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EFFECT OF L-CARNITINE ON MYOCARDIAL METABOLISM: RESULTS OF A BALANCED, PLACEBO-CONTROLLED, DOUBLE-BLIND STUDY IN PATIENTS UNDERGOING OPEN HEART SURGERY

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The effects of L-carnitine on cardiac performance after open heart surgery were evaluated in a balanced, placebo-controlled, double-blind study in 38 patients. Preoperative haemodynamic status was good in all of them. Seventeen subjects underwent mitral valve replacement and 19 patients coronary artery bypass grafting. Five grams L-carnitine were given intravenously over 2 h, twice daily for 5 consecutive days; moreover, 10 g L-carnitine in 1500 ml cardioplegia were administered through the aortic root after aortic cross-clamping. Surgery was always planned on treatment day 3. The post-ischaemic functional recovery of the heart was assessed by clinical parameters, as well as by biochemical and ultrastructure evaluations on biopsy specimens. No differences were found between the control and the treatment group with respect to all clinical parameters of cardiac performance after cardiopulmonary bypass. At anaesthesia induction, serum carnitine was significantly increased in treated patients, but carnitine concentrations in the right atrial biopsy obtained just before aortic declamping were similar in the two groups. In patients with mitral valve replacement, L-carnitine therapy was associated with significantly higher concentrations of pyruvate, ATP and creatine phosphate in papillary muscle. Glycogen levels were also higher in the treated group, but the difference was not statistically significant. Myocardial ultrastructure on septal biopsies, obtained within 5 min from weaning from extracorporeal circulation, showed better preservation scores for all considered parameters (nucleus, sarcoplasmic reticulum, mitochondria and cellular oedema) in the treated subjects, although the difference reached statistical significance only for nuclei. When biochemical and ultrastructural data are considered, these findings suggest that L-carnitine improves myocardial metabolism. However, it cannot be concluded that L-carnitine provides an advantageous support therapy for well-compensated patients requiring cardiac surgery. In contrast, the positive effects of L-carnitine on cardiac recovery after bypass might become clinically relevant in the surgical setting for haemodynamically compromised patients, in which further investigations are required.

KEY WORDS: L-carnitine; cardiac surgery; myocardial metabolism.

INTRODUCTION

Carnitine is the specific carrier for the transport of fatty acids from cell cytosol across the mitochondrial membrane for β-oxidation. The heart largely depends on β-oxidation for energy production, but it is unable to synthesise carnitine. However, under physiological conditions, myocardial carnitine concentrations are kept high via an active carnitine uptake from the blood [1]. In contrast, myocardial carnitine depletion has been reported during ischaemia, an

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observation raising questions on the practice of starting carnitine therapy in candidates for coronary artery surgery [2, 3]. In the canine myocardium, *in vivo*, carnitine treatment is associated with a significant reduction of long-chain acyl-CoA accumulation in the mitochondrial space induced by ischaemia. Since high levels of long-chain acyl-CoA inhibit the action of adenine nucleotide translocase, which is involved in the transport of ATP, carnitine protective effect on the ischaemic heart may be secondary to its action on mitochondrial long-chain acyl-CoA concentrations [4, 5].

In open heart procedures, it is of pivotal importance to realise an adequate myocardial preservation against the metabolic derangements occurring during ischaemia. Protection is achieved by limiting the ischaemic time and by using hyperkaliaemic cardioplegic solutions in order to induce a time-limited electromechanical quiescence of the heart. Recently, a number of studies have focused on the evaluation of various xenobiotics as therapeutic additives to commonly used cardioplegic solutions [6]. In isolated rat hearts, higher myocardial ATP concentrations and better morphometric scores for mitochondria were observed when carnitine had been added to the perfusate and cardioplegia [7].

On the basis of the experimental data and the good tolerability of L-carnitine in man [8], L-carnitine was given intravenously and as a supplement to cardioplegia in order to evaluate its effect on cardiac performance in patients undergoing cardiac surgery. The primary aim of the study was to determine if the time required for weaning from cardiopulmonary bypass (CPB) was shorter in patients treated with L-carnitine. Moreover, biochemical and ultrastructural data on cardiac biopsy were obtained in all patients to establish possible treatment-related differences in myocardial metabolism and morphometry.

PATIENTS AND METHODS

Study population

Overall, 38 patients (22 males and 16 females) aged 45–70 years took part in the study, which was approved by the local Ethics Committee. Each patient gave written informed consent to the study. Patients were scheduled for elective coronary artery bypass grafting or mitral valve replacement. Preoperatively, a New York Heart Association class > 2, an ejection fraction $< 40\%$ and a mean pulmonary artery pressure > 25 mmHg represented exclusion criteria from the study, as well as the occurrence of any clinically relevant non-cardiac disease.

Study design

After informed consent had been obtained, patients were randomised in a control or in a treated group according to a double-blind, placebo-controlled, study design. The L-carnitine group received 5 g L-carnitine intravenously over 2 h, twice daily for 5 consecutive days; moreover, 10 g L-carnitine in 1500 ml cardioplegia were given at surgery. The control group received the placebo formulation according to the same regimen.

Surgery planning, sample collection and clinical parameters recording

Surgery was always planned on treatment day 3. The surgical procedure, as well as the anaesthesia and the extracorporeal technique were standardised for all patients. General anaesthesia was induced and maintained with repeated bolus doses of fentanyl, flunitrazepam and pancuronium, the lungs being ventilated with an O_2/air mixture in order to maintain normocarbia and $SaO_2 > 97\%$. A 2 lead electrocardiogram (ECG) tracing (leads II and V_5), as well as an invasive arterial blood pressure were continuously monitored. A Swan-Ganz catheter for pulmonary pressure monitoring was placed only in selected patients. In all cases, the extracorporeal circuit was primed with Ringer's solution. On full CPB, mild systemic hypothermia (34 ± 1°C) was obtained. Then, the ascending aorta was crossclamped and a single 13 ml/kg dose of 5°C cardioplegia was delivered into the aortic root.

In patients with valve disease, the mitral valve and its apparatus were rapidly removed. In all patients, myocardial specimens from the right atrium and the interventricular septum were biopsied respectively just before the aortic declamping and immediately after weaning from CPB.

The papillary muscles and the right atrium samples were frozen in liquid nitrogen immediately after their collection. The septal tissue specimens were fixed in 0.5% Karnovsky's solution at 4°C for 3 h, washed in cacodylate buffer 0.2 M (pH 7.3), post-fixed with 1.5% OsO_4 in cacodylate buffer for 1 h at room temperature and then kept at 4°C until the analysis. In order to evaluate the post-ischaemic functional recovery of the heart, the following parameters were recorded: total bypass time, number and energy of DC shocks necessary to restore sinus rhythm after the aortic cross-clamp removal, drugs required to wean patients from CPB and their dosage, time required for weaning, ECG tracings. In all subjects, defibrillation was performed at a nasofaringeal temperature > 34°C according to the following scheme: first discharge, 10 J; second discharge, 20 J; third and fourth discharge, 20 J. The time required for weaning from CPB was defined as the lag-time between the graft perfusion during coronary artery surgery or the end of the de-airing manoeuvres after valve replacement and the discontinuation of bypass. ECG tracings were obtained preoperatively, on arrival in ICU and 24 h after the end of surgery. Each tracing was analysed for rhythm and deviation of ST

segment from isoelectric baseline. The ST segment level was measured 60 ms after the J-point and recorded in millimetres, using the standard calibration of 1 mV per 10 mm. Deviations > 0.1 mV were considered significant for myocardial ischaemia [9].

Biochemical assays

The serum and right atrial myocardium concentrations of free carnitine, long-chain carnitine and short-chain carnitine derivatives were determined in all patients.

Blood samples (5 ml) were obtained before the first carnitine or placebo dose administration and at general anaesthesia induction, which was scheduled 30 ± 5 min after the first drug infusion on the treatment day 3 had been delivered. Serum, obtained by immediate centrifugation at 1000 *g* for 10 min at room temperature, was kept frozen at -20°C until analysis. Carnitine and its fractions in the serum were determined according to the spectrophotometric method by Schäfer and Reichmann [10].

Upon removal from liquid nitrogen, the right atrium tissue was homogenised and diluted 1:10 with $HClO_4$ 0.3 M. The homogenate was then centrifuged at 5000 *g* for 15 min. Free carnitine and its short-chain derivative levels were determined on the supernatant fluid. The sediment was washed, diluted 1:10 with KOH, rehomogenised, incubated at 56°C for 1 h and centrifuged at 5000 *g* for 15 min. The long-chain acyl-carnitine was then determined on the supernatant fluid.

In patients with mitral valve disease, a quantitative determination of the glycolytic pathway metabolites, of the Krebs' cycle intermediates with the related amino acids and the energy mediators was performed on the papillary muscle. Once removed from liquid nitrogen, the papillary muscle was immediately powdered (Microdismembrator Braun; Melsungen AG) by two consecutive 45 s passages. The tissue was then diluted 1:10 with $HClO_4$ 0.6 N for acid deproteinisation. The homogenate, obtained by two 1-min passages in Ultra-Turrax (Ika-Werk), was split in two aliquots. After glycogen enzymatic hydrolysis on the first aliquot (0.2 ml), the papillary muscle glycogen level was measured according to the spectrophotometric method of Keppler and Decker [11]. The remaining homogenate was centrifuged at 1500 *g* for 15 min in a refrigerated centrifuge (Beckman J2-21; rotor JA-20). The supernatant was neutralised at pH 6 with $KHCO_3$ 2 M, and then recentrifuged at 1500 *g* for a further 15-min period. On the extract, kept at 0–4°C temperature, were immediately performed the determinations of pyruvate [12], lactate [13], citrate [14], α-ketoglutarate [15], malate [16], aspartate [17], glutamate [18], AMP and ADP [19], ATP and creatine phosphate [20]. Pyruvate, lactate, citrate, α-ketoglutarate, malate and aspartate were assayed spectrophotofluorimetrically (Perkin-Elmer LS5 spectrophotofluorimeter; Buckingamshire, UK), whereas the remaining metabolites were determined by a Beckman 35 spectrophotometer. Concentrations were expressed as μmol of glycosidic units g^{-1} of fresh tissue for glycogen and as μmol g^{-1} of fresh tissue for the other metabolites. For the energy store and mediators, the energy charge of the adenylate pool (ECP) was calcutated, according to Atkinson [21], as the value of the ratio $([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP])$.

Ultrastructure evaluation

After dehydration through graded concentrations of ethanol (from 2.5% to 100%) and propylenoxide, the interventricular septum biopsy specimens were embedded in Epon-Araldite. Twenty thin sections of each tissue specimen were obtained using a microtome. Each section was stained in uranyl acetate followed by lead citrate and examined in a Zeiss 902 electron microscope. A semi-quantitative analysis of morphologic changes in myocardial cells was performed blind by a single observer, using a previously published grading system with minor modifications [22]. Four parameters (mitochondria, nucleus, intracellular oedema and sarcoplasmic reticulum) were evaluated and graded separately on a scale of 0–4, grade 0 being considered normal and grades 3–4 representing an irreversible damage. For each parameter of the same specimen, the cumulative score was defined as the mean value of the overall scores for that parameter obtained from each single section.

Statistical analysis

Data are expressed as mean $\pm$ SD. Statistical comparison between and within groups was performed by the analysis of variance, when the considered quantitative variable showed a normal distribution. For non-parametric variables, a χ^2-test was performed. A P-value < 0.05 was accepted as statistically significant for both variables.

RESULTS

Clinical data

The study was carried out and completed on 38 and 36 patients, respectively. Two subjects (one patient in each group) dropped out because of a delay in the time of surgery. Nineteen patients underwent coronary artery bypass grafting, the remaining being scheduled for mitral valve replacement. Overall, 18 subjects received the treatment with L-carnitine, the study drug being given to 9 subjects undergoing mitral valve replacement and 9 subjects with coronary artery disease. In the treated group (10 males; 8 females), the mean age was 60 ± 8 years

(range: 45–69 years). In the control group (12 males; 6 females), the mean age was 58 ± 8 years (range: 41–69 years).

Anaesthesia was given in all cases according to the experimental protocol and no patient showed ischaemia or dysrhythmias in the pre-bypass period. Also the CPB was established and carried out without modifications from the study protocol, and no patient required blood derivatives before the bypass initiation. After cardioplegia delivery, the myocardial temperature decreased to 11°C on average (range: 8–13°C), allowing a good cardiac preservation. In patients with coronary artery grafting, weaning from the ECC was accomplished by infusing 0.5–1 $\mu g\ kg^{-1}\ min^{-1}$ glyceryl trinitrate. One subject in the control group developed a low output syndrome intraoperatively and required 0.5 $\mu g\ kg^{-1}\ min^{-1}$ epinephrine. In patients with mitral valve replacement, dopamine (range: 4–7 $\mu g\ kg^{-1}\ min^{-1}$) and sodium nitroprusside (range: 0.5–1, $\mu g\ kg^{-1}\ min^{-1}$) were routinarily co-administered in order to wean CPB.

The total bypass time and the time required for extracorporeal weaning in the study population are shown in Table I. No significant difference was found in patients with coronary artery disease, as well as in those with mitral valve disease, although total bypass time and time to wean from CPB were lower in the L-carnitine treated patients of the latter group.

Overall, spontaneous recovery of sinus rhythm occurred in 7 patients and only one 10 J discharge of DC shock was required to restore sinus rhythm in 17 subjects of the remaining patients. No differences were observed between treated and control patients with regard to spontaneous defibrillation, the number of DC shocks required to restore sinus rhythm and the analysis of cardiac rhythm and ischaemia throughout the peri-operative period. Preoperatively, all but one patient in the coronary artery bypass group were in sinus rhythm, while 16 of 17 subjects with mitral valve disease showed atrial fibrillation. Postoperatively, all but 2 mitral valve patients showed the same rhythm as preoperatively. A ST segment depression was detected in 4 grafted patients (3 of them in the control group) in the postoperative period; however, the CK-MB values were negative for myocardial ischaemia.

In all cases, L-carnitine was well tolerated and no drug related side effects were reported. The postoperative course was uneventful in 34 patients. One patient from the control group died on postoperative day 15 of multiple organ failure secondary to *Staphylococcus aureus* infection. Surgery was complicated by stroke in one subject of the treated group.

Biochemical data

Serum and right atrium concentrations of carnitine and its derivatives Basal free carnitine concentrations in serum ranged from 0.8 to 17.5 $\mu mol\ l^{-1}$, with similar mean values for the two groups of patients (Fig. 1). Before induction of anaesthesia, free carnitine in serum was on average 2.4 times higher in the carnitine treated subjects, mean concentrations being 23.7 ± 4.6, $\mu mol\ l^{-1}$ and 9.9 ± 2.3 $\mu mol\ l^{-1}$, respectively, in the treated and the control groups ($P < 0.001$). However, no significant difference was found between the two groups in the right atrium content of carnitine and its acyl derivatives (Fig. 1).

Papillary muscle metabolic profile The metabolic profile in the papillary muscle was assessed in 9 L-carnitine treated patients and 8 control subjects. Biochemical data are shown in Table II. With respect to the glycolytic pathway, mean pyruvate concentration was significantly higher in the carnitine treated patients. Since the lactate level was unchanged in the two groups, the lactate/pyruvate ratio value decreased significantly in the treated subjects. Glycogen concentrations were also higher in the treated population, but the difference did not reach statistical significance. Tissue concentration of Krebs' cycle intermediates and related aminoacids were similar in the carnitine and placebo patients, as

Table I
Clinical parameters of postbypass myocardial performance in patients receiving placebo ($n = 18$, 10 of them being scheduled for coronary artery bypass grafting) or L-carnitine ($n = 18$, 9 of them being scheduled for coronary artery bypass grafting)

Parameter	*Control group*	*Treated group*
Total bypass time (min)		
All patients	104 ± 7	104 ± 8
Patients with mitral valve disease	108 ± 26	82 ± 26
Patients with coronary artery disease	102 ± 29	121 ± 32
Time to wean ECC (min)		
All patients	16 ± 2	16 ± 2
Patients with mitral valve disease	17 ± 8	12 ± 6
Patients with coronary artery disease	15 ± 8	19 ± 10

Data are given as mean ± SD.

well as myocardial AMP, ADP and ECP. In contrast, the papillary muscle ATP and CP levels were significantly higher in the treated subjects.

Ultrastructural assessment

Interventricular septum biopsies for ultrastructure examination were obtained from 23 patients, 12 of them belonging to the treated group. In the remaining cases the biopsy was not performed because of severe cardiac arrhythmias during tissue harvesting. For all parameters, lower scores (indicative of a better myocyte preservation) were obtained in the L-carnitine treated patients, although the difference reached statistical significance only for nuclei (Table III). In particular, the indexes of mitochondrial changes did not reach statistical significance between the two groups. With respect to nuclear pattern, some nuclear clumping and nuclear chromatin margination were observed in the placebo but not in the treated group of patients (Fig. 2).

DISCUSSION

During cardiac ischaemia, intramitochondrial long-chain acyl-CoA concentrations increase. High levels of this compound inhibit the action of the mitochondrial enzyme adenine nucleotide translocase, which is involved in the ATP transport. As a consequence, cellular metabolism and viability deteriorate. During ischaemia, L-carnitine administration considerably reduces the accumulation of long-chain acyl CoA in the mitochondrial space. Moreover, in ischaemic canine myocardium, L-carnitine treatment is associated with improved mitochondrial function and β-oxidation of free fatty acids, as well as with a relative increase of glycolysis (compared to free fatty acids oxidation) for energy production [5]. These findings suggest a protective role of L-carnitine on the ischaemic myocardium and support the clinical use of L-carnitine, especially in patients with ischaemic heart disease [2, 3].

During open-heart surgery with cardioplegic arrest, myocardial ischaemia always occurs. Since such ischaemia is standardised, cardiac surgery with cardioplegic arrest represents an experimental model in man for the evaluation of the cardiac effects of xenobiotics [6], including carnitine [23, 24].

In patients with mitral valve disease, Sunamori *et al.* [24] have demonstrated that pretreatment with L-carnitine was effective in improving all clinical parameters of postbypass cardiac performance. In contrast, on the basis of the clinical results we can not conclude that L-carnitine improves myocardial function after cardiopulmonary bypass. In fact, the vasoactive drug requirements and the time necessary to wean CPB were similar in the treated and the placebo group. Our clinical data agree with the findings obtained by Demeyere *et al.* [23]. An intravenous 3 or 6 g L-carnitine or placebo infusion was given over a 10-min period before the onset of CPB to patients scheduled for elective coronary artery bypass grafting. Although plasma carnitine levels markedly increased in the treated patients, the haemodynamic profile after reperfusion was not favourably affected by L-carnitine. Moreover, the energy mediators content in transmural left ventricular biopsy specimens obtained at the beginning and the end of cardiopulmonary bypass did not differ significantly among the groups. On the contrary, the present study demonstrates in man that a multiple dose intravenous therapy with L-carnitine improves the myocardial content of ATP and creatine phosphate. In fact, the relative contribution of the various pathways addressed to energy production cannot be clearly elucidated, but a preferential activation of glycolysis may be inferred on the basis of the significant increase in pyruvate levels observed in the L-carnitine treated population. As far as this is concerned, the present findings provide further evidence that carnitine enhances glucose metabolism while decreasing the relative contribution of β-oxidation of free fatty acids during myocardial ischaemia in man [25]. However, when the biochemical data are considered, it might be surprising to detect the same right atrium carnitine concentrations in the L-carnitine treated and in the placebo group of patients. Recently, Nakagawa *et al.* [26] have demonstrated that the concentration of free carnitine and its fractions is markedly lower in the right atrial appendage than in the left ventricular muscle. Therefore, the right atrial tissue might be an inadequate tissue in order to establish whether carnitine positively affects myocardial performance. Moreover, the right atrial biopsies have been obtained just before aortic declamping, that is approximately 1 h after cardioplegic arrest. During ischaemia, tissue stores of carnitine and its fractions might have decreased, thus impairing the evaluation of any treatment-related difference in their concentrations. This hypothesis could be supported by recent findings in rats [27]. Labelled propionyl-L-carnitine given intravenously undergoes rapid clearance from plasma and it is extensively metabolised in the heart, the unchanged tracing representing only 2.4% of radioactivity 15 min after drug administration.

To date, there is a paucity of studies focused on the sequence of the ultrastructural changes during cardiac surgery and their results are often conflicting. However, on the basis of the available literature, it seems likely that nuclear abnormalities are more prominent during the cross-clamp period, whereas mitochondrial derangement occurs early in the reperfusion period and is often a partially reversible phenomenon. Overall in our patients, the

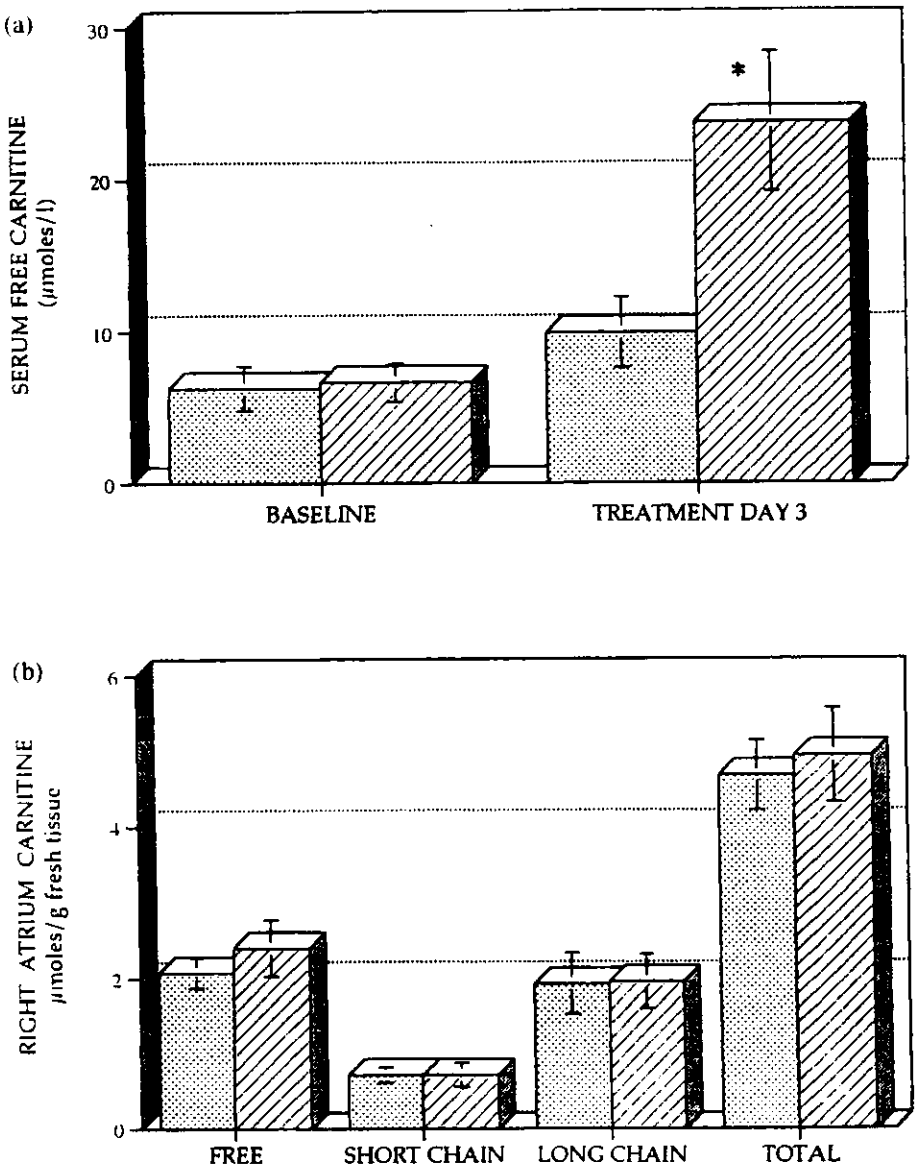

Fig. 1. (a) Serum free carnitine concentration (mean ± SE) in the control and in the treated group of patients at baseline and at anaesthesia induction (treatment day 3). $P < 0.001$. (b) Concentration of free carnitine, short-chain and long-chain carnitine derivatives (mean ± SE) in the right atrium specimens obtained at aortic crossclamp removal in the control and in the treated group of patients. ⊡, control group; ▨, treated group.

degree of morphologic injury was mild, with a score ranging from 0.6 to 1.4 on a scale from 0 to 4. Similar score values have been reported by Warner *et al.* [28] on biopsy specimens of left ventricle after cold potassium cardioplegic arrest, thus suggesting that the observed structural impairment might be shared by a number of cardiac pathologies (coronary artery disease, mitral valve pathology, aortic stenosis and insufficiency). Also, the time elapsed between initiation of reperfusion and the time of myocardial biopsy collection in our study population (16 ± 2 min) was similar to that reported by Warner *et al.* [28], as were the values for the nuclear and mitochondrial score at reperfusion in the placebo group of patients. A trend towards a better preservation of the cardiac myocyte was found in the carnitine treated patients. However, significant differences at ultrastructural examination were observed only for the nuclear chromatin. These findings are surprising, since, on the basis of the mechanism of action of carnitine, changes in mitochondrial rather than nuclear ultrastructure could have been expected.

Finally, a pharmacokinetic bias in the study planning is unlikely. In the healthy volunteer given 6 g L-carnitine intravenously over 10 min, the elimination half-life of carnitine is about 4 h [8]. In patients with heart disease, drugs kinetics may markedly change, and after multiple drug dosing the time required to reach the steady-state levels may be prolonged. In the present study, however, the preoperative drug administration could have been long enough to fulfil steady-state conditions, especially if

Table II
Papillary muscle concentration of glycogen, Krebs' cycle intermediates and energy mediators in patients with mitral valve disease receiving L-carnitine or placebo

Metabolite	*Control group (n = 9)*	*Treated group (n = 9)*
Glycogen	41.043 ± 36.802	65.140 ± 41.149
Pyruvate	0.176 ± 0.149	0.399 ± 0.312*
Lactate	5.603 ± 3.751	6.802 ± 3.016
Lactate/ pyruvate ratio	70.249 ± 91.852	39.202 ± 32.747*
Citrate	0.194 ± 0.100	0.261 ± 0.198
α-Ketoglutarate	0.038 ± 0.027	0.034 ± 0.021
Malate	0.318 ± 0.236	0.150 ± 0.084
Aspartate	0.313 ± 0.258	0.301 ± 0.072
Glutamate	1.915 ± 1.881	3.506 ± 2.616
Alanine	0.783 ± 0.609	0.621 ± 0.545
AMP	0.112 ± 0.123	0.116 ± 0.116
ADP	0.717 ± 0.327	0.772 ± 0.224
ATP	0.923 ± 0.325	1.523 ± 1.121*
Creatine phosphate	1.846 ± 0.975	3.010 ± 3.070*
ECP	0.626 ± 0.313	0.797 ± 0.081

Concentrations are expressed as μmol of glycosidic units g^{-1} of fresh tissue for glycogen and as μmol g^{-1} of fresh tissue for the other metabolites. Data are given as mean ± SD; *$P < 0.05$.

the patients' good pre-operative haemodynamic profile is considered.

In conclusion, in the present experimental conditions, our biochemical and ultrastructural data demonstrate that L-carnitine improves the myocyte metabolism in man; however, we cannot affirm that L-carnitine therapy may represent an advantageous support therapy in patients scheduled for open-heart operations when patients' preoperative haemodynamics are good. By contrast, the positive effects of L-carnitine on cardiac recovery after bypass might become clinically relevant in the setting of surgery on haemodynamically compromised patients, in which further investigations are required.

Finally, as far as the sample size is concerned, our results need confirmation with a larger sample of patients, since insufficient evidence to reject the standard null hypothesis of equivalence does not imply sufficient evidence to accept it.

Table III
Ultrastructural parameters on interventricular septum biopsy specimens obtained after weaning from CPB in patients receiving placebo or L-carnitine

Parameter	*Control group (n = 11)*	*Treated group (n = 12)*
Mitochondria	0.88 ± 0.83	0.63 ± 0.65
Nucleus	1.45 ± 0.39	0.76 ± 0.50*
Intracellular oedema	0.85 ± 0.58	0.71 ± 0.62
Sarcoplasmic reticulum	1.13 ± 0.72	1.09 ± 0.68

Data are given as mean ± SD; *$P = 0.002$.

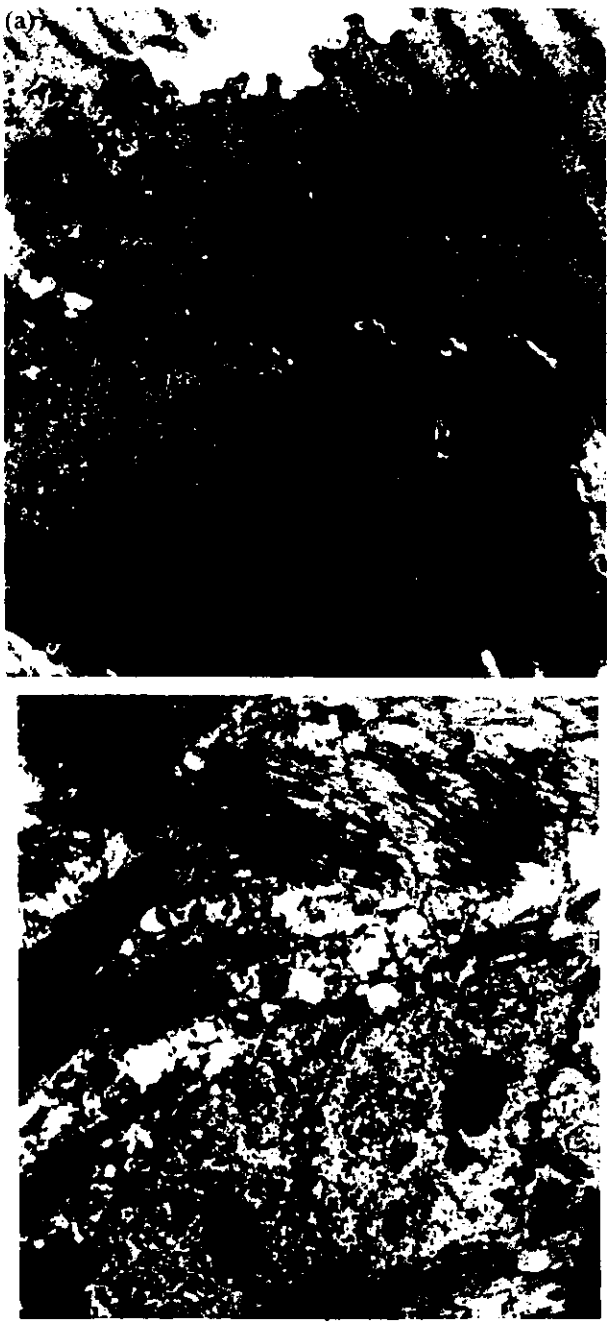

Fig. 2. (a) Electron micrograph showing well preserved myocyte nucleus, mitochondria and sarcomeres of a L-carnitine-treated patient. (b) Electron micrograph showing some chromatin clumping, myofibrillar lysis and mitochondrial cristolysis in the myocardium of a placebo-treated patient.

REFERENCES

1. Bieber LL. Carnitine. *Ann Rev Biochem* 1988; 57: 261–83.

2. Spagnoli LG, Corsi M, Villaschi S, Palmieri G, Maccari F. Myocardial carnitine deficiency in acute myocardial infarction. *Lancet* 1982; **i:** 1419–20.
3. Chierchia SL, Fragasso G. Metabolic management of ischemic heart disease. *Eur Heart J* 1993; *14(Suppl. G):* 2–5.
4. Suzuki Y, Kamikawa T, Kobayashi A, Masumura Y, Yamazaki N. Effects of L-carnitine on tissue levels of acyl carnitine, acyl coenzyme A and high energy phosphate in ischemic dog hearts. *Jpn Circ J* 1981; **45:** 687–94.
5. Kobayashi A, Fusjiawa S. Effect of L-carnitine on mitochondrial acyl CoA esters in the ischemic dog heart. *J Mol Cell Cardiol* 1994; **26:** 499–508.
6. Pastoris O, Dossena M, Vercesi L, Bruseghini M, Pagnin A, Ceriana P. Biochemical changes induced in the myocardial cell during cardioplegic arrest supplemented with creatine phosphate. *J Cardiothor Vasc Anesthes* 1991; **5:** 475–80.
7. Nakagawa T, Sunamori M, Suzuki A. The effect of L-carnitine on myocardial protection in cold cardioplegia followed by reperfusion. *Thorac Cardiovasc Surgeon* 1993; **42:** 85–9.
8. Harper P, Elwin CE, Cederblad G. Pharmacokinetics of bolus intravenous and oral doses of L-carnitine in healthy subjects. *Eur J Clin Pharmacol* 1988; **35:** 69–75.
9. Dorman BH, Zucker JR, Verrier ED, Gartman DM, Slachman FN. Clonidine improves perioperative myocardial ischemia, reduces anesthetic requirement, and alters hemodynamic parameters in patients undergoing coronary artery bypass surgery. *J Cardiothor Vasc Anesthes* 1993; **7:** 386–95.
10. Schäfer J, Reichmann H. A spectrophotometric method for the determination of free and esterified carnitine. *Clin Chim Acta* 1989; **182:** 87–9.
11. Keppler D, Decker K. Glycogen. Determination with amyloglucosidase. In: Bergmeyer HU, ed. *Methods of enzymatic analysis*. San Diego, CA, USA: Academic Press, 1974, pp. 1127–31.
12. Passonneau JV, Lowry OH. Pyruvate. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1974, pp. 1452–56.
13. Lowry OH, Passonneau JV, eds. Lactate. In: *A Flexible System of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1972, pp. 194–9.
14. Lowry OH, Passonneau JV. (eds). Citrate. In: *A Flexible System of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1972, pp. 157–8.
15. Narins RG, Passonneau JV. 2-Oxoglutarate. Fluorimetric determination. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1974, pp. 1580–84.
16. Lowry OH, Passonneau J (eds). Malate. In: *A Flexible System of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1972, pp. 201–3.
17. Bergmeyer HU, Bernt E, Möllering H, Pfleiderer G. L-aspartate and L-asparagine. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1974, pp. 1696–1700.
18. Witt I. L-glutamate. Determination with glutamate dehydrogenase and the 3-acetylpyridine analogue of NAD (APAD). In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1974, pp. 1713–5.
19. Jaworek D, Gruber W, Bergmeyer UH. Adenosine-5′-diphosphate and adenosine-5′-monophosphate. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1974, pp. 2127–31.
20. Lamprecht W, Stein P, Heinz P, Weisser H. Creatine phosphate. Determination with creatine kinase, hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. San Diego, CA, USA: Academic Press, 1974, pp. 1777–81.
21. Atkinson DE. The energy charge of the adenylate pool as regulatory parameter. Interaction with feedback modifiers. *Biochemistry* 1968; **7:** 4030–4.
22. Okamoto K, Kinoshita Y, Yoshioka T, Kawaguchi N, Onishi S, Sugimoto T. Myocardial preservation in brain-dead patients maintained with vasopressin and catecholamine. *Clin Transplant* 1992; **6:** 294–300.
23. Demeyere R, Lormans P, Weidler B, Minten J, Van Aken H, Flameng W. Cardioprotective effects of carnitine in extensive aortocoronary bypass grafting. *Anesth Analg* 1990; **71:** 520–8.
24. Sunamori M, Nakagawa T, Fujisawa S, Suzuki A. Myocardial response to pretreatment with L-carnitine in patients with cardiac valve replacement. *Vasc Surg* 1991; **25:** 607–17.
25. Neely JR, Morgan HE. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Ann Rev Physiol* 1974; **36:** 413–59.
26. Nakagawa T, Sunamori M, Suzuki A. The myocardial distribution and plasma concentration of carnitine in patients with mitral valve disease. *Jpn J Surg* 1994; **24:** 313–7.
27. Davenport RJ, Law MP, Pike VW, Osman S, Poole KG. Propionyl-L-carnitine: labelling in the *N*-methyl position with carbon-11 and pharmacokinetic studies in rats. *Nucl Med Biol* 1995; **22:** 699–709.
28. Warner KG, Khuri SF, Kloner RA, Josa M, Dalecki-Chipperfield KM, Butler MD, Assousa SN, Lee SS, Barsamian EM, Seiler M. Structural and metabolic correlates of cell injury in the hypertrophied myocardium during valve replacement. *J Thorac Cardiovasc Surg* 1987; **93:** 741–54.