven to be associated with the depletion of carnitine reserves and accumulation of toxic metabolites of fatty acid esterification, in consequence of restricted fatty-acids mitochondrial β-oxidation [25]. The result is a decrease of ATP concentration in the heart [26]. After only a few minutes of ischemia, free fatty acids, long-chain acyl-CoA esters and acyl-carnitine are all increased several times above the control level [27] and, after half an hour of ischemia, the free carnitine in the heart drops by half [26].

Long-chain acyl-carnitine esters are lipophylic and may readily damage membrane lipids and particularly, membrane bound enzymatic proteins [27]. This results in an increase of membrane fluidity and affects membrane ion transport. Inhibition of the sodium-potassium ATP-ase in sarcolemma results in a decrease of membrane rest-potential. In consequence, spontaneous action potentials may appear along with a delayed depolarization of action potential [28].

In the study of Silverman et al. carnitine supplementation had a positive and dose-dependent effect on preserving ventricle compliance and contractility of ischemic canine heart muscle, yet had no influence upon a decrease in ATP concentration induced by ischemia [25]. In another study, when oxygenated blood supply was restored, no recovery of lipid oxidation was observed unless carnitine deficiency had been compensated [29]. According to Liedtke et al. reduced carnitine reserves in the myocardium strengthen the negative influence of lipids and particularly of free fatty acids, on heart metabolism [29]. The positive carnitine influence seen in acute myocardial ischemia was explained by limiting a decrease in high-energy phosphates [30] and some improvement in glucose oxidation and lactate extraction [31]. Basic metabolic effects of carnitine supplementation during ischemia-reperfusion and its suggested clinical effects are presented in Fig. 2.

Following carnitine administration, some improvement in relaxation was observed in rat hearts with increased afterload [32]. However, when isolated rabbit hearts were studied, no improvement of contractility, heart rate, or coronary perfusion pressure was reported [32]. Broderick et al. demonstrated a more than two-fold increase in glucose metabolism after ischemia and reperfusion in rat heart preparations perfused with carnitine-enriched blood before the onset of ischemia [31].

View larger version:
In this page In a new window
Download as PowerPoint Slide

Fig. 2
Metabolic action of L-carnitine during myocardial ischemia-reperfusion, and its suggested clinical consequences.

A positive carnitine influence on myocardial metabolism during ischemia-reperfusion has been confirmed by increased ATP concentration in myocardium [33]. Another study demonstrated a beneficial effect of carnitine, added to cold cardioplegia, on the aorta flow velocity, the stores of ATP in myocardium, and the condition of mitochondria in rat hearts after reperfusion [34]. L-Carnitine supplementation beside causing an increase in ATP concentration, was associated with a lower amount of toxic esters [33], and with some improvement of systolic and diastolic function of diabetic rat hearts [35]. A similar therapy applied to a group of diabetes-free rats did not result in any change of heart function [35]. Experimental studies have demonstrated that in diabetic rats even a short-term carnitine supplementation caused an improvement of myocardial contractility after ischemia-reperfusion [36]. Administration of propionyl-L-carnitine to diabetic rats prior to ischemia and reperfusion resulted in a more complete recovery of post-reperfusion contractility [23].

In dogs without previous myocardial ischemia, supplementation of high carnitine doses was linked with a considerable increase (60-100%) in coronary blood flow [37]. In the same study a substantial increase in stroke volume and dP/dt for the left ventricle was reported.

When dogs with myocardial infarction were studied, a 50% lower ST elevation was observed in ECG, if L-carnitine was administered after the onset of ischemia [38]. Incubation of heart endothelium cells in a L-carnitine-rich environment causes a better recovery of their proper function during post-ischemic reoxygenation [39].

Protection of cell membranes and especially of membrane enzymes is a possible cause of electrophysiologic changes and antiarrythmic effect of carnitine. Studies on dogs treated with L-carnitine in doses of 40 and 80 mg/kg/min revealed a decrease in the heart rate by about 17 and 30%, respectively [31]. In canine heart preparations subjected to ischemia, a considerable carnitine-induced decrease in the frequency of ventricular arrhythmia, including ventricular fibrillation was observed [40]. The authors suggested it might have been caused by limiting the necrotic area rather than by direct antiarrythmic action. It has also been demonstrated that carnitine supplementation is connected to limiting heart rhythm disturbances that come from a high concentration of free fatty acids in dogs [41]. Carnitine administration to perfused isolated preparations of guinea pig hearts has substantially lowered the incidence of arrhythmia resulting from ischemia and reperfusion [42].

Propionyl-L-carnitine was demonstrated to be able to reduce the incidence of ventricle fibrillation during reoxygenation after ischemia in isolated preparations of guinea pig hearts [43].

Positive results of treatment with carnitine were observed in experimental

heart failure. Both in guinea pigs and dogs subjected to the toxin of *Corynebacterium diphtheriae* an improvement was observed in the left ventricle function and in the time of survival in the groups treated with L-carnitine [44,45]. Application of propionyl-L-carnitine to volume-loaded rat hearts was associated with an improvement in ventricle function without a simultaneous increase in oxygen consumption [46]. Schonekess et al. have reported an improvement in the function of hypertrophied myocardium and an increase in carbohydrate oxidation in rats treated with propionyl-L-carnitine [47]. Another study reported a decrease in mortality in hamsters with cardiomyopathy treated with carnitine [48]. There have also been contradictory reports, which presented a positive influence of inhibiting endogenic carnitine synthesis upon ischemic rat hearts [49]. In summary, relative myocardial carnitine deficiency is observed during ischemia and many experimental data suggest that some negative metabolic and biologic effects of ischemia, as well as heart failure are alleviated by carnitine supplementation.

3. Biological effects of carnitine and propionyl-carnitine administration

It has been suggested to treat metabolic dysfunction resulting from carnitine deficiency not only by administering L-carnitine, but also its derivative — propionyl-L-carnitine (Table 1). Some biologic properties of propionyl-L-carnitine suggest that beneficial clinical effects could be enhanced when compared to L-carnitine. Propionyl-L-carnitine has higher affinity for the plasma membrane transport system. It is more lipophylic and penetrates myocytes faster than L-carnitine [50]. Moreover, the propionyl residue of propionyl-L-carnitine can be metabolized to the succinate, a substrate of citric acid cycle [51]. Propionyl-L-carnitine has also protective properties for blood vessels and the energy reserves of ischemic striated muscles [52]. L-Carnitine and propionyl-L-carnitine were shown to protect the ischemic myocardium against oxidative stress, which is one of the basic mechanisms leading to a post-ischemic myocardial dysfunction known as 'stunning' [53]. Ferrari et al. have published interesting study concerning the maintenance of oxidative phosphorylation and prevention of calcium ion influx into mitochondria during reperfusion of rabbit hearts treated with propionyl-L-carnitine before the onset of ischemia [54]. Protection of the heart against oxidative stress during reperfusion was demonstrated after pre-ischemic administration of carnitine and its derivatives, particularly propionyl-L-carnitine [2]. Propionyl-L-carnitine does have a potential for inhibiting production of free hydroxyl radicals. Endothelial cellular membranes are better protected by propionyl-L-carnitine against Fe^{2+} and Fe^{3+} ions induced peroxide production, the protection being possibly due to ion chelating [39].

The protective effect of propionyl-L-carnitine in perfused rat hearts is dose-dependent and also depends on the time of administration, provided it is administered before post-ischemic reperfusion begins [55]. As suggested in the study, propionyl-L-carnitine may also have a role in stabilizing plasma membranes and in lowering the purine release from the perfused rat heart [39].

View this table: In this window In a new window

Table 1
Metabolic, experimental and clinical effects of carnitine and propionyl-L-carnitine supplementation on cardiovascular system

From the accumulated results it seems that positive biological effects observed after propionyl-L-carnitine are more evident that after L-carnitine administration. Better penetration into myocytes and supplying a substrate for the citric acid cycle can explain this observation in short-term supplementation. Inhibiting free-radicals generation and stabilizing plasma membranes may be other contributing factors.

4. Carnitine and circulatory diseases — clinical studies

4.1. Ischemic heart disease

A decreased carnitine concentration in the heart was observed in patients

who died of myocardial infarction [56]. In patients with acute myocardial infarction, a four-fold increase was observed in free carnitine elimination and almost a two-fold increase in the elimination of short-chain carnitine esters by kidney [20]. Arsenian et al. demonstrated a decrease in mortality and incidence of circulatory failure in a group of patients with acute myocardial infarction, who were administered 3 g of carnitine along with solution of glucose, insulin, potassium and magnesium [57]. Propionyl-L-carnitine used in doses of 15 mg/kg caused a slight decrease in peripheral vascular resistance in patients with stable coronary disease, but due to a simultaneous increase in stroke volume, no decrease in arterial blood pressure was observed [58].

A similar dose administered to patients with ischemic heart disease caused in a short time (5 min) a 43% increase in lactate uptake by myocardium and increase in stroke volume by 8% [59]. On the other hand, administration of L-carnitine in a single dose of 40 mg/kg to patients with stable coronary heart disease did not cause any change in the heart rate at rest or in systolic and diastolic arterial pressure [60].

Coronary artery sclerosis is particularly common in diabetic patients. Disturbances of myocardial contractility in diabetic patients are generally associated with a higher incidence of coronary heart disease in this population. Many diabetics, however, suffer from decreased myocardial contractility in spite of negative coronarographic examinations. The causes of impaired heart contractility in diabetic patients who present no changes detectable by coronarography include, among others, microangiopathy and metabolic disturbances. Carnitine deficiency is now the best recognized condition of the latter. Decreased level of free and total carnitine in diabetes, with a simultaneous increase in concentrations of long-chained acyl-CoA and long-chain carnitine esters has been shown [23].

Some correlation has been also demonstrated between the left ventricular contraction index and long-chain acyl-carnitine concentration in the myocardium during reperfusion in patients after mitral valve replacement [61]. These data suggest, in line with the results of experimental studies that carnitine and its derivatives protect human ischemic heart against oxidative stress not only by modifying carnitine acyl-transferase activity and metabolic effect, but by other mechanisms as well [21].

4.2. Arrhythmia

Heart electrophysiology after carnitine administration (30 mg per kg body weight over 3 min) did not show any changes either in the conductivity time or in refraction period. The cycle duration in the sinus node was shortened by 5%, while the arterial blood pressure remained unchanged [62]. It has been shown that the incidence of ventricular and supraventricular arrhythmia could be limited during hemodialysis in chronic renal failure [63]. A prolonged L-carnitine therapy in angina pectoris was associated with a considerable decrease in the frequency of ventricular arrhythmias [64]. Rizzon et al. noticed a statistically significant decrease of the frequency of ventricular arrhythmia in a group of patients with acute myocardial infarction who were administered 100 mg of carnitine per kg of body weight [65]. Although the studied groups of patients were small, L-carnitine administration to patients with ischemic heart disease appears to be a promising therapy of ischemia-induced arrhythmia as potentially addressed to restoring of membrane rest-potential.

4.3. Cardiopulmonary bypass surgery

Oral administration of carnitine (1 g) for 2 days before the coronary artery bypass graft (CABG) operation was associated with a higher ATP concentration and a more favourable ratio of free carnitine to long-chain acyl-carnitine in the atrial muscle [66]. Administration of high L-carnitine doses for 3 days prior to the extracorporeal circulation was not linked to an improvement of hemodynamic parameters, although a biopsy of septum revealed a better preserved cellular ultrastructure [67]. On the other hand, in patients with a low ejection fraction a dose of 360 mg of L-carnitine added to cardioplegia was demonstrated to have a positive influence on the stroke volume immediately after weaning from extracorporeal circulation [68]. In patients after CABG, L-carnitine supplementation has been shown to be associated with an increased

uptake of free fatty acids by myocardium and with their lower concentration in blood [2].

4.4. Circulatory failure

Early reports on carnitine in the treatment of circulatory failure were concerned with its application in cases of carnitine deficiency syndrome. Carnitine deficiency syndromes can be divided into systemic and myopathic in nature. The former is characterized by a low carnitine concentration both in muscles and the blood, and the latter is known for a low carnitine concentration in muscles coexisting with the proper carnitine concentration in blood. This division enables assessment of the rationale for using carnitine in the treatment of concurrent circulatory failure. Cardiomyopathies that coexist with a low blood carnitine level are generally well treatable with carnitine [69,70]. Myopathies and cardiomyopathies with proper blood carnitine concentration prevalently do have some acyl-CoA dehydrogenase deficiency or functional disturbances of carnitine transport proteins [71]. However, increased carnitine concentrations in blood have been also seen in cardiomyopathic patients [72]. In dilated cardiomyopathy patients who underwent heart transplantation for circulatory failure, a low carnitine concentration was found both in the blood and myocardium [73]. Myocardial carnitine concentration was also lower in rheumatic heart disease [74]. Administration of carnitine to children with diphtheria was found to have reduced substantially the incidence of myocarditis [75]. One-month propionyl-L-carnitine therapy in doses of 1.5 g for 24 h in patients with congestive heart failure resulted in an increased ejection fraction [76]. Hemodialysed patients were observed to have a lower free-carnitine concentration in blood, despite normal concentration of total carnitine. After a 12-month carnitine therapy at 500 mg/24 h dose the same patients had improved circulatory function in electrocardiographic examination [77].

Pugliese et al. observed a decrease in pulmonary arterial pressure, in the heart rate in patients with hepatic cirrhosis after administration of 30 mg of L-carnitine per kilogram body weight [78]. Both short- and long-term propionyl-L-carnitine supplementations, in patients with congestive heart failure resulted in decreased pressure in the pulmonary artery and the left ventricle diameter [79]. One study on patients with shock who were given high L-carnitine doses did demonstrate clinical improvement [80], while another report concluded that a 12-h acetyl-carnitine administration to patients in shock was associated only with a slight improvement in selected hemodynamic parameters [81].

In the light of these collected results, application of carnitine appears promising in circulatory failure. However, except for carnitine deficiency syndromes, this has not been satisfactorily proven.

4.5. Peripheral blood vessel diseases

The data on the influence of carnitine and its derivatives in cases of peripheral blood vessel disease generally come from experimental studies. Accordingly, propionyl-carnitine has a potential for inhibiting thrombosis induced in the rat's tail [82]. Corsico et al. observed the protective properties of propionyl-L-carnitine for the condition and function of muscles as well as the condition of vessels that were damaged with sodium lauryl-sulphate [53]. Patients with peripheral blood vessel disease were found to have on exertion an increased blood concentration of total carnitine as well as long- and short-chain acyl-carnitine [83]. Following a 3-week carnitine therapy, these patients had a longer claudication distance [84]. Beneficial effects of propionyl-L-carnitine in peripheral blood vessel disease can be not only related to an improved metabolism of ischemic muscles. They are also possibly linked to some potential for limiting a negative influence of endothelin on blood vessels [85] or to increasing tissue concentration of plasminogen activator, or to an improvement in the rheological properties of erythrocytes [86]. Studies have also proven that propionyl-L-carnitine has a vasodilative action independent of nitric oxide, but mediated by prostaglandins [87].

An increasing amount of data supports the thesis of the beneficial effects of carnitine in patients with ischemic heart disease and ischemia-related arrhythmia, circulatory failure and peripheral vascular disease. The results of studies on hemodynamic effects of carnitine and its derivatives

administration could possibly be more univocal, if the studied groups were more homogenic concerning poor ventricular function and coexisting state of carnitine deficiency.

5. Side effects

Carnitine preparations administered orally can occasionally cause heart-burn and dyspepsia. Two patients treated with intravenous carnitine in doses of 6 g complained of blurred vision and one reported a headache [88]. Fairly high doses of carnitine administered orally may produce an unpleasant body odor that is similar to that of rotten fish. There are no reports to date of serious side effects caused by L-carnitine and its derivatives, and most clinical studies have claimed there were not any undesired effects.

6. Conclusions

The results reviewed in this article indicate that carnitine and propionyl-L-carnitine exert a positive metabolic and functional effect on myocardium in ischemic heart disease and in heart failure. The accumulated data resulting from experimental and clinical studies and general knowledge of myocardial metabolism in ischemia and reperfusion, allows us to expect encouraging clinical results especially in carnitine-deficient patients. However, some conflicting results do not permit definitive conclusion to be drawn about the efficacy of the action of carnitine and its derivatives on ischemic and reperfused myocardium. Additional studies performed under well-controlled conditions are needed to further elucidate the rationale for carnitine administration in ischemic heart disease and before cardiopulmonary bypass surgery.

R Lango[a,*], R.T Smolenski[b], M Narkiewicz[c], J Suchorzewska[a] and W Lysiak-Szydlowska[d]
+ Author Affiliations

* Corresponding author. Tel.: +48-58-349-2482; fax: +48-58-349-4858 rlango@amg.gda.pl

Received November 28, 2000.
Accepted April 9, 2001.

Journal of the American College of Cardiology
© 2009 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 54, No. 18, 2009
ISSN 0735-1097/09/$36.00
doi:10.1016/j.jacc.2009.08.012

Micronutrient Deficiencies

An Unmet Need in Heart Failure

Victor Soukoulis, MD, PHD,* Jamil B. Dihu, DO,‡ Michael Sole, MD,§
Stefan D. Anker, MD, PHD,‖¶ John Cleland, MD,# Gregg C. Fonarow, MD,**
Marco Metra, MD,†† Evasio Pasini, MD,‡‡ Theresa Strzelczyk, APN, CNS,*
Heinrich Taegtmeyer, MD, DPHIL,§§ Mihai Gheorghiade, MD†

Chicago, Illinois; Toronto, Ontario, Canada; Berlin, Germany; Roma and Brescia, Italy; Kingston upon Hull, United Kingdom; Los Angeles, California; and Houston, Texas

Heart failure (HF) is a common, disabling, and costly disease. Despite major advances in medical therapy, morbidity and mortality remain high, in part because current pharmacological regimens may not fully address some unique requirements of the heart for energy. The heart requires a continuous supply of energy-providing substrates and amino acids in order to maintain its function. In HF, defects in substrate metabolism and cardiac energy and substrate utilization may contribute to contractile dysfunction. HF is often accompanied by a deficiency in key micronutrients required for unimpeded energy transfer. Correcting these deficits has been proposed as a method to limit or even reverse the progressive myocyte dysfunction and/or necrosis in HF. This review summarizes the existing HF literature with respect to supplementation trials of key micronutrients involved in cardiac metabolism: coenzyme Q10, L-carnitine, thiamine, and amino acids, including taurine. Studies using a broader approach to supplementation are also considered. Although some of the results are promising, none are conclusive. There is a need for a prospective trial to examine the effects of micronutrient supplementation on morbidity and mortality in patients with HF. (J Am Coll Cardiol 2009;54:1660–73) © 2009 by the American College of Cardiology Foundation

Heart failure (HF) is a common, disabling, and costly disease throughout the world (1,2). Annual costs related to the treatment of HF in the U.S. are estimated at $38 billion, and account for 5.4% of the health care budget (3). The treatment of HF is based on medical guidelines published by the professional societies (4–7). The recommended pharmacological therapy includes the use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, beta-blockers, and aldosterone antagonists. Additional nonpharmacologic measures, such as cardiac resynchronization therapy, implantable cardioverter-defibrillators, and exercise training, have shown beneficial outcomes in quality of life, morbidity, and/or mortality of HF patients (7).

Despite improvements in mortality, HF hospitalizations continue to rise (8). Hospitalization for HF is a strong predictor of poor prognosis and is associated with post-discharge mortality and readmission rates that can be as high as 15% and 30%, respectively, within 60 to 90 days (9,10). Although new therapeutic options have undoubtedly improved morbidity and mortality, additional interventions are needed to prevent progression of HF and improve outcomes (10,11).

Although current therapies have addressed hemodynamics, neurohormonal modulation, and electrophysiological aspects of HF, these therapies have not targeted the metabolic needs of the failing heart (12).

From the *Department of Medicine and †Center for Cardiovascular Quality and Outcomes, Northwestern University Feinberg School of Medicine, Chicago, Illinois; ‡Department of Medicine, University of Chicago (North Shore), Pritzker School of Medicine, Chicago, Illinois; §Division of Cardiology, University Health Network, Toronto, Ontario, Canada; ‖Department of Cardiology, Charite, Campus Virchow-Klinikum, Berlin, Germany; ¶Centre for Clinical and Basic Research, IRCCS San Raffaele, Roma, Italy; #Department of Cardiology, Castle Hill Hospital, Hull York Medical School, Kingston upon Hull, United Kingdom; **Division of Cardiology, David Geffen School of Medicine at UCLA, Los Angeles, California; ††Section of Cardiovascular Diseases, Department of Experimental and Applied Sciences, University of Brescia, and ‡‡Fondazione "S Maugeri," Medical Centre of Lumezzane, Brescia, Italy; and the §§Department of Medicine/Cardiology, University of Texas Houston Medical School, Houston, Texas. Dr. Sole has a share in patents on a nutritional supplement for heart failure (containing any effective combination of coenzyme Q10, L-carnitine or its derivatives, and taurine) issued by the U.S., Canada, and most Western European countries. He has licensed a supplement in Canada to Seaford Pharmaceuticals. Dr. Anker receives research grants and honoraria for speaking and consultancy from Fresenius (Germany) as well as honoraria for consultancy from Professional Dietetics (Italy). Dr. Cleland has a consultancy with NUMICO (recently taken over by DANONE). Dr. Fonarow receives research funding from the National Heart, Lung, and Blood Institute, GlaxoSmithKline, and Pfizer, and is a consultant for or receives honoraria from GlaxoSmithKline, Debiopharm, Novartis, Merck, and Scios. Dr. Taegtmeyer receives research funding from the National Heart, Lung, and Blood Institute and the American Heart Association. Dr. Gheorghiade performs consultant work with Otsuka, Solvay Pharma, Novartis, Bayer, Sigma-Tau, Debiopharm, Medtronic, Merck, Astellas Pharma, Cytokinetics, Corthera, PeriCor Therapeutics, GlaxoSmithKline, Johnson & Johnson, Abbott, Errekappa Terapeutici, PDL: BioPharma, AstraZeneca, and Sanofi-Aventis.

Manuscript received July 16, 2009; revised manuscript received August 24, 2009, accepted August 25, 2009.

Normal Cardiac Metabolism

The adult human heart, which weighs between 200 and 425 g, is a highly efficient converter of chemical energy to mechanical energy (13). This relatively small mass uses more energy, in the form of adenosine triphosphate (ATP), than any other organ as it pumps 5 l of blood per minute, 7,200 l per day, and over 2.6 million liters per year (14). It is estimated that over 6 kg of ATP is hydrolyzed by the heart daily for this pumping function (15). To maintain this essential level of efficiency, the enzymes, membranes, and structural elements of the heart undergo constant turnover and rebuilding. Every 30 days, an entire heart is reconstructed with brand-new protein components, using a steady supply of nutritional building blocks in the form of amino acids, lipids, and carbohydrates (16,17).

Energy transfer in the beating heart can be visualized as a system of interconnected cycles that receives nutrients through the circulation and that transfers energy from nutrients to ATP, which, in turn, is used to support cyclic contractions (Fig. 1). Under normal conditions, the system readily responds to environmental changes by either increasing or decreasing the rate of energy turnover. The schematic refers to yet another biological principle: cycles consist of moieties (e.g., blood in the circulation, enzymes in cells) that provide the necessary machinery for energy transfer.

The heart has also been described as a metabolic omnivore, because it has the capacity to oxidize fat and carbohydrates simultaneously or interchangeably for its energy needs (18,19). *Macronutrients* are defined here as fats and carbohydrates that constitute the main sources of ATP for contraction of the mammalian heart. *Micronutrients*, such as coenzyme Q10 (CoQ10), L-carnitine, thiamine, amino acids, including taurine, and other small molecules are defined as essential cofactors for energy transfer, biochemical maintenance, and physiological heart function (20,21).

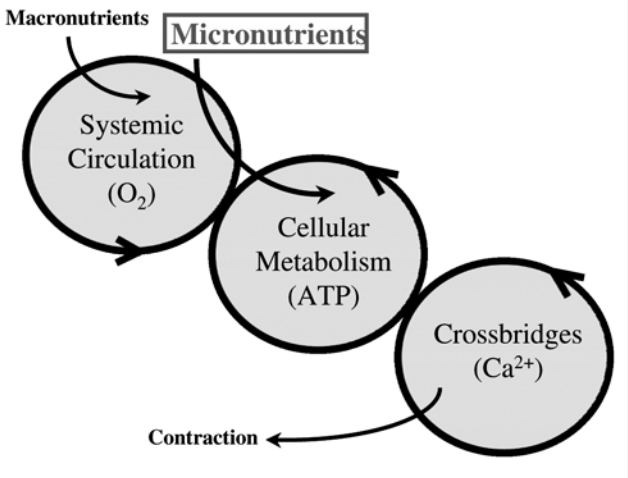


Figure 1 **Cycles of Energy Transfer and the Place of Micronutrients**

Energy transfer in the heart as a system of interconnected cycles. Micronutrients are essential components of moiety-conserved cycles in the cell. See the Normal Cardiac Metabolism section for further discussion.

Abbreviations and Acronyms

ATP = adenosine triphosphate
CoQ10 = coenzyme Q10
EF = ejection fraction
HF = heart failure
LV = left ventricle/ventricular
LVEDV = left ventricular end-diastolic volume
LVESV = left ventricular end-systolic volume
MRI = magnetic resonance imaging
NYHA = New York Heart Association

Metabolism in HF

Deficiencies in CoQ10, L-carnitine, thiamine and other B vitamins, and taurine are all well documented in the failing myocardium (22,23). Deficiencies of L-carnitine, thiamine, and taurine alone are well-established causes of cardiomyopathy (23). Animal models have similarly revealed that micronutrient deficiencies are present in HF, that genetically induced deficiencies lead to HF, and that correction of these deficits can improve heart function (24–30). Thus, in HF, it has been proposed that the human heart is deficient in adequate amounts of key nutrient cofactors or micronutrients (Fig. 1) (23,31–36). However, the heart is "not out of fuel" as it has been suggested (34), because the coronary circulation provides substrates in excess of their rate of utilization. Indeed, a large part of the morbidity of HF appears to be the result of impaired energy substrate metabolism (37). ATP turnover in the failing myocardium may be reduced by as much as 30%, although it is not clear whether the reduced rate of ATP turnover is the cause or the consequence of HF (38).

When the heart is stressed, as seen in HF, it returns to the fetal gene program, switching from the dominant fatty acid metabolism to a more efficient use of carbohydrates in an attempt to limit further damage (39–44). It is also of interest that infants have a reduced ability to biosynthesize L-carnitine and taurine. Hence, these nutrients are mandated for inclusion in infant formulas. Whether the fetal gene program contributes to the deficiencies of L-carnitine and taurine seen in HF is unknown. In any case, the early adaptation of the heart eventually becomes maladaptive as HF progresses (17,45). The evolution of HF usually occurs over a number of years; therefore, only a very small percentage of cardiomyocytes at a given time may be irreversibly injured. These myocytes retain a relatively preserved structure and are in a "vegetative state" equal to viable, but dysfunctional, myocardium as a result of metabolic imbalance, in part related to excessive and continuous activation of the neuroendocrine system (31,32,46,47). Therapy with micronutrients when deficiencies exist has the potential to prevent myocyte death and restore function.

In HF, maladaptive changes appear to occur at all steps of energy production and transfer: substrate utilization, oxidative phosphorylation, and ATP utilization (37). Inadequate energy conversion in already overworked cardiomyocytes may potentially result in cell damage or death mediated by oxidative stress, resulting in mitochondrial damage and

JACC Vol. 54, No. 18, 2009
October 27, 2009:1660–73

Table 1 Guideline Recommendations for Nutritional Support in HF

Guideline	Recommendation	Level of Evidence
ACC/AHA (7)	Routine use of nutritional supplements of unproved value and not recommended	Class III, Level of Evidence: C
HFSA (5)	Patients with HF, especially those on diuretic therapy and restricted diets, should be considered for daily multivitamin-mineral supplementation to ensure adequate intake of the recommended daily value of essential nutrients	Level of Evidence: C
ESC (4)	Omitted in 2008 guidelines	N/A
CCS (6)	CoQ10, vitamin and herbal supplements are not recommended as HF therapy	Class III, Level of Evidence: C

ACC = American College of Cardiology; AHA = American Heart Association; CCS = Canadian Cardiovascular Society; CoQ10 = coenzyme Q10; ESC = European Society of Cardiology; HF = heart failure; HFSA = Heart Failure Society of America; N/A = not available.

cytochrome c release (23,48). Decreased capacity for energy conversion makes the damaged heart more susceptible to ischemia, accelerating the process of HF. In Figure 1, it is proposed that micronutrients allow for the efficient and appropriate utilization of fuel for the preservation of normal structure and function of the heart. It should also be recognized that plasma levels of compounds such as L-carnitine, taurine, and CoQ10 may not reflect tissue levels because of large transmembrane cellular gradients (23,49–53).

The lack of sufficient micronutrients may be intensified by medical interventions. Commonly prescribed medications, such as the cholesterol-lowering HMG-CoA reductase inhibitors, have been shown to cause reductions in serum CoQ10 levels, thus potentially exacerbating already-present nutritional deficiencies and limiting long-term treatment success (54–59). Similarly, loop diuretics have been shown to substantially lower thiamine levels in patients with HF (60–63).

Micronutrient Supplementation

Micronutrients may work synergistically with standard therapies in patients with HF by correcting defects in energy metabolism and providing the failing heart with deficient cofactors that are limiting cellular energy transfer (Fig. 1). Despite the appeal of such a strategy, large clinical trials evaluating therapy with micronutrients are lacking (Table 1). However, this is a potentially promising new paradigm for the treatment of HF therapy, deserving further investigation. Micronutrient supplementation offers the opportunity to correct deficiencies in critical myocyte pathways, including those associated with the provision of ATP (CoQ10, L-carnitine, thiamine and the B vitamins, amino acids), protein production (amino acids), intracellular calcium balance (taurine), and the reduction of oxidative stress (CoQ10 and taurine).

To date, clinical research of a single micronutrient in the treatment of HF has been the usual investigative approach. However, this method may potentially shift the rate-limiting step to another pathway of the energy cascade. There are only a few studies evaluating a broader, multiple-micronutrient approach. Such an approach may be a more comprehensive method of supplementation and overcome some of the limitations with a single-supplement method. As described below, nutritional studies to date as a whole have significant design drawbacks, indicating an important need for larger, more comprehensive micronutrient trials in HF.

The Need for More Clinical Trials

The subsequent sections will summarize the existing data in this supplementation field, identify the limitations of current studies, and propose how to move forward with research to fill critical gaps in knowledge. Here, the focus is primarily on CoQ10, L-carnitine, thiamine, and taurine. These nutrients were chosen because: 1) they are known essential components for metabolic pathways that participate in energy production, myocardial calcium balance, and oxidative defenses; 2) there is evidence for a reduction in the level of each in HF; 3) there is evidence that a deficiency in each of them, alone, may result in cardiac or skeletal muscle pathology; and 4) there is possibly pathological reversal (27,64,65).

CoQ10

CoQ10, or ubiquinone, is an obligatory component of the respiratory chain in mitochondria. It serves as a carrier for electrons flowing through complexes I, II, and III. As such, CoQ10 plays an essential role in ATP formation in most tissues, including the heart, skeletal muscle, brain, kidney, and liver. CoQ10 is localized in the inner mitochondrial (and other intracellular) membrane, where it serves to stabilize these structures, control electron flow, and regulate the flow of reducing equivalents (66,67). In addition to its role in energy transfer, CoQ10 also functions as an antioxidant and protects circulating low-density lipoprotein particles from oxidation (68). Its inhibition of the mitochondrial permeability transition pore prevents the activation of apoptotic cascades and the oxidative inactivation of key proteins involved in ATP production (69,70). The diverse roles of CoQ10 in energy metabolism are important in the failing heart, where oxidation of energy-providing substrates becomes inadequate (54). Deficiency of CoQ10 can be caused by inhibition of its synthesis. Mevalonate, a precursor of CoQ10, is formed in a pathway dependent on HMG-CoA reductase. Not surprisingly, statins, which inhibit HMG-CoA reductase, have been shown to reduce the production of CoQ10 (54–58).

CoQ10 is widely available in dietary sources such as meat, poultry, and oils, which together provide an average daily intake of 5 mg (71). However, the majority of CoQ10 is produced by the human body from tyrosine and mevalonate through endogenous pathways. Trials involving CoQ10 supplementation in HF have primarily used dosage ranges from 60 to 300 mg/day. No significant toxicity has been observed in human studies, although there have been adverse side effects, including diarrhea, nausea, epigastric discomfort, and elevated liver enzyme levels. Gastrointestinal side effects are decreased by administration in divided doses no greater than 100 mg (55,72).

The first study on the role of CoQ10 in HF was published in 1976 (73). Serum and tissue levels of CoQ10 are lower in HF patients compared with normal control subjects (74,75). Additionally, the degree of CoQ10 deficiency may correlate with New York Heart Association (NYHA) functional class, left ventricular (LV) function, and mortality (76–79). This apparent deficiency of CoQ10 in HF has led to numerous trials seeking to establish the efficacy of CoQ10 supplementation in varying etiologies of HF (Table 2). These trials are promising because they have shown statistically significant improvement in a variety of functional, structural, and hemodynamic parameters, including ejection fraction (EF), stroke volume, cardiac output, pulmonary artery pressure, exercise capacity, and quality of life (72,75,80–86). Additionally, decreases in end-diastolic volume, NYHA functional class, hospital admissions, dyspnea, and fatigue have also been documented. However, results have been mixed (87,88). The largest trial conducted with CoQ10 in an HF population was not a double-blind, placebo-controlled study (72). Conversely, several of the studies showing no effect have been criticized for not achieving adequate CoQ10 serum levels, in addition to being too short in duration (87–89). Meta-analyses to evaluate the overall findings of CoQ10 trials appear to support many of the aforementioned benefits of CoQ10 in HF (90–92). In short, CoQ10 appears to be a safe and potentially effective nutritional supplement for the treatment of HF, based primarily, however, on small studies and meta-analyses. Larger trials of CoQ10 in combination with other micronutrients are needed to show a reduction in morbidity and mortality for HF patients already receiving standard therapy.

L-Carnitine

L-carnitine is an amino acid derivative synthesized primarily from the amino acids lysine and methionine (93). It plays a critical role in fatty acid transport into the mitochondria (94). Additionally, L-carnitine reverses the inhibition of pyruvate dehydrogenase, allowing for improved coupling between glycolysis and glucose oxidation (95). Genetic carnitine deficiency, secondary to a plasma membrane transporter defect, results in a cardiomyopathy (28). Lastly, an alternate form of the molecule, propionyl-L-carnitine, has a high penetration rate into myocytes, and its products can serve as a substrate of the Krebs cycle (93). Propionyl-L-carnitine has been shown to improve contractile function in the isolated working rat heart (96) and also to reduce the lactate and hydrogen burden in the hypertrophied human heart by increasing glucose oxidation (95).

L-carnitine is either supplied in the diet or produced endogenously, although daily consumption far exceeds endogenous production (97). L-carnitine deficiency in the failing heart has been well documented (36,98–100). As noted previously, infants have a reduced ability to biosynthesize L-carnitine; whether the fetal gene program contributes to the deficiency of L-carnitine seen in HF is unknown. Studies have also shown that plasma levels of L-carnitine often do not reflect the tissue level of L-carnitine because a significant intracellular-to-extracellular gradient is maintained by sodium-dependent pumps (23,100). Most trials conducted with L-carnitine supplements in HF have used doses of 1.5 to 3 g/day, which seem to be well tolerated (101–105).

There have been several promising studies evaluating the role of L-carnitine in cardiac diseases, including HF (Table 3). However, their value has been limited by their design and inconsistent results. Although multicenter trials, such as the CEDIM (L-Carnitine Ecocardiografia Digitalizzata Infarto Miocardico) study, have shown a benefit of L-carnitine on cardiac remodeling after myocardial infarction (106), less is known about L-carnitine in the treatment of HF of nonischemic causes. Supplementation of L-carnitine or its analog, propionyl-L-carnitine, in HF has led to statistically significant increases in exercise capacity, maximum exercise time, peak heart rate, and peak oxygen consumption (101–104). Small studies on hemodynamic and echocardiographic effects of supplementation also have shown promising results, reducing pulmonary artery pressure, as well as LV systolic, diastolic, left atrial, and end-diastolic dimensions (102). Improvements in EF have also been observed, albeit not consistently (102,104). Interpretation has been limited due to lack of control groups and high dropout rates in certain trials (102,103). A larger European study relied on subgroup analyses to observe an effect (101). One trial revealed a potentially interesting benefit of L-carnitine on mortality by demonstrating a significantly improved 3-year survival in patients with dilated cardiomyopathy and NYHA functional class III to IV HF (105). It is therefore proposed that both carnitine and propionyl-L-carnitine warrant further study in patients with HF.

Thiamine (Vitamin B_1) and Other B Vitamins

Thiamine (vitamin B_1) is a water-soluble B vitamin that plays an important role as a coenzyme in carbohydrate metabolism. Through the addition of magnesium and ATP, thiamine is converted to thiamine pyrophosphate by the enzyme thiamine pyrophosphokinase (107). As the metabolically active form of thiamine, thiamine pyrophosphate

Table 2 Selected Trials With CoQ10 Supplementation in HF

Study Author (Ref. #)	Year	No. of Patients	Study Design	Primary End Point	NYHA Functional Class	Results	Dose	Side Effects Related to CoQ10	Remarks
Baggio et al. (72)	1994	2,664	Post-marketing surveillance study	Assessment of clinical signs and symptoms using a 7-point scale	II–III	Improvement of at least 3 symptoms seen in 54% of patients	50–150 mg/day	None	Not a double-blind, placebo-controlled study. Doses varied. Before modern therapy was available. Used point system for clinical improvement.
Langsjoen et al. (75)	1985	19	Double-blind, placebo double-crossover	Evaluation of EF, SV, CoQ10 serum level, weight, and clinical status over 28 weeks	III–IV	↑ EF and SV, ↑ CoQ10 serum level	33.3 mg BID	None	Small size and before modern therapy
Belardinelli et al. (80)	2006	23	Double-blind, placebo-controlled crossover	At 4 weeks effects of CoQ10 and exercise training on Vo_2 max, EF, and endothelium-dependent dilation of brachial artery	II–III	Increase in Vo_2 max, increase in LVEF at rest and peak, improvement in endothelium-dependent dilation of brachial artery	100 mg TID	None	Small study size. Also evaluated patients in exercise training regimen.
Morisco et al. (81)	1993	191	Double-blind placebo-controlled	Incidence of hospitalization and life-threatening pulmonary edema	III–IV	↓ Hospitalization and pulmonary edema	2 mg/kg/day	NA	Small size and before modern therapy
Munkholm et al. (85)	1999	22	Double-blind, placebo-controlled	Right heart catheterization for RAP, RVSP, EDP, PAP, and PCWP at rest, 1, or 3 min of work done at 12 weeks	II–III	↑ Stroke index at rest and work, ↓ PAWP at rest. ↓ PCWP at work. Otherwise no changes.	100 mg BID	NA	Small study size
Keogh et al. (86)	2003	39	Double-blind, placebo-controlled	Physician-assessed NYHA symptom functional class at 3 months	II–III	Small but statistically significant improvement in NYHA functional class	150 mg/day	NA	Small study size. Treated with ACEI but not beta-blockers.
Khatta et al. (87)	2000	46	Double-blind, placebo-controlled	At 6 months change in EF, as assessed by nuclear ventriculography, and change in peak O_2 consumption	III–IV	No effect on EF, peak O_2 consumption	200 mg/day	None	Small study size, lower CoQ10 serum levels attained
Watson et al. (88)	1999	30	Double-blind, placebo-controlled crossover	At 3 months evaluation of LVEDV, LVESV, and EF. Right heart catheterization for CO and PCWP.	Not reported; EF <35%	No significant change	33 mg TID	None	Small size, used lower dose of CoQ10

ACEI = angiotensin-converting enzyme inhibitor; BID = twice daily; CO = cardiac output; EDP = end-diastolic pressure; EF = ejection fraction; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; NYHA = New York Heart Association; PAP = pulmonary artery pressure; PAWP = pulmonary artery wedge pressure; PCWP = pulmonary capillary wedge pressure; RAP = right atrial pressure; RVSP = right ventricular systolic pressure; SV = stroke volume; TID = three times daily; other abbreviations as in Table 1.

JACC Vol. 54, No. 18, 2009
October 27, 2009:1660–73

Table 3 Selected Trials With Carnitine in HF

Study Author (Ref. #)	Year	No. of Patients	Study Design	Primary End Point	NYHA Functional Class	Results	Dose	Side Effects Related to Carnitine	Remarks
Study investigators (101)	1999	353	Double-blind, placebo-controlled	At 6 months and evaluation of exercise capacity using bicycle exercise	II–III	No difference in maximum exercise duration	1 g BID	None	No BB used (only ACEI and diuretic), non-a priori subgroup analysis. ↑ Max HR in patients with higher EF (30%–40%).
Anand et al. (102)	1998	30	Single-blind, placebo-controlled	At days 1, 15, and 30 measurement of LV function, hemodynamics, hormone levels, exercise capacity, and peak O_2 consumption	II–III	No change in LV function. ↓ End-diastolic dimensions and end point septal separation. ↓ PAP and PAWP on days 1 and 30. No change in hormone levels. ↑ Peak O_2 consumption, exercise time, exercise HR.	Initial bolus 30 mg/kg, then 500 mg TID	None	Single blind and small study size
Loster et al. (103)	1999	41	Double-blind, placebo-controlled	Bicycle ergometer test to determine maximum performance, systolic and diastolic pressure, HR, and ST changes at up to 180 days	II–III	Improved performance. Trend toward improved hemodynamic parameters.	1 g TID	NA	Small size and limited end point assessments, high dropout rate
Mancini et al. (104)	1992	60	Double-blind, placebo-controlled	LVEF and maximum exercise time on ergometer bicycle at up to 180 days	II–III	↑ Maximum exercise time and EF	500 mg TID	NA	Small size, before current treatments available
Rizos (105)	2000	80	Double-blind, placebo-controlled	Measurement of LVEF, maximum exercise time, peak Vo_2 consumption, arterial and pulmonary BP, CO, and 3-year mortality	III–IV dilated cardiomyopathy	↑ 3-yr survival. Improved maximum exercise time, peak O_2 consumption, CO, arterial/pulmonary BP	2 g/day	GI discomfort in 3 patients (all completed)	Limited to dilated cardiomyopathy
Iliceto et al. (106)	1995	472	Double-blind, placebo-controlled	LV volume and EF at 12 months after MI	NA	↓ LVEDV and LVESV. No difference in LVEF.	9 g/day IV for 5 d, then 6 g/day PO for 12 months	NA	Evaluated results after acute MI. Not in HF.

BB = beta-blocker; BP = blood pressure; EDV = end-diastolic volume; ESV = end-systolic volume; GI = gastrointestinal; HR = heart rate; IV = intravenous; LV = left ventricular; MI = myocardial infarction; PO = by mouth; other abbreviations as in Tables 1 and 2.

JACC Vol. 54, No. 18, 2009
October 27, 2009:1660–73

serves as a cofactor for the pyruvate dehydrogenase complex and for transketolase, both key mediators of energy substrate metabolism. Thiamine deficiency results in decreased ATP production and increased cellular acidosis on a metabolic level (108).

Thiamine itself is stored in the body in only small amounts and cannot be produced endogenously (23). Adequate nutritional intake through diet (whole grains, legumes, nuts) or supplements is therefore critical in preventing deficiency. Doses used in trials have ranged from 1.5 to 200 mg/day.

The cardiac effects of severe thiamine deficiency are clinically referred to as wet beriberi (109). A chronic disease develops consisting of a peripherally vasodilated state that leads to fluid retention through activation of the renin-angiotensin system. The end result of thiamine deficiency is high-output HF (110). In the Western world, however, wet beriberi is rarely encountered. Instead, concern focuses on moderate thiamine deficiency in patients with HF. Animal models have shown that thiamine deficiency can lead to cardiac dysfunction, hypertrophy, and arrhythmias without the presence of beriberi (111–121). In patients with HF, the incidence of thiamine deficiency ranges from 13% to 93% (60–62,122–125). Patients with NYHA functional class III/IV HF appear to have more severe deficiency than their class I/II counterparts (60). Furosemide, commonly used in such patients, may exacerbate this problem through urinary excretion of thiamine (60–63). There also appears to be an increased need for thiamine in the setting of HF, as similar nutritional amounts are inadequate in HF patients compared with controls (60).

Given the proposed role of thiamine deficiency in HF, several small studies have been conducted to examine the clinical utility of supplementation. Thiamine deficiency appears to respond to oral supplements as small as 1.5 mg/day, as shown in 1 study of 100 hospitalized HF patients (60). In one of the most promising trials, 30 patients with HF receiving furosemide were given either thiamine (200 mg/day) or placebo (126). After only 1 week, thiamine levels had increased, with statistically significant improvements in diuresis and EF noted. By the end of the 7-week study, improvement in cardiac function was observed with a 22% increase in EF. Although results from other trials have been mixed (124,127), the known effects of thiamine deficiency on the heart suggest that supplementation may be of therapeutic benefit if examined in larger HF trials.

Riboflavin (vitamin B_2) and pyridoxine (vitamin B_6) play critical roles in carbohydrate energy metabolism and the production of red blood cells. These B vitamins, like thiamine, are water soluble, are subject to renal excretion, have limited tissue storage, and are dependent on intake. Therefore, their status may also be adversely affected by the use of loop diuretics. Indeed, similar deficiencies in HF have been reported (22). The prevalence of having a deficiency of any one B vitamin in HF was recently reported to be 68% (35). Vitamin B_{12} and folate have also been shown to be deficient in a small subset of HF patients (128). Although these vitamins have been postulated to play a role in endothelial dysfunction through homocysteine action, studies to support an effect in HF are currently lacking.

Amino Acids

Amino acids play a dual role in cardiac metabolism. First, they are the "building blocks" of proteins. Second, they are intermediary metabolites in energy substrate metabolism (16,33). A relevant role is played by taurine, which comprises 25% of the cardiomyocyte amino acid pool in humans. Taurine is not a substrate for protein synthesis or intermediary metabolism, but rather functions both as an antioxidant and as an important endogenous regulator of intracellular calcium homeostasis (129–132). Taurine modulates voltage-dependent calcium channels, sodium-calcium exchange, and sodium-taurine cotransport. The net effect is to protect heart muscle cells from calcium overload, on the one hand, and low calcium states on the other. Since myocyte calcium levels increase in HF and contribute to cellular injury, maintenance of appropriate taurine levels would seem to be critical. Taurine, like CoQ10, is also a potent antioxidant and reacts with a variety of potentially toxic intracellular aldehydes (133). Cytokine activity, particularly TNF-alpha, is increased in HF; TNF-alpha has been shown to decrease taurine levels (134). Lastly, the adverse actions of angiotensin II are potentiated in taurine-deficient cardiomyocytes (135).

Although taurine can be synthesized from methionine or cysteine, and as such is not an essential amino acid, the majority is obtained from dietary sources such as fish and milk (136,137). The heart extracts its supply through active transport by a taurine transporter (138). In infants and the elderly, taurine biosynthesis is reduced; thus, there was an increasing dependence on dietary sources (137). It is an essential ingredient in infant formulas, as taurine deficiency can produce HF. Taurine, dosed at up to 1 g 3 times per day, appears to be well tolerated (137). Indeed, large doses of taurine are a central component of many popular caffeine energy drinks.

Taurine levels are reduced in ischemic cardiomyopathy (137,139,140). Animal studies have shown that supplemental amino acids, including taurine, are beneficial in several cardiac injury and HF models (24,25,30,141,142). In humans with HF, amino acid mixtures have resulted in improvements in exercise capacity (143–145). There is only 1 small study examining taurine supplementation specifically (129). After 6 weeks, a significant improvement in EF was observed in the taurine-treated group. Additional studies are needed to confirm any beneficial effects of amino acid supplementation in HF.

Other Micronutrients

There are many other micronutrients of potential interest with respect to HF. Although the evidence surrounding them is not as robust as for the molecules we have mentioned above, and some may not play a role in energy metabolism, it is worthwhile to briefly mention them to provide a complete picture of the field.

Creatine is a key regulator of energy metabolism in all muscle tissues of the body, including the heart. Mitochondrial energy stored in the phosphate bond of ATP is transferred to phosphocreatine by creatine kinase (146). In the failing myocardium, there is evidence that this system becomes dysfunctional and that the level of deficiency may correlate with the severity of HF (147–150). There are few studies specifically examining creatine supplementation in HF, however, and analysis is complicated by its effects on skeletal muscle and the alterations in creatinine levels that occur with supplementation that confuse monitoring of renal function (151–153).

Vitamin D is a molecule that plays an important role in the homeostasis of calcium, a key player in cardiac contractility. It is also an inhibitor of renin production (154). Low vitamin D levels have been observed in HF patients (155). Early experiments have suggested that low vitamin D levels may contribute to cardiac dysfunction through both calcium-dependent and calcium-independent processes (156,157).

Multiple cations, such as magnesium, potassium, zinc, and selenium, have been associated with HF to varying degrees (158). Magnesium and potassium deficiencies have been primarily related to arrhythmias in HF, although the prognostic significance of this finding is unclear (159–161). Zinc is an antioxidant that has been found to be deficient in HF patients, although the exact importance of this finding remains unclear (162). Finally, selenium is a component of glutathione peroxidase, an antioxidant enzyme that protects against endothelial dysfunction (163). Dietary deficiency in China has been associated with a cardiomyopathy in children, and there are case reports suggesting a role of severe selenium deficiency in the development of HF after bariatric surgery in the U.S. (164).

Multiple-Micronutrient Supplementation

A limitation of many of the clinical trials reviewed here has been the use of only a single supplement. Although this allows researchers to focus on the effects of each micronutrient individually, it may potentially lead to minimal clinical outcomes if it simply shifts rate-limiting steps from one to another component of the complex energy-providing pathways. Correcting 1 deficiency thus, in theory, would unmask 1 of the many other deficiencies present. Also, the need for a given nutrient may not be apparent, as blood levels do not always reflect a deficiency or increased requirements in the diseased myocardium (e.g., L-carnitine). Therefore, given the multiple-nutrient deficits in HF patients, an alternative strategy is to use a combination of supplements to attempt to maximize clinical effects, as performed in animal experiments (27,36,165).

One study showed that cardiomyopathic hamsters had deficiencies in CoQ10, L-carnitine, and taurine at the myocardial level during the late stages of their disease (27). Supplementation for 3 months versus a placebo diet improved the actual structure of myocyte sarcomeres and mitochondria, contractility, and cardiac function. In a more recent study, rats pre-treated with this combination before coronary artery ligation exhibited markedly improved survival, cardiac function, and reduced infarct size when compared with placebo (165).

Few studies have included multiple-nutrient supplementation in human HF (Table 4). One study (79) examined the effects of a nutrient drink. Although this supplement contains many nutrients, the ones relevant to this review include CoQ10, carnitine, thiamine, and taurine. Forty-one patients with ischemic cardiomyopathy (EF <40%) scheduled for elective bypass surgery were randomized to either the nutrient supplement or a similar-tasting placebo. The groups were relatively well matched except for a slightly higher age and lower digoxin use in the control group. The patients were then followed for 40 to 45 days until their surgery, at which point LV biopsies were obtained. These tissue samples were used to confirm the primary end points: increases in myocardial levels of taurine, carnitine, and CoQ10. Indeed, tissue samples revealed 40% to 144% higher levels of each of these nutrients in the treated group. Secondary end point analysis showed a decrease in left ventricular end-diastolic volume (LVEDV) when compared with placebo and a trend toward reduced left ventricular end-systolic volume (LVESV). However, EF was unaffected. Adverse effects of the supplement were minimal. The primary complaint was gastrointestinal, possibly due to the large undivided dose of CoQ10 administered. An isolated increase in creatinine, not blood urea nitrogen, was felt to likely be secondary to the breakdown of creatine consumed through the supplement. Overall, whereas the results were promising, the study did face several limitations. The small sample size and short duration of the trial made detecting significant differences more difficult. Additionally, many patients in the study did not have clinical symptoms of HF at the onset of the trial. Thus, no comment could be made on symptom improvement with supplementation. The confirmation, however, of increased levels of certain key nutrients in the myocardium with oral administration did reaffirm the validity of such a delivery method for future trials.

Another study evaluating the role of multiple-micronutrient supplementation in elderly HF patients has also showed promising results (166). In this trial, patients with stable, ischemic HF (EF <35%) were randomized to either capsules containing multiple nutrients or placebo. Although many vitamins and minerals were administered,

JACC Vol. 54, No. 18, 2009
October 27, 2009:1660–73

Table 4 Selected Trials Utilizing Multiple-Micronutrient Supplementation in HF

Study Author (Ref. #)	Year	No. of Patients	Study Design	Primary End Point	Patient Population	Results	Dose	Side Effects Related to Supplement	Remarks
Jeejeebhoy et al. (79)	2002	41	Double-blind, placebo-controlled	Comparison of the myocardial levels of taurine, carnitine, and CoQ10 for 30–45 days	HF with CAD (EF ≤40%)	↑ Myocardial levels of carnitine, CoQ10, and taurine	CoQ10 150 mg/day, carnitine 3 g/day, thiamine 25 mg/day, taurine 3 g/day	1 patient exited study due to diarrhea	Short study duration and small size. No assessment of symptoms. Secondary end point improvement in LVED volume. No change in EF.
Witte et al. (166)	2005	30	Double-blind, placebo-controlled	Evaluation of LV function, pro-inflammatory cytokines, and QoL at end of therapy (~1 yr)	HF with CAD (EF ≤35% + fatigue or breathlessness)	↑ EF. ↓ LVEDV, LVESV. No difference in cytokine levels. Improvement in QoL scores.	CoQ10 150 mg/day, thiamine 200 mg/day	None	Small study size. Limited supplement cocktail.

CAD = coronary artery disease; QoL = quality of life; other abbreviations as in Tables 1 and 2.

the substances relevant to this review were CoQ10 and thiamine. No significant adverse effects were noted during the study. Patients were followed for 9 months, at which point the primary end point, a change in EF, was evaluated by cardiac magnetic resonance imaging (MRI). The active compound, but not placebo, significantly improved EF, with a significant decrease in LVESV and LVEDV. There was an improvement in quality-of-life scores at 6 months, reaffirming the need for long-term observation to witness improvement from supplementation. However, there were no changes in NYHA functional class or exercise capacity, which the authors attributed to the small sample size. Overall, this study did show improvement in cardiac function parameters on MRI, which is highly promising. Yet again, a larger study population would have been helpful for detecting more subtle differences, as noted by the authors. Also, the inclusion of key missing nutrients, such as carnitine and amino acids, would have minimized the chance of unmasking these deficiencies when administering a more limited supplement cocktail.

Both of the trials described here, in their own way, illustrate the promise for long-term improvement of cardiac function in HF patients when key nutrients are administered simultaneously. Future studies are needed to first confirm these results and to then lead to a better definition of the ideal regimen to maximize cardiac benefits.

Outlook and Challenges

The optimal treatment of HF remains a formidable challenge, as suggested by the disappointing outcomes of recent trials (10,59,167–169). There is a clear need for new therapies that can work synergistically with standard treatments to reverse the progression of the disease. This calls for a more detailed examination of the role of nutrition in the treatment of HF. The recent GISSI-HF (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardio–Heart Failure) trial, for example, has shown the promise of such a complementary approach with respect to fatty acids (170).

In the failing heart, the presence of viable, but dysfunctional, myocardium is relatively common and is an important predictor of improvement of systolic function (reverse remodeling) in response to medical and revascularization therapies (171–174). Certain causes of dysfunctional myocardium are known, such as stunning hibernation due to chronic ischemia (175). Perturbed energetics due to micronutrient deficiencies may also be responsible for dysfunctional myocardium. The chronic improvement or normalization of contractility of dysfunctional myocardium may be used as a surrogate end point to assess the effects of micronutrients in HF (171,173,174,176).

An underexplored therapeutic field involves micronutrients and their effects on the energy dynamics of the heart. The nutritional studies summarized here show interesting results in the treatment of HF. It is well known that the

Table 5 Limitations of Existing Trials and Suggested Improvements in Methodology

Pitfalls in Previous Study Methodologies	Suggested Improvements
Supplementation of only a single nutrient	Supplementation of multiple nutrients previously studied individually
Small sample size (largest multinutrient trial has 41 patients)	Large patient population in a double-blind, placebo-controlled trial
No pre-trial assessment of viable, but dysfunctional, myocardium	Use of MRI or other modalities to assess for viable, but dysfunctional, myocardium pre-trial and at specified intervals thereafter
Conducted prior to the use of current standard-of-care therapy for HF	Use of current guideline-directed standard-of-care therapy, including ACEI and BB
Short duration of study	Long-term outcome trial (>1 yr)
Evaluation of limited end points	Evaluation of symptom improvement, cardiac structure/function (e.g., reverse remodeling, EF), and survival

MRI = magnetic resonance imaging; other abbreviations as in Tables 1, 2, and 3.

heart has unique and significant energy requirements due to its mechanical pumping function and that a failing heart is in a perpetual state of increased stress due to neurohormonal activation. Defects in complex pathways, such as those involved in ATP production and driving calcium cycling, result in further energy imbalances and lead to additional contractile impairment in HF. Results from animal models and small human trials suggest a potential cardiac benefit to correcting micronutrient deficiencies. To varying degrees, CoQ10, L-carnitine, thiamine, taurine, and amino acids have all been shown to improve functional, structural, and hemodynamic parameters in HF patients, mostly by improving flux through the cycles of energy transfer in the heart (Fig. 1). Micronutrients have been shown to improve EF (90,91,104,126,129). However, there are substantial limitations to the research in its current state. First, studies have often been small, with inconsistent end points, and short in duration. A few trials were not randomized, and most did not examine key end points such as improvement in pump function and clinical outcomes. Lastly, the focus on replenishment of only a single nutrient has possibly masked therapeutic effects by simply shifting rate-limiting steps to other energy pathways. Given the complex nature of myocardial energy metabolism (19), it is not likely that a single substance alone will be able to reverse the widespread deficits present in HF, similar to a starving individual requiring diverse nutrition from multiple food groups for survival. These design limitations have together contributed to the often inconsistent results evident in the existing literature (Table 5). Animal and human studies that have attempted to overcome the single-nutrient restriction have yielded interesting, but not conclusive, results (79,166). Current advances in imaging technology (e.g., MRI, echocardiography) have also not yet been adequately utilized in this field (171). No single trial conducted to date has met the necessary criteria to demonstrate a significant clinical benefit when 1 or more micronutrients were added to standard therapy for HF (Table 5). Such a study should show conclusive improvement in important outcomes such as hospitalizations and mortality in HF when micronutrients are added to standard therapy. Compared with the landmark multicenter trials of existing HF therapies, the studies of nutritional supplementation are in a relative state of infancy (177–179). If proven to be beneficial, the widespread availability of these individual nutritional compounds from generic manufacturers may offer patients a lower-cost therapy not burdened by development and marketing expenses.

In summary, the potential importance of any novel therapy in a disease with increasing prevalence, such as HF, necessitates a timely and detailed examination of efficacy in a clinical trial population. The unmet energy requirements of the failing heart have been carefully detailed, and the molecules controlling energy and substrate utilization are equally recognized from extensive laboratory work. Unfortunately, the effort dedicated up to now to testing the theoretical benefits of these micronutrients in a scientifically rigorous clinical trial fashion has been insufficient to yield any conclusions that can be applied to widespread practice.

Reprint requests and correspondence: Dr. Heinrich Taegtmeyer, Department of Internal Medicine, Division of Cardiology, University of Texas Houston Medical School, 6431 Fannin, MSB 1.246, Houston, Texas 77030. E-mail: Heinrich.Taegtmeyer@uth.tmc.edu.

REFERENCES

1. McCullough PA, Philbin EF, Spertus JA, Kaatz S, Sandberg KR, Weaver WD. Confirmation of a heart failure epidemic: findings from the Resource Utilization Among Congestive Heart Failure (REACH) study. J Am Coll Cardiol 2002;39:60–9.
2. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics—2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25–146.
3. O'Connell JB. The economic burden of heart failure. Clin Cardiol 2000;23:III6–10.
4. Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). Eur Heart J 2008;29:2388–442.
5. Heart Failure Society of America. HFSA 2006 comprehensive heart failure practice guideline. J Card Fail 2006;12:e1–2.
6. Arnold JM, Liu P, Demers C, et al. Canadian Cardiovascular Society consensus conference recommendations on heart failure 2006: diagnosis and management. Can J Cardiol 2006;22:23–45.
7. Hunt SA, Abraham WT, Chin MH, et al. 2009 focused update incorporated into the ACC/AHA 2005 guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2009;53:e1–90.

8. Fang J, Mensah GA, Croft JB, Keenan NL. Heart failure-related hospitalization in the U.S., 1979 to 2004. J Am Coll Cardiol 2008;52:428–34.
9. Ahmed A, Allman RM, Fonarow GC, et al. Incident heart failure hospitalization and subsequent mortality in chronic heart failure: a propensity-matched study. J Card Fail 2008;14:211–8.
10. Gheorghiade M, Pang PS. Acute heart failure syndromes. J Am Coll Cardiol 2009;53:557–73.
11. Butler J, Kalogeropoulos A. Worsening heart failure hospitalization epidemic we do not know how to prevent and we do not know how to treat! J Am Coll Cardiol 2008;52:435–7.
12. Taegtmeyer H, Razeghi P, Young ME. Alterations in Cardiac Metabolism in Heart Failure. Philadelphia, PA: Saunders, 2004.
13. Taegtmeyer H. Cardiac metabolism as a target for the treatment of heart failure. Circulation 2004;110:894–6.
14. Kumar V, Abbas AK, Fausto N, Robbins SL, Cotran RS. Robbins and Cotran Pathologic Basis of Disease. 7th edition. Philadelphia, PA: Elsevier Saunders, 2005.
15. Ingwall JS. ATP and the Heart. Boston, MA: Kluwer Academic, 2002:16.
16. Taegtmeyer H, Harinstein ME, Gheorghiade M. More than bricks and mortar: comments on protein and amino acid metabolism in the heart. Am J Cardiol 2008;101:3E–7E.
17. Razeghi P, Taegtmeyer H. Cardiac remodeling: UPS lost in transit. Circ Res 2005;97:964–6.
18. Taegtmeyer H. Carbohydrate interconversions and energy production. Circulation 1985;72:IV1–8.
19. Taegtmeyer H. Energy metabolism of the heart: from basic concepts to clinical applications. Curr Probl Cardiol 1994;19:59–113.
20. Taegtmeyer H, Hems R, Krebs HA. Utilization of energy-providing substrates in the isolated working rat heart. Biochem J 1980;186:701–11.
21. Witte KK, Clark AL. Micronutrients and their supplementation in chronic cardiac failure. An update beyond theoretical perspectives. Heart Fail Rev 2006;11:65–74.
22. Keith ME, Walsh NA, Darling PB, et al. B-vitamin deficiency in hospitalized patients with heart failure. J Am Diet Assoc 2009;109:1406–10.
23. Sole MJ, Jeejeebhoy KN. Conditioned nutritional requirements and the pathogenesis and treatment of myocardial failure. Curr Opin Clin Nutr Metab Care 2000;3:417–24.
24. Azari J, Brumbaugh P, Barbeau A, Huxtable R. Taurine decreases lesion severity in the hearts of cardiomyopathic hamsters. Can J Neurol Sci 1980;7:435–40.
25. Hamaguchi T, Azuma J, Awata N, et al. Reduction of doxorubicin-induced cardiotoxicity in mice by taurine. Res Commun Chem Pathol Pharmacol 1988;59:21–30.
26. Ito T, Kimura Y, Uozumi Y, et al. Taurine depletion caused by knocking out the taurine transporter gene leads to cardiomyopathy with cardiac atrophy. J Mol Cell Cardiol 2008;44:927–37.
27. Keith ME, Ball A, Jeejeebhoy KN, et al. Conditioned nutritional deficiencies in the cardiomyopathic hamster heart. Can J Cardiol 2001;17:449–58.
28. Scholte HR, Rodrigues Pereira R, de Jonge PC, et al. Primary carnitine deficiency. J Clin Chem Clin Biochem 1990;28:351–7.
29. Takahashi R, Asai T, Murakami H, et al. Pressure overload-induced cardiomyopathy in heterozygous carrier mice of carnitine transporter gene mutation. Hypertension 2007;50:497–502.
30. Takihara K, Azuma J, Awata N, et al. Beneficial effect of taurine in rabbits with chronic congestive heart failure. Am Heart J 1986;112:1278–84.
31. Anker SD, Coats AJ. Cardiac cachexia: a syndrome with impaired survival and immune and neuroendocrine activation. Chest 1999;115:836–47.
32. Anker SD, Steinborn W, Strassburg S. Cardiac cachexia. Ann Med 2004;36:518–29.
33. Kalantar-Zadeh K, Block G, Horwich T, Fonarow GC. Reverse epidemiology of conventional cardiovascular risk factors in patients with chronic heart failure. J Am Coll Cardiol 2004;43:1439–44.
34. Neubauer S. The failing heart—an engine out of fuel. N Engl J Med 2007;356:1140–51.
35. Allard ML, Jeejeebhoy KN, Sole MJ. The management of conditioned nutritional requirements in heart failure. Heart Fail Rev 2006;11:75–82.
36. Sole MJ, Jeejeebhoy KN. Conditioned nutritional requirements: therapeutic relevance to heart failure. Herz 2002;27:174–8.
37. Olson RE, Schwartz WB. Myocardial metabolism in congestive heart failure. Medicine (Baltimore) 1951;30:21–41.
38. Ingwall JS. On the hypothesis that the failing heart is energy starved: lessons learned from the metabolism of ATP and creatine. Curr Hypertens Rep 2006;8:457–64.
39. Remondino A, Rosenblatt-Velin N, Montessuit C, et al. Altered expression of proteins of metabolic regulation during remodeling of the left ventricle after myocardial infarction. J Mol Cell Cardiol 2000;32:2025–34.
40. Taegtmeyer H, Wilson CR, Razeghi P, Sharma S. Metabolic energetics and genetics in the heart. Ann N Y Acad Sci 2005;1047:208–18.
41. Rajabi M, Kassiotis C, Razeghi P, Taegtmeyer H. Return to the fetal gene program protects the stressed heart: a strong hypothesis. Heart Fail Rev 2007;12:331–43.
42. Depre C, Shipley GL, Chen W, et al. Unloaded heart in vivo replicates fetal gene expression of cardiac hypertrophy. Nat Med 1998;4:1269–75.
43. Doenst T, Goodwin GW, Cedars AM, Wang M, Stepkowski S, Taegtmeyer H. Load-induced changes in vivo alter substrate fluxes and insulin responsiveness of rat heart in vitro. Metabolism 2001;50:1083–90.
44. Nascimben L, Ingwall JS, Lorell BH, et al. Mechanisms for increased glycolysis in the hypertrophied rat heart. Hypertension 2004;44:662–7.
45. Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH, Taegtmeyer H. Metabolic gene expression in fetal and failing human heart. Circulation 2001;104:2923–31.
46. Brixius K, Reuter H, Bloch W, Schwinger RH. Altered hetero- and homeometric autoregulation in the terminally failing human heart. Eur J Heart Fail 2005;7:29–35.
47. Tang WH, Francis GS. Neurohormonal upregulation in heart failure. Heart Fail Clin 2005;1:1–9.
48. Zhang M, Shah AM. Role of reactive oxygen species in myocardial remodeling. Curr Heart Fail Rep 2007;4:26–30.
49. Korang K, Milakofsky L, Hare TA, Hofford JM, Vogel WH. Levels of taurine, amino acids and related compounds in plasma, vena cava, aorta and heart of rats after taurine administration. Pharmacology 1996;52:263–70.
50. Barshop BA, Gangoiti JA. Analysis of coenzyme Q in human blood and tissues. Mitochondrion 2007;7 Suppl:S89–93.
51. Debska S, Kawecka A, Wojnarowski K, et al. Correlation between plasma carnitine, muscle carnitine and glycogen levels in maintenance hemodialysis patients. Int J Artif Organs 2000;23:90–6.
52. Harper P, Wadstrom C, Cederblad G. Carnitine measurements in liver, muscle tissue, and blood in normal subjects. Clin Chem 1993;39:592–9.
53. Steele PE, Tang PH, DeGrauw AJ, Miles MV. Clinical laboratory monitoring of coenzyme Q10 use in neurologic and muscular diseases. Am J Clin Pathol 2004;121 Suppl:S113–20.
54. Pepe S, Marasco SF, Haas SJ, Sheeran FL, Krum H, Rosenfeldt FL. Coenzyme Q10 in cardiovascular disease. Mitochondrion 2007;7 Suppl:S154–67.
55. Sarter B. Coenzyme Q10 and cardiovascular disease: a review. J Cardiovasc Nurs 2002;16:9–20.
56. Strey CH, Young JM, Molyneux SL, et al. Endothelium-ameliorating effects of statin therapy and coenzyme Q10 reductions in chronic heart failure. Atherosclerosis 2005;179:201–6.
57. Langsjoen PH, Langsjoen AM. The clinical use of HMG CoA-reductase inhibitors and the associated depletion of coenzyme Q10. A review of animal and human publications. Biofactors 2003;18:101–11.
58. Ghirlanda G, Oradei A, Manto A, et al. Evidence of plasma CoQ10-lowering effect by HMG-CoA reductase inhibitors: a double-blind, placebo-controlled study. J Clin Pharmacol 1993;33:226–9.
59. Gissi HFI, Tavazzi L, Maggioni AP, et al. Effect of rosuvastatin in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. Lancet 2008;372:1231–9.
60. Hanninen SA, Darling PB, Sole MJ, Barr A, Keith ME. The prevalence of thiamin deficiency in hospitalized patients with congestive heart failure. J Am Coll Cardiol 2006;47:354–61.

61. Seligmann H, Halkin H, Rauchfleisch S, et al. Thiamine deficiency in patients with congestive heart failure receiving long-term furosemide therapy: a pilot study. Am J Med 1991;91:151–5.
62. Zenuk C, Healey J, Donnelly J, Vaillancourt R, Almalki Y, Smith S. Thiamine deficiency in congestive heart failure patients receiving long term furosemide therapy. Can J Clin Pharmacol 2003;10:184–8.
63. Yui Y, Itokawa Y, Kawai C. Furosemide-induced thiamine deficiency. Cardiovasc Res 1980;14:537–40.
64. Harinstein ME, Berliner JI, Shah SJ, Taegtmeyer H, Gheorghiade M. Normalization of ejection fraction and resolution of symptoms in chronic severe heart failure is possible with modern medical therapy: clinical observations in 11 patients. Am J Ther 2008;15:206–13.
65. Razeghi P, Myers TJ, Frazier OH, Taegtmeyer H. Reverse remodeling of the failing human heart with mechanical unloading. Emerging concepts and unanswered questions. Cardiology 2002;98:167–74.
66. Crane FL, Sun IL, Crowe RA, Alcain FJ, Low H. Coenzyme Q10, plasma membrane oxidase and growth control. Mol Aspects Med 1994;15 Suppl:s1–11.
67. Villalba JM, Navarro F, Cordoba F, et al. Coenzyme Q reductase from liver plasma membrane: purification and role in trans-plasma-membrane electron transport. Proc Natl Acad Sci U S A 1995;92:4887–91.
68. Littarru GP, Tiano L. Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. Mol Biotechnol 2007;37:31–7.
69. Di Lisa F, Canton M, Menabo R, Dodoni G, Bernardi P. Mitochondria and reperfusion injury. The role of permeability transition. Basic Res Cardiol 2003;98:235–41.
70. Papucci L, Schiavone N, Witort E, et al. Coenzyme q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property. J Biol Chem 2003;278:28220–8.
71. Weber C, Bysted A, Holmer G. Coenzyme Q10 in the diet—daily intake and relative bioavailability. Mol Aspects Med 1997;18 Suppl:S251–4.
72. Baggio E, Gandini R, Plancher AC, Passeri M, Carmosino G. Italian multicenter study on the safety and efficacy of coenzyme Q10 as adjunctive therapy in heart failure. CoQ10 Drug Surveillance Investigators. Mol Aspects Med 1994;15 Suppl:s287–94.
73. Ishiyama T, Morita Y, Toyama S, Yamagami T, Tsukamoto N. A clinical study of the effect of coenzyme Q on congestive heart failure. Jpn Heart J 1976;17:32–42.
74. Folkers K, Langsjoen P, Langsjoen PH. Therapy with coenzyme Q10 of patients in heart failure who are eligible or ineligible for a transplant. Biochem Biophys Res Commun 1992;182:247–53.
75. Langsjoen PH, Vadhanavikit S, Folkers K. Response of patients in classes III and IV of cardiomyopathy to therapy in a blind and crossover trial with coenzyme Q10. Proc Natl Acad Sci U S A 1985;82:4240–4.
76. Folkers K, Vadhanavikit S, Mortensen SA. Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q10. Proc Natl Acad Sci U S A 1985;82:901–4.
77. Jameson S. Statistical data support prediction of death within 6 months on low levels of coenzyme Q10 and other entities. Clin Investig 1993;71:S137–9.
78. Molyneux SL, Florkowski CM, George PM, et al. Coenzyme Q10: an independent predictor of mortality in chronic heart failure. J Am Coll Cardiol 2008;52:1435–41.
79. Jeejeebhoy F, Keith M, Freeman M, et al. Nutritional supplementation with MyoVive repletes essential cardiac myocyte nutrients and reduces left ventricular size in patients with left ventricular dysfunction. Am Heart J 2002;143:1092–100.
80. Belardinelli R, Mucaj A, Lacalaprice F, et al. Coenzyme Q10 and exercise training in chronic heart failure. Eur Heart J 2006;27:2675–81.
81. Morisco C, Trimarco B, Condorelli M. Effect of coenzyme Q10 therapy in patients with congestive heart failure: a long-term multicenter randomized study. Clin Investig 1993;71:S134–6.
82. Langsjoen H, Langsjoen P, Willis R, Folkers K. Usefulness of coenzyme Q10 in clinical cardiology: a long-term study. Mol Aspects Med 1994;15 Suppl:s165–75.
83. Langsjoen PH, Folkers K. Isolated diastolic dysfunction of the myocardium and its response to CoQ10 treatment. Clin Investig 1993;71:S140–4.
84. Langsjoen PH, Langsjoen A, Willis R, Folkers K. Treatment of hypertrophic cardiomyopathy with coenzyme Q10. Mol Aspects Med 1997;18 Suppl:S145–51.
85. Munkholm H, Hansen HH, Rasmussen K. Coenzyme Q10 treatment in serious heart failure. Biofactors 1999;9:285–9.
86. Keogh A, Fenton S, Leslie C, et al. Randomised double-blind, placebo-controlled trial of coenzyme Q_1 therapy in class II and III systolic heart failure. Heart Lung Circ 2003;12:135–41.
87. Khatta M, Alexander BS, Krichten CM, et al. The effect of coenzyme Q10 in patients with congestive heart failure. Ann Intern Med 2000;132:636–40.
88. Watson PS, Scalia GM, Galbraith A, Burstow DJ, Bett N, Aroney CN. Lack of effect of coenzyme Q on left ventricular function in patients with congestive heart failure. J Am Coll Cardiol 1999;33:1549–52.
89. Sinatra ST. Coenzyme Q10 and congestive heart failure. Ann Intern Med 2000;133:745–6.
90. Sander S, Coleman CI, Patel AA, Kluger J, White CM. The impact of coenzyme Q10 on systolic function in patients with chronic heart failure. J Card Fail 2006;12:464–72.
91. Soja AM, Mortensen SA. Treatment of congestive heart failure with coenzyme Q10 illuminated by meta-analyses of clinical trials. Mol Aspects Med 1997;18 Suppl:S159–68.
92. Mortensen SA. Overview on coenzyme Q10 as adjunctive therapy in chronic heart failure. Rationale, design and end-points of "Q-symbio"—a multinational trial. Biofactors 2003;18:79–89.
93. Siliprandi N, Di Lisa F, Menabo R. Propionyl-L-carnitine: biochemical significance and possible role in cardiac metabolism. Cardiovasc Drugs Ther 1991;5 Suppl 1:11–5.
94. Arsenian MA. Carnitine and its derivatives in cardiovascular disease. Prog Cardiovasc Dis 1997;40:265–86.
95. Schonekess BO, Allard MF, Lopaschuk GD. Propionyl L-carnitine improvement of hypertrophied heart function is accompanied by an increase in carbohydrate oxidation. Circ Res 1995;77:726–34.
96. Russell RR 3rd, Mommessin JI, Taegtmeyer H. Propionyl-L-carnitine-mediated improvement in contractile function of rat hearts oxidizing acetoacetate. Am J Physiol 1995;268:H441–7.
97. Kendler BS. Supplemental conditionally essential nutrients in cardiovascular disease therapy. J Cardiovasc Nurs 2006;21:9–16.
98. Masumura Y, Kobayashi A, Yamazaki N. Myocardial free carnitine and fatty acylcarnitine levels in patients with chronic heart failure. Jpn Circ J 1990;54:1471–6.
99. Regitz V, Shug AL, Fleck E. Defective myocardial carnitine metabolism in congestive heart failure secondary to dilated cardiomyopathy and to coronary, hypertensive and valvular heart diseases. Am J Cardiol 1990;65:755–60.
100. Siliprandi N, Di Lisa F, Pivetta A, Miotto G, Siliprandi D. Transport and function of L-carnitine and L-propionylcarnitine: relevance to some cardiomyopathies and cardiac ischemia. Z Kardiol 1987;76 Suppl 5:34–40.
101. The Investigators of the Study on Propionyl-L-Carnitine in Chronic Heart Failure. Study on propionyl-L-carnitine in chronic heart failure. Eur Heart J 1999;20:70–6.
102. Anand I, Chandrashekhan Y, De Giuli F, et al. Acute and chronic effects of propionyl-L-carnitine on the hemodynamics, exercise capacity, and hormones in patients with congestive heart failure. Cardiovasc Drugs Ther 1998;12:291–9.
103. Loster H, Miehe K, Punzel M, Stiller O, Pankau H, Schauer J. Prolonged oral L-carnitine substitution increases bicycle ergometer performance in patients with severe, ischemically induced cardiac insufficiency. Cardiovasc Drugs Ther 1999;13:537–46.
104. Mancini M, Rengo F, Lingetti M, Sorrentino GP, Nolfe G. Controlled study on the therapeutic efficacy of propionyl-L-carnitine in patients with congestive heart failure. Arzneimittelforschung 1992;42:1101–4.
105. Rizos I. Three-year survival of patients with heart failure caused by dilated cardiomyopathy and L-carnitine administration. Am Heart J 2000;139:S120–3.
106. Iliceto S, Scrutinio D, Bruzzi P, et al. Effects of L-carnitine administration on left ventricular remodeling after acute anterior myocardial infarction: the L-Carnitine Ecocardiografia Digitalizzata Infarto Miocardico (CEDIM) trial. J Am Coll Cardiol 1995;26:380–7.

107. Voskoboyev AI, Ostrovsky YM. Thiamin pyrophosphokinase: structure, properties, and role in thiamin metabolism. Ann N Y Acad Sci 1982;378:161–76.
108. Pannunzio P, Hazell AS, Pannunzio M, Rao KV, Butterworth RF. Thiamine deficiency results in metabolic acidosis and energy failure in cerebellar granule cells: an in vitro model for the study of cell death mechanisms in Wernicke's encephalopathy. J Neurosci Res 2000;62: 286–92.
109. Sica DA. Loop diuretic therapy, thiamine balance, and heart failure. Congest Heart Fail 2007;13:244–7.
110. Roman GC. Nutritional disorders of the nervous system. In: Shils ME, Shike M, editors. Modern Nutrition in Health and Disease. 10th edition. Philadelphia, PA: Lippincott Williams & Wilkins, 2006:1368–9.
111. Yoshitoshi Y, Shibata N, Yamashita S. Experimental studies on the beriberi heart. I. Cardiac lesions in thiamine deficient rats. Jpn Heart J 1961;2:42–64.
112. Singleton CK, Martin PR. Molecular mechanisms of thiamine utilization. Curr Mol Med 2001;1:197–207.
113. Iwata H. Catecholamine metabolism in thiamine-deficient rats. J Nutr Sci Vitaminol (Tokyo) 1976;22 Suppl:25–7.
114. Boullin DJ. Pharmacological responses of thiamine-deficient rat tissues. Br J Pharmacol Chemother 1963;20:190–203.
115. Yamashita S. Relationship between thiamine deficient cardiac lesions and myocardial and blood pyruvic acid contents. Jpn Heart J 1971;12:474–85.
116. Aldinger EE. Effect of thiamine deficiency on potential myocardial contractility. Circ Res 1965;16:238–43.
117. Hirota Y, Kane RL, Abelmann WH. Cardiovascular effects of exercise in hamsters with experimental thiamine deficiency. Jpn Circ J 1979;43:99–106.
118. Cohen EM, Abelmann WH, Messer JV, Bing HL. Mechanical properties of rat cardiac muscle during experimental thiamine deficiency. Am J Physiol 1976;231:1390–4.
119. Cappelli V, Bottinelli R, Polla B, Reggiani C. Altered contractile properties of rat cardiac muscle during experimental thiamine deficiency and food deprivation. J Mol Cell Cardiol 1990;22:1095–106.
120. Davies MJ, Jennings RB. The ultrastructure of the myocardium in the thiamine-deficient rat. J Pathol 1970;102:87–95.
121. Zangen A, Shainberg A. Thiamine deficiency in cardiac cells in culture. Biochem Pharmacol 1997;54:575–82.
122. Brady JA, Rock CL, Horneffer MR. Thiamin status, diuretic medications, and the management of congestive heart failure. J Am Diet Assoc 1995;95:541–4.
123. Kwok T, Falconer-Smith JF, Potter JF, Ives DR. Thiamine status of elderly patients with cardiac failure. Age Ageing 1992;21:67–71.
124. Pfitzenmeyer P, Guilland JC, d'Athis P, Petit-Marnier C, Gaudet M. Thiamine status of elderly patients with cardiac failure including the effects of supplementation. Int J Vitam Nutr Res 1994;64:113–8.
125. Yue QY, Beermann B, Lindstrom B, Nyquist O. No difference in blood thiamine diphosphate levels between Swedish Caucasian patients with congestive heart failure treated with furosemide and patients without heart failure. J Intern Med 1997;242:491–5.
126. Shimon I, Almog S, Vered Z, et al. Improved left ventricular function after thiamine supplementation in patients with congestive heart failure receiving long-term furosemide therapy. Am J Med 1995;98: 485–90.
127. Smithline HA. Thiamine for the treatment of acute decompensated heart failure. Am J Emerg Med 2007;25:124–6.
128. Witte KK, Desilva R, Chattopadhyay S, Ghosh J, Cleland JG, Clark AL. Are hematinic deficiencies the cause of anemia in chronic heart failure? Am Heart J 2004;147:924–30.
129. Azuma J, Sawamura A, Awata N. Usefulness of taurine in chronic congestive heart failure and its prospective application. Jpn Circ J 1992;56:95–9.
130. Satoh H, Sperelakis N. Review of some actions of taurine on ion channels of cardiac muscle cells and others. Gen Pharmacol 1998;30: 451–63.
131. Kramer JH, Chovan JP, Schaffer SW. Effect of taurine on calcium paradox and ischemic heart failure. Am J Physiol 1981;240: H238–46.
132. Schaffer SW, Kramer J, Chovan JP. Regulation of calcium homeostasis in the heart by taurine. Fed Proc 1980;39:2691–4.
133. Ogarsawa M, Nakamura T, Koyoma I, Nemoto M, Yoshida T. Reactivity of taurine with aldehydes and its physiologic role. In: Huxtable R, Michallk DV, editors. Taurine in Health and Disease. Advances in Experimental Biology. New York, NY: Plenum, 1994.
134. Grimble RF, Jackson AA, Persaud C, Wride MJ, Delers F, Engler R. Cysteine and glycine supplementation modulate the metabolic response to tumor necrosis factor alpha in rats fed a low protein diet. J Nutr 1992;122:2066–73.
135. Schaffer S, Solodushko V, Pastukh V, Ricci C, Azuma J. Possible cause of taurine-deficient cardiomyopathy: potentiation of angiotensin II action. J Cardiovasc Pharmacol 2003;41:751–9.
136. Huxtable RJ, Chubb J, Azari J. Physiological and experimental regulation of taurine content in the heart. Fed Proc 1980;39:2685–90.
137. Schuller-Levis GB, Park E. Taurine: new implications for an old amino acid. FEMS Microbiol Lett 2003;226:195–202.
138. Ganapathy V, Leibach FH. Expression and regulation of the taurine transporter in cultured cell lines of human origin. Adv Exp Med Biol 1994;359:51–7.
139. Crass MF 3rd, Lombardini JB. Loss of cardiac muscle taurine after acute left ventricular ischemia. Life Sci 1977;21:951–8.
140. Schaffer SW, Allo S, Mozaffari M. Potentiation of myocardial ischemic injury by drug-induced taurine depletion. Adv Exp Med Biol 1987;217:151–8.
141. Corsetti G, Pasini E, D'Antona G, et al. Morphometric changes induced by amino acid supplementation in skeletal and cardiac muscles of old mice. Am J Cardiol 2008;101:26E–34E.
142. Pasini E, Scarabelli TM, D'Antona G, Dioguardi FS. Effect of amino acid mixture on the isolated ischemic heart. Am J Cardiol 2004;93:30A–34A.
143. Scognamiglio R, Testa A, Aquilani R, Dioguardi FS, Pasini E. Impairment in walking capacity and myocardial function in the elderly: is there a role for nonpharmacologic therapy with nutritional amino acid supplements? Am J Cardiol 2008;101:78E–81E.
144. Aquilani R, Viglio S, Iadarola P, et al. Oral amino acid supplements improve exercise capacities in elderly patients with chronic heart failure. Am J Cardiol 2008;101:104E–10E.
145. Aquilani R, Opasich C, Gualco A, et al. Adequate energy-protein intake is not enough to improve nutritional and metabolic status in muscle-depleted patients with chronic heart failure. Eur J Heart Fail 2008;10:1127–35.
146. Brosnan JT, Brosnan ME. Creatine: endogenous metabolite, dietary, and therapeutic supplement. Annu Rev Nutr 2007;27:241–61.
147. Beer M, Seyfarth T, Sandstede J, et al. Absolute concentrations of high-energy phosphate metabolites in normal, hypertrophied, and failing human myocardium measured noninvasively with (31)P-SLOOP magnetic resonance spectroscopy. J Am Coll Cardiol 2002; 40:1267–74.
148. Neubauer S, Horn M, Cramer M, et al. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. Circulation 1997;96:2190–6.
149. Shen W, Spindler M, Higgins MA, et al. The fall in creatine levels and creatine kinase isozyme changes in the failing heart are reversible: complex post-transcriptional regulation of the components of the CK system. J Mol Cell Cardiol 2005;39:537–44.
150. Smith CS, Bottomley PA, Schulman SP, Gerstenblith G, Weiss RG. Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium. Circulation 2006;114:1151–8.
151. Ferraro S, Codella C, Palumbo F, et al. Hemodynamic effects of creatine phosphate in patients with congestive heart failure: a double-blind comparison trial versus placebo. Clin Cardiol 1996;19: 699–703.
152. Gordon A, Hultman E, Kaijser L, et al. Creatine supplementation in chronic heart failure increases skeletal muscle creatine phosphate and muscle performance. Cardiovasc Res 1995;30:413–8.
153. Kuethe F, Krack A, Richartz BM, Figulla HR. Creatine supplementation improves muscle strength in patients with congestive heart failure. Pharmazie 2006;61:218–22.
154. Weber KT, Simpson RU, Carbone LD. Vitamin D and calcium dyshomoeostasis-associated heart failure. Heart 2008;94:540–1.
155. Zittermann A, Schleithoff SS, Tenderich G, Berthold HK, Korfer R, Stehle P. Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure? J Am Coll Cardiol 2003;41: 105–12.

156. Maiya S, Sullivan I, Allgrove J, et al. Hypocalcaemia and vitamin D deficiency: an important, but preventable, cause of life-threatening infant heart failure. Heart 2008;94:581–4.
157. Weishaar RE, Simpson RU. Involvement of vitamin D3 with cardiovascular function. II. Direct and indirect effects. Am J Physiol 1987;253:E675–83.
158. Weber KT, Weglicki WB, Simpson RU. Macro- and micronutrient dyshomeostasis in the adverse structural remodelling of myocardium. Cardiovasc Res 2009;81:500–8.
159. Eichhorn EJ, Tandon PK, DiBianco R, et al. Clinical and prognostic significance of serum magnesium concentration in patients with severe chronic congestive heart failure: the PROMISE Study. J Am Coll Cardiol 1993;21:634–40.
160. Gottlieb SS, Baruch L, Kukin ML, Bernstein JL, Fisher ML, Packer M. Prognostic importance of the serum magnesium concentration in patients with congestive heart failure. J Am Coll Cardiol 1990;16: 827–31.
161. Iezhitsa IN. Potassium and magnesium depletions in congestive heart failure—pathophysiology, consequences and replenishment. Clin Calcium 2005;15:123–33.
162. Ripa S, Ripa R, Giustiniani S. Are failured cardiomyopathies a zinc-deficit related disease? A study on Zn and Cu in patients with chronic failured dilated and hypertrophic cardiomyopathies. Minerva Med 1998;89:397–403.
163. de Lorgeril M, Salen P. Selenium and antioxidant defenses as major mediators in the development of chronic heart failure. Heart Fail Rev 2006;11:13–7.
164. Boldery R, Fielding G, Rafter T, Pascoe AL, Scalia GM. Nutritional deficiency of selenium secondary to weight loss (bariatric) surgery associated with life-threatening cardiomyopathy. Heart Lung Circ 2007;16:123–6.
165. Briet F, Keith M, Leong-Poi H, et al. Triple nutrient supplementation improves survival, infarct size and cardiac function following myocardial infarction in rats. Nutr Metab Cardiovasc Dis 2008;18: 691–9.
166. Witte KK, Nikitin NP, Parker AC, et al. The effect of micronutrient supplementation on quality-of-life and left ventricular function in elderly patients with chronic heart failure. Eur Heart J 2005;26: 2238–44.
167. Fox K, Ford I, Steg PG, Tendera M, Ferrari R. Ivabradine for patients with stable coronary artery disease and left-ventricular systolic dysfunction (BEAUTIFUL): a randomised, double-blind, placebo-controlled trial. Lancet 2008;372:807–16.
168. Gheorghiade M, Konstam MA, Burnett JC Jr., et al. Short-term clinical effects of tolvaptan, an oral vasopressin antagonist, in patients hospitalized for heart failure: the EVEREST Clinical Status Trials. JAMA 2007;297:1332–43.
169. McMurray JJ, Teerlink JR, Cotter G, et al. Effects of tezosentan on symptoms and clinical outcomes in patients with acute heart failure: the VERITAS randomized controlled trials. JAMA 2007;298: 2009–19.
170. Tavazzi L, Maggioni AP, Marchioli R, et al. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. Lancet 2008;372:1223–30.
171. Bello D, Shah DJ, Farah GM, et al. Gadolinium cardiovascular magnetic resonance predicts reversible myocardial dysfunction and remodeling in patients with heart failure undergoing beta-blocker therapy. Circulation 2003;108:1945–53.
172. Cleland JG, Pennell DJ, Ray SG, et al. Myocardial viability as a determinant of the ejection fraction response to carvedilol in patients with heart failure (CHRISTMAS trial): randomised controlled trial. Lancet 2003;362:14–21.
173. Seghatol FF, Shah DJ, Diluzio S, et al. Relation between contractile reserve and improvement in left ventricular function with beta-blocker therapy in patients with heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 2004;93:854–9.
174. Shors SM, Cotts WG, Pavlovic-Surjancev B, et al. Non-invasive cardiac evaluation in heart failure patients using magnetic resonance imaging: a feasibility study. Heart Fail Rev 2005;10:265–73.
175. Rahimtoola SH. Concept and evaluation of hibernating myocardium. Annu Rev Med 1999;50:75–86.
176. Klein L, Gattis WA, Borrello F, Wu E, O'Connor CM, Gheorghiade M. Effects of amino acid supplementation on left ventricular remodeling in patients with chronic heart failure with decreased systolic function and diabetes mellitus: rationale and design of a magnetic resonance imaging study. Am J Cardiol 2004;93:44A–6A.
177. Packer M, Fowler MB, Roecker EB, et al. Effect of carvedilol on the morbidity of patients with severe chronic heart failure: results of the carvedilol prospective randomized cumulative survival (COPERNICUS) study. Circulation 2002;106:2194–9.
178. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med 1999;341:709–17.
179. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF). Lancet 1999;353:2001–7.

Key Words: heart failure ■ nutrition ■ energy ■ micronutrients ■ metabolism.

DOCTOR'S FORMULAS
ΦΟΡΜΟΥΛΕΣ ΥΓΕΙΑΣ

Διάθεση - Διανομή: **Ν.Α. Φικιώρης & ΣΙΑ Ε.Π.Ε.**

Μέρλιν 11 & Κανάρη, Κολωνάκι, τ.κ. 106 71, Αθήνα,
τηλ.: 210 36 04 223, 210 33 88 229, **fax:** 210 33 88 829
www.doctorsformulas.com

in touch health