ABSTRACT: To define the skeletal muscle abnormalities in patients undergoing exercise deconditioning and evaluate the metabolic effect of propionyl-L-carnitine (PLC), muscle biopsies were obtained from 28 patients with effort angina and 31 control subjects. Coronary artery disease patients received either placebo (n = 12), PLC (1.5 g IV followed by infusion of 1 mg/kg/min for 30 min, n = 10), or L-carnitine (1 g IV followed by infusion of 0.65 mg/kg/min for 30 min, n = 6) for 2 days. Exercise deconditioned patients treated with placebo showed normal muscle content of total carnitine and glycogen, and decrease in percentage of type 1 fibers ($P < 0.01$) and in the activity of citrate synthase ($P < 0.05$), succinate dehydrogenase ($P < 0.05$), and cytochrome oxidase ($P < 0.05$), as compared to controls. Both PLC and L-carnitine did not modify muscle fiber composition or enzyme activities, but significantly increased muscle levels of total carnitine by 42% and 31%, respectively ($P < 0.05$). Moreover, PLC significantly increased glycogen muscle content ($P < 0.01$), while the equimolar dose of L-carnitine did not. This effect, probably due to the anaplerotic activity of the propionic group of PLC, suggests that this drug may be effective in improving energy metabolism of muscles with impaired oxidative capacity. *Muscle Nerve* **20:** 1115–1120, 1997
Key words: propionyl-L-carnitine; skeletal muscle; energy metabolism; physical deconditioning; coronary artery disease

CHANGES IN SKELETAL MUSCLE HISTOLOGY AND METABOLISM IN PATIENTS UNDERGOING EXERCISE DECONDITIONING: EFFECT OF PROPIONYL-L-CARNITINE

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Exercise deconditioning affects fiber composition and oxidative metabolism in skeletal muscle.[12,16,22] These alterations have been well documented in patients with chronic heart failure,[9,30,31] and several studies indicate that skeletal muscle biochemical and histological changes are correlated to exercise intolerance in such patients.[9,28,31] Patients with coronary artery disease (CAD) and effort angina tend to avoid voluntary effort and thus may undergo muscle physical deconditioning. In fact, they have a reduced percentage of type 1 fibers, increased percentage of type 2 fibers, and low oxidative capacity of the thigh muscles.[14,15] The present study was designed to: (1) define the structural and metabolic changes that occur in muscle of patients undergoing physical deconditioning because of effort angina; (2) evaluate the metabolic effect of propionyl-L-carnitine (PLC), a metabolic enhancer that increases carnitine content and may improve metabolism of ischemic muscle.[2,5,7]

PATIENTS AND METHODS

Patients. Biopsy specimens of sartorius muscle were obtained from male patients with CAD during coronary artery bypass grafting. Written informed consent was obtained from all patients and control subjects before the study began. Twenty-two patients

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were randomly allocated to receive placebo (n = 12) or PLC (1.5 g IV as a single bolus, followed by an infusion of 1 mg/kg/min for 30 min, n = 10), for 2 days before surgery. Their degree of cardiovascular disability was assessed by the Canadian Cardiovascular Society functional classification.

All patients were taking calcium antagonists and nitrates at the time of the study. Additional treatments were: β-blockers in 6 patients of the placebo and in 3 of the treated group; angiotensin converting enzyme (ACE) inhibitors in 1 patient in each of the two groups. None of the patients had a documented history of heart failure or had peripheral edema, intermittent claudication, or neuromuscular disorder. Seven patients in the placebo group and 4 in the treated group suffered from myocardial infarction at least 1 year before the study.

In order to compare the metabolic effect observed with PLC to those with an equimolar dose of L-carnitine, we studied 6 additional patients, matched to those of the placebo group for age, disease duration, left ventricular ejection fraction, and Canadian Cardiovascular Society functional class. In these patients, muscle biopsies were obtained after treatment with L-carnitine (1 g IV as a single bolus, followed by an infusion of 0.65 mg/kg/min for 30 min), for 2 days before surgery. All patients were taking nitrates and calcium antagonists at the time of the study. Additional treatments were: β-blockers in 5 and ACE inhibitors in 1 patient. Muscle biopsies were obtained also from 5 normal age-matched subjects who received PLC at the dosage indicated above. The data of the present study were compared with those obtained from muscles of 31 normal, untrained age-matched males previously studied in our laboratory.

Histochemical Analysis. Muscle specimens were immediately frozen in isopentane cooled in liquid nitrogen and stored at −70°C. Serial 10-μm-thick cross sections of muscle tissue were stained for routine myosin adenosine triphosphatases (ATPases) (pH = 4.35 and 4.6), NADH-TR, cytochrome oxidase (COX), and succinate dehydrogenase (SDH). These stains were used to classify muscle fibers into type 1 or 2, for the detection of angulated or grouped fibers, and to analyze subsarcolemmal rims of mitochondria. The calculation of the mean fiber diameter and of fiber type atrophy factor was performed according to methods described elsewhere.[19]

Biochemical Analysis. Glycogen was assayed by the method of Hassid and Abraham.[13] Free carnitine was assayed by a radiochemical method[6] in the presence of 0.5 mmol/L N-ethylmaleimide. Short- and long-chain acylcarnitines were measured after alkaline hydrolysis, as described by Pearson et al.[20] Noncollagen proteins (NCP) were determined according to the method of Lowry.[17] SDH,[26] citrate synthase,[27] and COX[32] activities were determined using established spectrophotometric methods. Muscle specimens were homogenized in 50 mmol/L Tris, 150 mmol/L KCl buffer medium, pH = 7.4, and the 600 g supernatant, devoid of myofibrillar fraction, was used for determination of enzyme activities.

Statistical Analysis. Group values are expressed as mean ± SEM. Group comparisons were performed by the analysis of variance and the Tukey post hoc test. Statistical significance was set at $P < 0.05$.

RESULTS

The clinical characteristics of patients treated with placebo, PLC, and L-carnitine were similar (Table 1). As specified in Table 1, all patients were at least in functional class II of the Canadian Cardiovascular Society, and thus presented limitation in ordinary activity because of anginal pain, dyspnea, or fatigue.

The muscle samples from all patients of the three groups showed atrophic fibers with minimal or no angular features and occasional type grouping (Fig. 1). No NADH-TR reductase hyperreactive atrophic fibers were seen.

The percentage of type 1 fibers in controls was 60.6 ± 3.2, in placebo-treated patients 46.2 ± 2.6, in PLC-treated 37.7 ± 2.5, and in L-carnitine-treated patients 46.2 ± 3.5. In all three groups of patients it was significantly decreased ($P < 0.01$) compared with controls. Fiber type distribution and atrophy factor, and other histochemical findings observed in patients and controls are shown in Table 2.

In the placebo group and in the PLC- and L-carnitine-treated groups, the biochemical activities

Table 1. Clinical characteristics.

	Placebo (n = 12)	Propionyl-L-carnitine (n = 10)	L-carnitine (n = 6)
Age (years)	59.8 ± 2.3	63.7 ± 2.2	60.7 ± 0.9
Disease duration (years)	2.3 ± 0.6	2.9 ± 1.0	2.2 ± 0.3
Left ventricular ejection fraction (%)	46.2 ± 2.7	49.4 ± 2.8	50.3 ± 5.2
CCSFC II	3 (25%)	2 (20%)	2 (33%)
CCSFC III	8 (67%)	7 (70%)	3 (50%)
CCSFC IV	1 (8%)	1 (10%)	1 (17%)
Myocardial infarction	7 (58%)	4 (40%)	2 (33%)

CCSFC, Canadian Cardiovascular Society functional class.

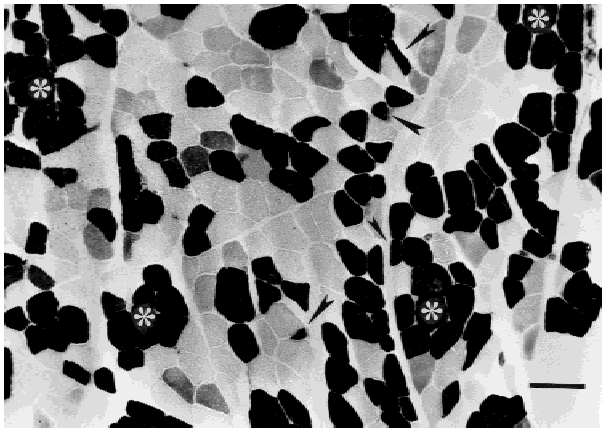

FIGURE 1. Muscle biopsy from a patient with coronary artery disease who underwent physical deconditioning. Note type 1 atrophic fibers (arrowheads) and initial type grouping of either type I (asterisks) or type II fibers. Acid ATPase stain (pH = 4.6). Bar = 0.1 mm.

of SDH, COX, and citrate synthase were significantly lower than in controls ($P < 0.05$) (Table 3).

As shown in Figure 2, the muscle total carnitine content in placebo-treated patients was similar to that of the control group (22.2 ± 1.3 and 21.1 ± 0.6 nmol/mg NCP, respectively). On the contrary, the muscle total carnitine content in PLC- and L-carnitine-treated patients was significantly higher ($P < 0.05$) than in controls (29.7 ± 3.4 and 27.8 ± 3.4 nmol/mg NCP, respectively). The increase in muscle total carnitine observed in patients who received active treatments was due to a significant increase in free carnitine ($P < 0.05$) (Table 4). In the PLC-treated group, even the muscle concentration of short-chain acylcarnitines was significantly higher than in controls ($P < 0.01$). It is noteworthy that PLC did not modify the muscle levels of carnitine and its fractions in the 5 control subjects treated before biopsy (Table 4).

Table 2. Histochemical findings.

	Controls	Placebo	PLC	LC
Type 1 fiber distribution (%)	60.6 ± 3.2	46.2 ± 2.6*	37.7 ± 2.5*	46.2 ± 3.5*
Type 1 fiber diameter (μm)	41.1 ± 0.7	49.7 ± 2.8	42.3 ± 1.7	46.9 ± 3.6
Type 2 fiber distribution (%)	39.4 ± 1.9	53.8 ± 2.4*	62.3 ± 2.2*	53.8 ± 2.6*
Type 2 fiber diameter (μm)	40.8 ± 0.8	56.2 ± 2.2	48.5 ± 2.5	53.2 ± 3.4
Patients with increased type 1 atrophy factor	0	8 (67%)	8 (80%)	4 (67%)
Patients with increased type 2 atrophy factor	0	3 (25%)	7 (70%)	3 (50%)

PLC, propionyl-L-carnitine; LC, L-carnitine.
**P < 0.01 vs. controls.*

Table 3. Enzyme activities in control subjects and patients undergoing physical deconditioning.

	Controls	Placebo	PLC	LC
Citrate synthase	240.1 ± 10	194.4 ± 17*	187.6 ± 15†	169.7 ± 21†
Succinate dehydrogenase	11.7 ± 1.1	7.2 ± 1.7*	5.1 ± 1.8†	8.1 ± 1.4*
Cytochrome C oxidase	37.6 ± 2.8	21.4 ± 2.7*	17.4 ± 5.2†	23.3 ± 5.6*

Values are expressed as nmol/min/mg noncollagen protein.
PLC, propionyl-L-carnitine; LC, L-carnitine.
**Significantly lower than in control subjects, P < 0.05.*
†Significantly lower than in control subjects, P < 0.01.

As shown in Figure 3, muscle glycogen levels of placebo-treated patients were similar to those of controls (0.93 ± 0.10 and 0.88 ± 0.08 g%, respectively). Treatment with PLC increased significantly the muscle glycogen content (1.49 ± 0.21 g%) as compared to the control group ($P < 0.01$). This effect was not observed in patients treated with L-carnitine, their muscle glycogen level being 0.78 ± 0.11 g%. In the 5 control subjects treated before biopsy, PLC did not modify the muscle glycogen content, which was 0.94 ± 0.2 g%.

DISCUSSION

Although angina is the cardinal symptom of CAD, patients often complain of leg fatigue and exhaustion during exercise. Results of the present study suggest that muscle fatigue may be due to changes in skeletal muscle histology and biochemistry which include reduction in the proportion of type 1 fibers, increase in type 2 fibers, fiber atrophy, and reduction in oxidative enzyme activity.

The fiber atrophy described in the present study has not been previously reported in patients with CAD, while a type 2 fiber atrophy has been described in patients with heart failure.[10,18]

The mean fiber diameter of type 2 fibers in placebo-, PLC-, and L-carnitine-treated groups was similar to controls. However, there was a high proportion of patients receiving either PLC or L-carnitine who showed an increase in type 2 fiber atrophy factor. This may be due to selective type 2 fibers involvement resulting in the presence of atrophic type 2 fibers. In the calculation of atrophy factor, the presence of some fibers with marked atrophy has a determining role.

Our patients were at least in functional class II of the Canadian Cardiovascular Society and thus presented a more or less severe limitation in ordinary physical activity. Therefore, it is conceivable that exercise deconditioning may be responsible, at least in part, for the histological and biochemical alterations

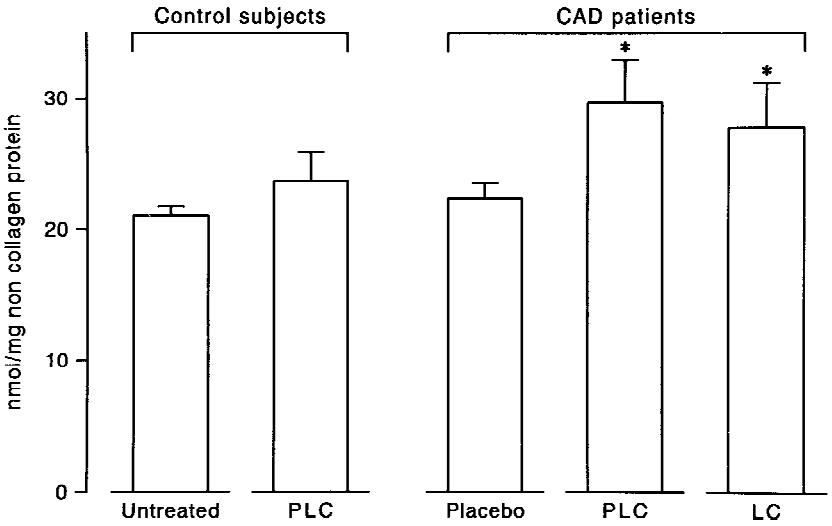

FIGURE 2. Total carnitine levels in the skeletal muscle of control subjects and patients with coronary artery disease (CAD). PLC, propionyl-L-carnitine; LC, L-carnitine. *Significantly higher than in normal subjects and placebo-treated patients ($P < 0.05$).

observed in the present study. Indeed, inactivity can alter fiber type composition,[12] causes generalized fiber atrophy,[23] and reduces the activity of oxidative enzymes.[8,29] Another mechanism which may induce muscle abnormalities similar to those seen in our patients is an inadequate muscle blood flow. In effect, an impaired vasodilator reserve has been reported in patients with angina.[24] In our patients, we did not study skeletal muscle blood flow; however, we found a high proportion of type 2 fibers, a feature reported to be associated with high peripheral circulatory resistance.[11] Finally, the muscle alterations described in this report could be a feature of aging, but they were not seen in our age-matched controls.

Short-term treatment with PLC or L-carnitine did not modify the muscle histochemical profile or enzyme activities. On the contrary, PLC significantly increased muscle content of glycogen and total carnitine. Also, short-chain acylcarnitines and free carnitine were significantly increased. This indicates that part of the administered PLC was taken up by the muscle and that a consistent portion was transformed into free carnitine. In effect, within the mitochondria, PLC is readily converted into free carnitine and propionyl CoA.[25]

The increase in glycogen muscle content may be related to the metabolic action of propionyl-CoA rather than to the increased availability of free carnitine. In fact, an equimolar dose of L-carnitine increased free carnitine muscle content, but did not modify glycogen levels. Propionyl-CoA may increase glycogen muscle content either by promoting glycogen synthesis or by blocking its utilization. In effect, propionyl-CoA is a gluconeogenic substrate,[25] and thus it can generate glucose-6-phosphate, which can become glycogen. Alternatively, propionyl-CoA, by entering into the Krebs' cycle as succinyl-CoA,[25] could provide additional substrates for energy metabolism through an anaplerotic mechanism[21] and thus lead to a glycogen sparing effect.

The increase in glycogen content observed in the sartorius muscle cannot be extrapolated to other skeletal muscles (i.e., trunk or distal limb muscles) that have different metabolic activity and fiber type

Table 4. Muscle levels of carnitine and its fractions in control subjects and patients undergoing physical deconditioning.

	Control subjects		Deconditioned patients		
	Untreated	PLC	Placebo	PLC	LC
Total carnitine	21.1 ± 0.6	23.9 ± 2.0	22.2 ± 1.3	29.7 ± 3.4*	27.8 ± 3.4*
Free carnitine	18.8 ± 0.6	21.1 ± 2.4	19.1 ± 1.3	24.4 ± 3.6*	24.7 ± 3.3*
Short-chain acylcarnitine	1.95 ± 0.2	2.4 ± 0.7	2.6 ± 0.4	5.0 ± 1.7†	2.6 ± 0.5
Long-chain acylcarnitine	0.33 ± 0.1	0.40 ± 0.1	0.46 ± 0.1	0.33 ± 0.1	0.50 ± 0.04

Values are expressed as nmol/mg noncollagen protein.
PLC, propionyl-L-carnitine; LC, L-carnitine.
**Significantly higher than in untreated control subjects,* $P < 0.05$.
†*Significantly higher than in untreated control subjects,* $P < 0.01$.

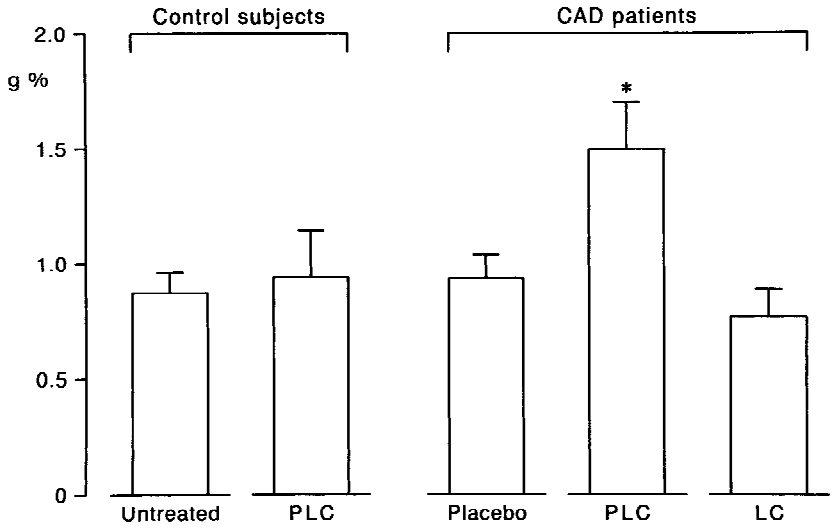

FIGURE 3. Glycogen levels in the skeletal muscle of control subjects and patients with coronary artery disease (CAD). PLC, propionyl-L-carnitine; LC, L-carnitine. *Significantly higher than in normal subjects and placebo-treated patients ($P < 0.05$).

composition. Interestingly, PLC did not modify the muscle content of carnitine and glycogen in normal subjects. This finding is consistent with the previous observation that PLC increases the muscle content of carnitine in patients with severe peripheral arterial insufficiency, while it does not in controls.[2] Therefore, it is conceivable that PLC, when given IV for a short period, is taken up only by muscles with impaired oxidative capacity.

This study was not designed to investigate the functional significance of the metabolic changes induced by PLC. Further studies need to clarify whether the increase in muscle carnitine and glycogen may or may not affect exercise capacity. However, previous findings indicate that PLC and L-carnitine improve exercise tolerance in patients with intermittent claudication.[1,3,4] This beneficial effect is related to a metabolic effect, since both the drugs do not affect regional hemodynamics.[1,3] Indeed, increased carnitine availability is associated with improvement in oxidative phosphorylation efficiency.[1] Accordingly, results of the present study may have relevant clinical implications, especially for patients with CAD and chronic heart failure, in whom exertional fatigue is a major limiting symptom. These patients have muscle histologic and biochemical adaptations similar to those observed in our patients[30] and, in addition, may present reduced glycogen levels in the skeletal muscle.[30] The feasibility of increasing both carnitine and glycogen content in skeletal muscle of such patients by PLC provides the basis for a rational treatment of their exercise intolerance.

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