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Propionyl L-carnitine improvement of hypertrophied rat heart function is associated with an increase in cardiac efficiency

Brett O. Schönekess [a], Michael F. Allard [b], Gary D. Lopaschuk [a,*]

[a] *Departments of Pharmacology and Pediatrics, University of Alberta, Edmonton, Canada*
[b] *Department of Pathology and Laboratory Medicine, University of British Columbia and St. Paul's Hospital, Vancouver, Canada*

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Abstract

Although propionyl L-carnitine improves contractile function of hypertrophied rat hearts, the mechanism(s) by which it does this are not known. One postulated mechanism is that propionyl L-carnitine reverses the alterations in energy metabolism that occur secondary to the carnitine deficiency seen in hypertrophied myocardium. This study determined the effects of chronic propionyl L-carnitine administration on myocardial carnitine content and energy metabolism in hypertrophic hearts from male Wistar Kyoto rats. Pressure-overload hypertrophy was produced by constriction of the abdominal aorta in juvenile rats. Propionyl L-carnitine was administered to the rats via the drinking water for an 8 week period (60 $mg \cdot kg^{-1} \cdot day^{-1}$). Myocardial function and metabolic analysis was determined in isolated working hearts obtained from aortic-banded and sham-operated (control) animals at the end of the 8 week study period. Carnitine content was significantly decreased in hypertrophied hearts compared to control hearts, but was normalized by propionyl L-carnitine treatment. Propionyl L-carnitine treatment also prevented the decrease in cardiac work that occurred in hypertrophied hearts compared to control hearts. The primary change in energy substrate use in hypertrophied hearts was a decrease in fatty acid oxidation rates. Glucose and lactate oxidation were similar in control and hypertrophied hearts. While glycolytic rates were slightly higher at moderate workloads, this was not seen at high workloads. Surprisingly, propionyl L-carnitine treatment did not reverse the depression of fatty acid oxidation seen in hypertrophied rat hearts. In fact, a further significant decrease in fatty acid oxidation occurred, such that the contribution of fatty acid oxidation to ATP production decreased from 35 to 26%. Since propionyl L-carnitine treatment increased cardiac work in hypertrophied hearts despite an overall decrease in ATP production rates, an increase in cardiac efficiency was seen. In treated vs. untreated hypertrophied hearts efficiency (cardiac work/ATP produced) increased from 0.23 to 0.40 $ml \cdot mm\ Hg \cdot \mu mol\ ATP^{-1} \cdot g$ dry weight at high workloads. These data suggest that the beneficial effect of propionyl L-carnitine on mechanical function in the hypertrophied heart does not result from a normalization of fatty acid oxidation, but rather from an increase in the efficiency of translating ATP production into cardiac work.

Keywords: Hypertrophy; L-Carnitine; Fatty acid; Cardiac efficiency

1. Introduction

Pressure-overload myocardial hypertrophy is associated with changes in myocardial contractility that include diastolic dysfunction and impairment of relaxation (Capasso et al., 1981; Taegetmeyer and Overturf, 1988; Micheletti et al., 1994b). Although the increased duration of isometric contraction and relaxation is paralleled by a shift in myosin isoenzymes from a faster (V_1) to slower (V_3) enzymatic form (Cappelli et al., 1989; Mercardier et al., 1981), the mechanisms responsible for these changes in contractile function are not yet fully characterized. A number of investigators have implicated alterations in myocardial energy metabolism as contributing to the depression in contractile function (Bishop and Aultshuld, 1971; Ingwall, 1984; Cunningham et al., 1990; Kayaga et al., 1990; El Alaoui-Talibi et al., 1992; Schwartz et al., 1992; Allard et al., 1994; Taegetmeyer and Overturf, 1988; Micheletti et al., 1994b; Yang et al., 1992).

One prominent change in energy substrate metabolism that occurs in the hypertrophied heart is a de-

* Corresponding author. 423 Heritage Medical Research Building, The University of Alberta, Edmonton, Alberta, Canada T6G 2S2. Tel. (403) 492-2170, fax (403) 492-9753, e-mail glopasch@gpu.srv.ualberta.ca.

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crease in the rates of fatty acid oxidation (El Alaoui-Talibi et al., 1992; Allard et al., 1994). This is accompanied by an increase in glycolytic rates (Allard et al., 1994) and an increase in the activity of enzymes associated with anaerobic metabolism (Taegetmeyer and Overturf, 1988; Ingwall, 1984). The decrease in fatty acid oxidation has been suggested to occur secondary to the decreased carnitine content seen in hypertrophied myocardium (El Alaoui-Talibi et al., 1992; Reibel et al., 1983). Carnitine is a required co-factor for the translocation of long chain fatty acids into the mitochondria where they subsequently undergo β-oxidation. Since fatty acid oxidation is the primary source of ATP production in the heart, a decrease in fatty acid oxidation may result in a depression of contractile function secondary to a decrease in ATP production.

Propionyl L-carnitine is a naturally occurring derivative of carnitine that cannot only increase tissue carnitine levels (Paulson et al., 1986), but also has the potential to replenish key mitochondrial tricarboxylic acid cycle intermediates (Davies et al., 1980; Tassani et al., 1994). Several studies have shown beneficial effects of propionyl L-carnitine on functional and hemodynamic parameters measured in vivo and in vitro (Motterlini et al., 1992; Yang et al., 1992). Propionyl L-carnitine has also been shown to be beneficial to cardiac function in humans (Bartels et al., 1992). Recently Micheletti et al. (1994a) have shown beneficial hemodynamic effects of long-term propionyl L-carnitine treatment on hypertrophied hearts exposed to an enhanced preload stress, as well as a correction of the mechanical abnormalities seen in pressure overloaded rat papillary muscle and skinned trabeculae (Micheletti et al., 1994b). As shown in the preceding study, propionyl L-carnitine treatment can also improve hemodynamic function in vivo in aortic-banded rats. The exact mechanism of action of propionyl L-carnitine has not been delineated.

Short term administration of propionyl L-carnitine and the beneficial effect on mechanical and hemodynamic behavior of hypertrophied hearts has been suggested to involve an improvement of metabolism (Motterlini et al., 1992; Yang et al., 1992). However, the effects of chronic propionyl L-carnitine treatment on whole heart energy substrate metabolism is not known. We therefore investigated the effects of long term propionyl L-carnitine treatment on mechanical function and overall energy substrate metabolism in isolated working hearts obtained from aortic-banded rats.

2. Materials and methods

2.1. Materials

D-[5-^{3}H]glucose, D-[U-^{14}C]glucose, [U-^{14}C]lactate and [9,10-^{3}H(N)]palmitate were purchased from Du Pont-New England Nuclear. Bovine serum albumin (fraction V) was obtained from Boehringer Mannheim, Germany. Hyamine hydroxide (methylbenzethonium; 1 M in methanol solution) was obtained from Sigma Chemical Company. Dowex 1-X4 anion exchange resin (200–400 mesh chloride form) was obtained from Bio-Rad. ACS counting scintillant was purchased from Amersham Canada. Ecolite counting scintillant was obtained from ICN Biomedicals Canada. All other chemicals were reagent grade.

2.2. Hypertrophy model

A mild pressure overload hypertrophy was produced as previously described (Allard et al., 1994). Briefly, male Wistar-Kyoto rats weighing 60–70 g, were anesthetized with a 50 $mg \cdot kg^{-1}$ intraperitoneal injection of methohexital sodium. A lateral incision of the abdominal wall was used to expose the abdominal aorta. The aorta was isolated and a minimally occlusive (0.4 mm) hemoclip was applied in a suprarenal position. In control animals the aorta was isolated but not banded. The incision was then closed and the animals were allowed to recover.

2.3. Propionyl L-carnitine feeding protocol

Propionyl L-carnitine (lot No. 920068) was obtained from Prassis Sigma Tau Research Institute (Milano, Italy). Following surgery, sham-operated (control) and aortic-banded animals were randomly divided into one of two groups. A group of sham-operated and a group of aortic-banded animals were fed food and water ad libitum for the 8 week study period. The second group of control and aortic-banded animals underwent a similar protocol except that propionyl L-carnitine was present in the drinking water throughout the eight week period. The final concentration of the drinking water was adjusted such that the animals consumed 60 $mg \cdot kg^{-1} \cdot day^{-1}$ of propionyl L-carnitine. Following the 8 week treatment period, the animals were anesthetized with pentobarbital (60 $mg \cdot kg^{-1}$ i.p.) and the hearts quickly removed for experimentation.

2.4. Isolated working heart perfusions

Hearts were immediately cannulated, and an aortic retrograde perfusion was initiated. The left atrium was subsequently cannulated, following which the hearts were switched to the working mode. The perfusate consisted of a Krebs-Henseleit buffer (pH 7.4) gassed with a 95% O_2-5% CO_2 air mixture, with a free Ca^{2+} concentration of 1.25 mM. The perfusion buffer also contained 11 mM glucose, 0.5 mM lactate, 0.4 mM palmitate prebound to 3% bovine serum albumin, and 100 $\mu U \cdot ml^{-1}$ insulin.

The initial perfusion pressures of the working hearts were set at a 11.5 mm Hg left atrial preload and an 80 mm Hg aortic afterload (moderate work). All hearts were paced at a rate of 280 beats · min^{-1}. This was accomplished by delivering a 5 V pulse through the perfusion cannulae (0.5 ms pulse duration). Hearts were perfused for a 30 min period under these conditions, following which time they were exposed to a 15 min period in which the aortic outflow line was clamped (high work). Perfusion buffer samples required for metabolic analysis were taken at 10 min intervals during the first 30 min, and at 5 min intervals during the high work period. Peak systolic pressure was recorded on a Grass 79-D physiograph with a Spectramed p 23XL pressure transducer in the aortic outflow line. The pressure transducer was in line just above the aortic valve. As a result, even though the hearts were perfused against a 80 mm Hg hydrostatic afterload, peak pressure in the aortic line during systole exceeded 80 mm Hg. Developed pressure was calculated as the difference between peak systolic pressure and peak diastolic pressure in the aortic line. Cardiac output and aortic flow were measured using Transonic ultrasonic flow probes present in the atrial preload line and the aortic outflow line, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. Perfusate oxygen content was measured using YSI micro oxygen electrodes in the preload line and in the line originating from the cannulated pulmonary artery.

2.5. Measurement of glycolysis and glucose oxidation

Glycolysis and glucose oxidation were simultaneously measured in isolated working hearts by the incorporation of radiolabelled [5-^{3}H/U-^{14}C]glucose into the perfusion buffer. The measurement of glycolysis was achieved by determining the amount of ^3H_2O produced (which is liberated from [5-^{3}H]glucose at the enolase step of glycolysis). Separation of ^3H_2O from [^{3}H/^{14}C]glucose was achieved as described earlier (Saddik and Lopaschuk, 1991; Allard et al., 1994) Measurements of glucose oxidation was achieved by quantitatively collecting $^{14}CO_2$ produced (which is liberated from [U-^{14}C]glucose at the level the pyruvate dehydrogenase complex and in the tricarboxylic acid cycle) as described previously (Saddik and Lopaschuk, 1991). Briefly the $^{14}CO_2$ produced as a gas was collected from the sealed perfusion system through a 1 M methylbenzethonium hydroxide solution, which acted as a $^{14}CO_2$ trap. Perfusate samples containing [^{14}C]bicarbonate were also collected and stored under mineral oil to prevent the liberation of $^{14}CO_2$, and subsequently injected into closed metabolic reaction flasks containing 9 N H_2SO_4. The $^{14}CO_2$ released from the perfusion buffer, was trapped in center wells filled with 1 M methylbenzethonium hydroxide. Afterwards the center wells were removed and counted in ACS scintillant using standard counting procedures. Therefore, total $^{14}CO_2$ production was determined by analyzing samples of the methylbenzethonium hydroxide used as a $^{14}CO_2$ gas trap, and perfusate samples which contained [^{14}C]bicarbonate.

2.6. Measurement of lactate and palmitate oxidation

The measurement of lactate and palmitate oxidation was achieved by incorporating [U-^{14}C]lactate and [9,10-^{3}H]palmitate into the perfusion buffer of a parallel series of hearts. Lactate oxidation was measured in a manner similar to that described for glucose oxidation. Palmitate oxidation was quantified by measuring ^3H_2O production from [^{3}H]palmitate. The ^3H_2O in the perfusate was separated from [^{3}H]palmitate as described previously (Saddik and Lopaschuk, 1991). Briefly, this method entails the extraction of perfusion buffer samples with a mixture of chloroform, methanol, and a KCl-HCl solution. The aqueous phase was taken and extracted again using chloroform, methanol and a KCl-HCl solution. Samples of the aqueous phase were taken and counted to determine the content of ^3H_2O water. This method results in a greater than 99.7% extraction and separation of ^3H_2O from the [^{3}H]palmitate. The spill-over of [^{14}C]lactate into the aqueous phase of the extract was taken into account by subtracting the amount of spill-over counts from the ^{14}C counting window of the scintillation counter into the ^{3}H counting window.

2.7. Tissue analysis

At the end of the perfusions, heart ventricles were freeze-clamped using Wollenberger clamps cooled to the temperature of liquid nitrogen. The frozen tissue was weighed to determine total wet ventricular weight. The atria were dried, weighed and used in the calculation of whole heart weight.

Frozen ventricular tissue was powdered using a mortar and pestle cooled to the temperature of liquid nitrogen. A portion of the tissue was dried and used to calculate the dry-to-wet ratio of the tissue. The remainder of the tissue was stored in a −70°C freezer. The dry-to-wet ratio as well as the wet ventricular weight and the atria dry weight were used to calculate the total dry weight of the heart.

2.8. Determination of coenzyme A esters, ATP and creatine-P

Myocardial coenzyme A esters were determined in perchloric acid extracts of ventricular tissues as described previously (Saddik et al., 1993). This involved a

Table 1
Characteristics of aortic-banded and control rats treated with propionyl L-carnitine (60 mg · kg^{-1} · day^{-1} orally) for an 8 week period

Experimental group	Heart weight (g wet weight)	Body weight (g)	Heart weight to body weight ratio ($\times 10^{-3}$)
Control			
Vehicle ($n = 18$)	1.501 ± 0.028	320 ± 3	4.69 ± 0.08
Propionyl L-carnitine ($n = 26$)	1.459 ± 0.043	314 ± 5	4.65 ± 0.11
Hypertrophy			
Vehicle ($n = 22$)	1.777 ± 0.074 [a]	304 ± 6	5.86 ± 0.24 [a]
Propionyl L-carnitine ($n = 26$)	1.907 ± 0.083 [a]	318 ± 3	6.03 ± 0.28 [a]

Values are the means ± S.E.M. of the number of hearts shown in parentheses. Heart and body weights were obtained 8 weeks following sham operation (Control) or aortic banding (Hypertrophy). [a] Significantly different from corresponding control rats.

modified high performance liquid chromatography procedure based on the procedure of King et al. (1988). ATP and creatine-P were extracted from hearts and measured spectrophotometrically (Lopaschuk et al., 1993).

2.9. Determination of myocardial and plasma carnitine content

Total, free, short and long chain carnitine ester content was determined following perchloric acid extracts of ventricular tissue as described previously (Broderick et al., 1992). A radiometric assay method which involves the transfer of [^{3}H]-acetyl-coenzyme A to free L-carnitine was used to measure the carnitine content of the tissue (McGarry and Foster, 1976).

Carnitine levels were also determined in blood samples taken from the chest cavities of the animals immediately after the removal of the heart. The blood samples where centrifuged and the plasma was collected and analyzed for carnitine content. Plasma carnitine content was measured using a kit from Boehringer Mannheim (Cat. No. 1242008).

2.10. Statistical analysis

The data are represented as the mean ± S.E.M. Analysis of variance, followed by the Neuman-Keuls test was used in the determination of statistical difference between groups. A value of $P \leq 0.05$ was regarded as significant.

3. Results

3.1. Characteristics of control and aortic banded rats

Heart and body weights in the different experimental groups are shown in Table 1. The banding procedure resulted in an 18% increase in wet heart weight of

Table 2
Effects of long term propionyl L-carnitine treatment (60 mg · kg^{-1} · day^{-1} orally) on tissue levels of carnitine esters in hearts from control and aortic-banded rats

Experimental group	Carnitine ester			
	Free	Short chain	Long chain	Total
Control				
Vehicle ($n = 8$)	4009 ± 439	1448 ± 412	423 ± 157	5465 ± 474
Propionyl L-carnitine ($n = 10$)	6151 ± 333 [a]	772 ± 340	387 ± 116	7156 ± 276 [a]
Hypertrophy				
Vehicle ($n = 10$)	3290 ± 258	815 ± 156	468 ± 108	4526 ± 197 [b]
Propionyl L-carnitine ($n = 8$)	5196 ± 427 [a]	1139 ± 245	360 ± 103	6411 ± 305 [a]

Values are the means ± S.E.M. (nmol · g dry weight^{-1}) of the number of hearts shown in parentheses. Carnitine levels were measured in hearts frozen following the 15 min period of high work. [a] Significantly different from comparable vehicle-treated hearts. [b] Significantly different from vehicle-treated control hearts.

Table 3
Effects of long term propionyl L-carnitine treatment (60 mg · kg^{-1} · day^{-1} orally) on mechanical function in isolated working hearts from control and aortic-banded rats perfused at a moderate workload

Perfusion condition	Heart rate (beats · min^{-1})	Peak systolic pressure (mm Hg)	Developed pressure (mm Hg)	Heart rate ×peak systolic pressure (×10^{-3})	Heart rate × developed pressure (×10^{-3})	Cardiac output (ml · min^{-1})	Coronary flow (ml · min^{-1})	Cardiac work (ml · min · mm Hg · 10^{-2})
Control								
Vehicle (n = 16)	280 [c]	103.9 ± 2.4	41.2 ± 2.7	29.1 ± 0.7	11.5 ± 0.8	37.1 ± 3.2	26.4 ± 2.3	39.4 ± 3.8
Propionyl L-carnitine (n = 17)	280	105.3 ± 2.1	44.0 ± 2.8	29.5 ± 0.6	12.3 ± 0.8	39.1 ± 2.9	28.2 ± 1.9	41.9 ± 3.5
Hypertrophy								
Vehicle (n = 18)	263 ± 12 [d]	91.4 ± 5.1	39.1 ± 2.8	24.3 ± 1.9 [a]	10.1 ± 0.7	28.0 ± 2.6 [a]	21.9 ± 1.9	26.5 ± 3.3 [a]
Propionyl L-carnitine (n = 21)	273 ± 5	96.4 ± 4.8	41.4 ± 3.4	26.5 ± 1.5	11.4 ± 1.0	35.9 ± 3.4 [b]	29.2 ± 3.1	36.0 ± 3.7 [b]

Values are the means ± S.E.M. of the number of hearts shown in parentheses. Heart function shown was measured in hearts perfused at a 11.5 mm Hg left atrial preload and 80 mm Hg afterload. Values were obtained at the end of the 30 min period of moderate work. [a] Significantly different from control hearts. [b] Significantly different from hypertrophied hearts perfused in the absence of propionyl L-carnitine. [c] Hearts were paced at 280 beats · min^{-1}. [d] Not all hypertrophied hearts could be paced at 280 beats · min^{-1}.

banded animals compared to control animals that did not receive propionyl L-carnitine. Propionyl L-carnitine treatment did not significantly alter heart weight or body weight in either the control or aortic-banded animals.

3.2. Myocardial carnitine and plasma carnitine content

The effects of propionyl L-carnitine treatment on myocardial carnitine content is shown in Table 2. Total myocardial carnitine content was depressed in the hy-

Table 4
Effects of long term propionyl L-carnitine treatment (60 mg · kg^{-1} · day^{-1} orally) on mechanical function in isolated working hearts from control and aortic-banded rats perfused at high workloads

Perfusion condition	Heart rate (beats · min^{-1})	Peak systolic pressure (mm Hg)	Developed pressure (mm Hg)	Heart rate ×peak systolic pressure (×10^{-3})	Heart rate × developed pressure (×10^{-3})	Cardiac output (ml · min^{-1})	Coronary flow (ml · min^{-1})	Cardiac work (ml · min · mm Hg · 10^{-2})
Control								
Vehicle (n = 16)	280 [c]	128.9 ± 4.5	34.4 ± 2.4	36.1 ± 1.3	9.6 ± 0.7	30.4 ± 2.5	30.4 ± 2.5 [e]	39.8 ± 4.0
Propionyl L-carnitine (n = 17)	280	130.5 ± 5.6	36.2 ± 2.6	36.6 ± 1.6	10.2 ± 0.7	32.8 ± 2.2	32.8 ± 2.2 [e]	44.1 ± 3.8
Hypertrophy								
Vehicle (n = 18)	258 ± 13 [d]	110.8 ± 8.2	33.7 ± 2.4	29.3 ± 2.8 [a]	8.4 ± 0.6	24.9 ± 2.3	24.9 ± 2.3 [e]	28.5 ± 3.6 [a]
Propionyl L-carnitine (n = 21)	280 ± 1	120.7 ± 7.2	37.0 ± 3.2	33.8 ± 2.1	10.4 ± 0.9	34.1 ± 3.0 [b]	34.1 ± 3.0 [b,e]	42.3 ± 4.2 [b]

Values are the means ± S.E.M. of the number of hearts shown in parentheses. Heart function shown was measured in hearts perfused at a 11.5 mm Hg left atrial preload and in which the aortic afterload line was clamped. Values shown were obtained at the end of the 15 min period of high work. [a] Significantly different from control hearts. [b] Significantly different from hypertrophied hearts obtained from rats not administered propionyl L-carnitine. [c] Hearts were paced at 280 beats · min^{-1}. [d] Not all hypertrophied hearts could be paced. [e] Coronary flow equals cardiac output when the aortic afterload line is clamped.

pertrophied rat hearts from untreated animals compared to control animals. This was primarily due to a decrease in the free and short chain esters of carnitine. Propionyl L-carnitine treatment resulted in a significant increase in total carnitine levels in both control and hypertrophied hearts. As a result, the decrease in carnitine content observed in hypertrophied hearts was completely reversed by propionyl L-carnitine treatment. The primary effect of propionyl L-carnitine treatment in both hypertrophied and control hearts was an increase in the free carnitine content of hearts.

Plasma carnitine levels were increased in both the control and aortic-banded animals treated with propionyl L-carnitine. The plasma levels in control animals increased from 5.5 ± 2.1 to 9.5 ± 1.8 mg · ml^{-1} ($n = 3$), and the aortic banded animals went from 7.5 ± 1.7 to 11.5 ± 1.0 mg · ml^{-1} ($n = 3$).

3.3. Mechanical function of isolated working control and hypertrophied hearts

Mechanical function in isolated working hearts perfused at a moderate workload is shown in Table 3. Throughout the perfusion protocol an attempt was made to pace the hearts at 280 beats · min^{-1}. Although the control hearts could be successfully paced, 3 of the 18 vehicle-treated hypertrophied hearts and 6 of the 21 propionyl L-carnitine-treated hypertrophied hearts would not accept the pace and were therefore allowed to beat spontaneously. In hearts from untreated animals, rate-pressure product, cardiac output, and cardiac work were all depressed in hypertrophied hearts compared to control. In contrast, no difference in heart function was seen between hearts obtained from propionyl L-carnitine-treated aortic-banded animals, and hearts from either the propionyl L-carnitine-treated or untreated control animals. Propionyl L-carnitine treatment increased both cardiac output and cardiac work in hypertrophied hearts subjected to moderate workloads.

Mechanical function in hearts perfused at high workloads is shown in Table 4. In all groups the occlusion of aortic outflow to produce high work resulted in an increased peak systolic pressure produced by the hearts. However, in hypertrophied hearts from untreated animals a significant decrease in rate pressure product, coronary flow and cardiac work compared to control hearts (note that under these conditions the entire cardiac output consists of coronary flow). The hypertrophied hearts of propionyl L-carnitine-treated animals showed a significant improvement in coronary flow and cardiac work compared to hypertrophied hearts from untreated animals. Function in hypertrophied hearts from propionyl L-carnitine-treated rats did not differ from that of either of the two control groups.

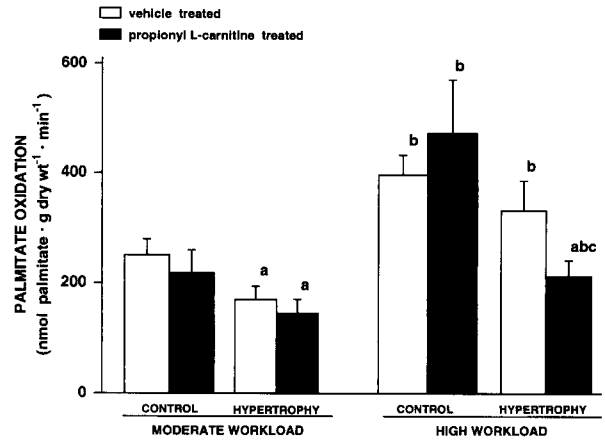

Fig. 1. Effects of long term propionyl L-carnitine treatment (60 mg·kg^{-1}·day^{-1} orally) on fatty acid oxidation rates in isolated working hearts from control and aortic-banded rats perfused at moderate and high workloads. Values are the means ± S.E.M. of 7–12 hearts in each group. Hearts were perfused at either a moderate workload or high workload, as described in Methods. [a] Significantly different from vehicle treated control hearts. [b] Significantly different from comparable hearts at a moderate workload. [c] Significantly different from comparable vehicle-treated heart.

3.4. Energy substrate metabolism in control and hypertrophied hearts

Fig. 1 shows the steady state rates of palmitate oxidation at both moderate and high workloads in both control and hypertrophied hearts. In the untreated group, hypertrophied hearts had significantly depressed rates of fatty acid oxidation compared to control hearts. Propionyl L-carnitine treatment did not have any effect on fatty acid oxidation rates in control hearts. Surprisingly, propionyl L-carnitine treatment did not increase fatty acid oxidation rates in the hypertrophied hearts. In fact, a tendency towards a decrease in fatty acid oxidation rates was seen (not significant). This occurred despite the fact that propionyl L-carnitine significantly increased myocardial carnitine content. When the untreated control and hypertrophied hearts were subjected to high work, rates of fatty acid oxidation increased. Once again, propionyl L-carnitine treatment did not increase rates of fatty acid oxidation in the hypertrophied hearts, but rather resulted in a significant decrease in rates.

Fig. 2 shows the steady state rates of glucose oxidation in control and hypertrophied hearts. At moderate workloads no difference in glucose oxidation was seen between control and hypertrophied hearts. Propionyl L-carnitine treatment significantly increased glucose oxidation in control, but not in hypertrophied hearts. At high workloads a dramatic increase in glucose oxidation was observed in all groups. No significant difference in rates between the experimental groups were observed.

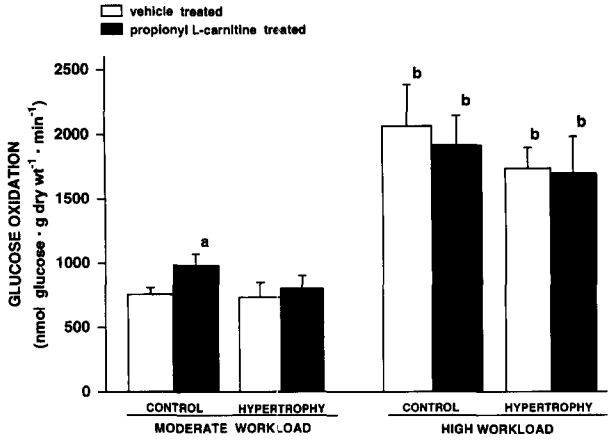

Fig. 2. Effects of long term propionyl L-carnitine treatment (60 $mg \cdot kg^{-1} \cdot day^{-1}$ orally) on glucose oxidation rates in isolated working hearts from control and aortic-banded rats perfused at moderate and high workloads. Values are the means ± S.E.M. of 7–11 hearts in each group. Hearts were perfused at either a moderate workload or high workload, as described in Methods. [a] Significantly different from vehicle treated control hearts. [b] Significantly different from comparable hearts at a moderate workload.

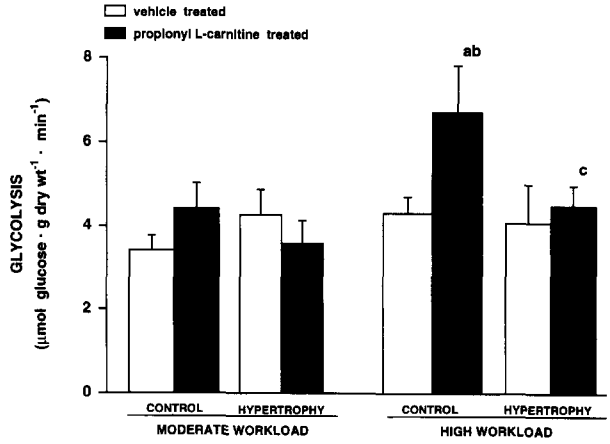

Fig. 4. Effects of long term propionyl L-carnitine treatment (60 $mg \cdot kg^{-1} \cdot day^{-1}$ orally) on glycolytic rates in isolated working hearts from control and aortic-banded rats perfused at moderate and high workloads. Values are the means ± S.E.M. of 7–10 hearts in each group. Hearts were perfused at either a moderate workload or high workload, as described in Methods. [a] Significantly different from vehicle treated control hearts. [b] Significantly different from comparable hearts at a moderate workload. [c] Significantly different from comparable vehicle treated hypertrophied hearts.

Fig. 3 shows the steady state rates of lactate oxidation in control and hypertrophied hearts. Rates at moderate work were comparable in all experimental groups. Propionyl L-carnitine treatment did result in a significant increase in lactate oxidation in control hearts at high workloads, but not in hypertrophied hearts.

Fig. 4 shows the steady state rates of glycolysis in control and hypertrophied hearts. Glycolytic rates were found to be substantially higher than glucose oxidation rates (Fig. 2) in all experimental groups. No major differences in glycolytic rates were seen between control and hypertrophied hearts at either moderate or high workloads. Propionyl L-carnitine treatment did result in an increase in glycolysis in control hearts at high workloads, which again was not seen in the hypertrophied hearts.

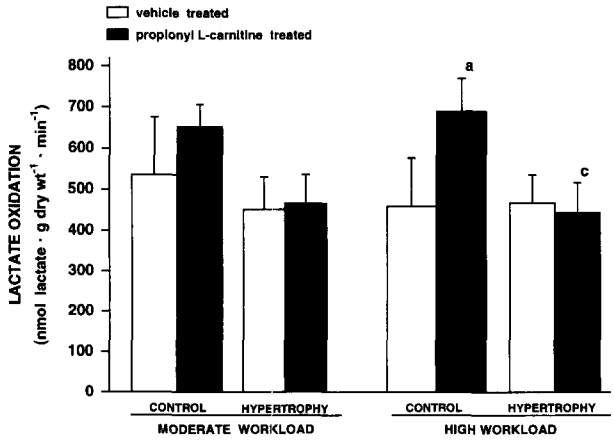

Fig. 3. Effects of long term propionyl L-carnitine treatment (60 $mg \cdot kg^{-1} \cdot day^{-1}$ orally) on lactate oxidation rates in isolated working hearts from control and aortic-banded rats perfused at moderate and high workloads. Values are the means ± S.E.M. of 6–14 hearts in each group. Hearts were perfused at either a moderate workload or high workload, as described in Methods. [a] Significantly different from vehicle treated control hearts. [c] Significantly different from comparable vehicle-treated hypertrophied hearts.

3.5. ATP production rates in control and hypertrophied hearts

Fig. 5 shows the rates of ATP production calculated from the steady state rates of substrate utilization shown in Figs. 1–4. Standard errors are not included in this figure since rates were obtained from different hearts in each of the perfusion series. In control hearts perfused at moderate work approximately 50% of the ATP production was derived from the oxidation of fatty acids. ATP production rates were slightly decreased in hypertrophied hearts obtained from untreated animals. This may reflect the slight decrease in mechanical function seen in the hypertrophied hearts. As expected overall ATP production rates in all groups increased when workload was increased. The additional ATP produced during the increase in workload was primarily derived from glucose oxidation. Again, at the high workload ATP production rates were slightly decreased in hypertrophied hearts vs control hearts obtained from untreated animals.

In the propionyl L-carnitine-treated control group, hearts showed a slight increase in ATP production compared to untreated control hearts, at both moderate and high workloads. However, in hypertrophied hearts from propionyl L-carnitine-treated animals, ATP

production either did not change (moderate workload) or decreased (high workload) when compared to hypertrophied hearts obtained from untreated rats.

At the end of the perfusions, hearts were frozen and a portion of the tissue used to measure ATP levels. ATP levels were similar in vehicle treated and propionyl L-carnitine-treated control hearts (23.46 ± 1.27 and 22.48 ± 1.27 μmol · g dry weight^{-1}, respectively). In hypertrophied hearts, propionyl L-carnitine also did not alter ATP levels (25.26 ± 1.76 and 21.90 ± 0.98 μmol · g dry weight^{-1} in vehicle-treated and propionyl L-carnitine-treated hypertrophied hearts, respectively). Creatine-P levels were also similar in all perfusion groups (36.48 ± 1.48, 36.42 ± 1.61, 34.54 ± 1.63 and 32.22 ± 1.60 μmol · g dry weight^{-1} in vehicle-treated control, propionyl L-carnitine-treated control, vehicle-treated hypertrophied and propionyl L-carnitine-treated hypertrophied hearts, respectively).

3.6. Cardiac efficiency in control and hypertrophied hearts

Because propionyl L-carnitine treatment improved cardiac work without an increase in myocardial ATP production, we also calculated the cardiac work performed per ATP produced (i.e. cardiac efficiency). As shown in Fig. 6 (upper panel), cardiac work per ATP produced in hypertrophied hearts was slightly lower than control hearts at moderate workloads. At high workloads, cardiac efficiency decreased in both groups, with the hypertrophied hearts again being slightly lower than control hearts.

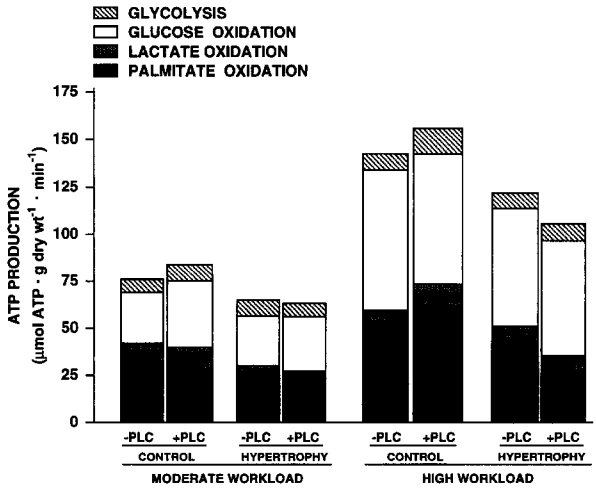

Fig. 5. Effects of long term propionyl L-carnitine treatment (60 mg·kg^{-1}·day^{-1} orally) on ATP production rates in isolated working hearts from control and aortic-banded rats perfused at moderate and high workloads. Values were calculated from the data from Figs. 1–4. A value of 2 ATP molecules produced per molecule of glucose passing through glycolysis, 36 ATP molecules produced per molecule of glucose oxidized, 18 ATP molecules produced per molecule of lactate oxidized, and 129 ATP molecules produced per molecule of fatty acid oxidized were used in the calculations.

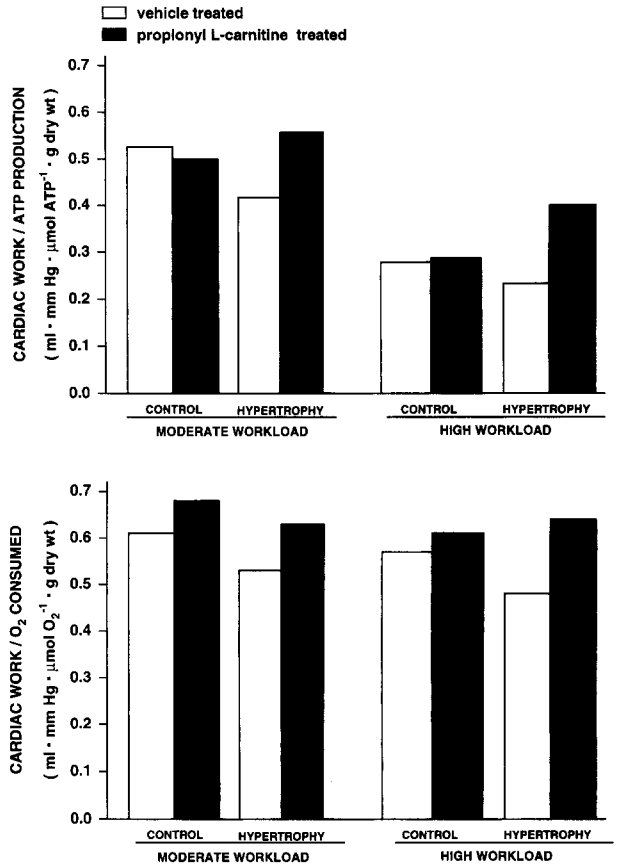

Fig. 6. Effects of long term propionyl L-carnitine treatment (60 mg·kg^{-1}·day^{-1} orally) on cardiac efficiency expressed as cardiac work per ATP produced (upper panel) and cardiac work per O_2 consumed (lower panel) of isolated working hearts from control and aortic-banded rats perfused at moderate and high workloads. Values in Fig. 6 (upper panel) were calculated by dividing cardiac work performed by the hearts in Tables 3 and 4 from the ATP production rates shown in Fig. 5. Values for Fig. 6 (lower panel) were obtained by dividing cardiac work performed by the hearts in Tables 3 and 4 from O_2 consumption rates shown in Table 5.

Propionyl L-carnitine treatment did not alter cardiac work per ATP produced in control hearts at either moderate or high workloads. However, propionyl L-carnitine treatment did dramatically increase efficiency in the hypertrophied hearts at both moderate and high workload. This suggests that the primary metabolic effect of chronic propionyl L-carnitine treatment is to reduce the ATP requirements necessary for contractile function.

The effect of propionyl L-carnitine on myocardial oxygen consumption is shown in Table 5. In hypertrophied hearts perfused at moderate or high workloads, the improvement in heart function observed in propionyl L-carnitine treated hearts was not accompanied by a significant increase in oxygen consumption at either moderate or high workloads. Propionyl L-carnitine re-

sulted in a 48% increase in cardiac work at high workloads ($P < 0.05$) in the hypertrophied hearts, even though oxygen consumption increased by only 11% (P = N.S.). As a result, the beneficial effects of propionyl L-carnitine in the hypertrophied heart are accompanied by an increase in cardiac work per oxygen consumed (Fig. 6, lower panel).

3.7. Acetyl coenzyme A, succinyl coenzyme A and malonyl coenzyme A esters in the hypertrophied heart

Because propionyl L-carnitine treatment did not improve fatty acid oxidation in the hypertrophied hearts we measured acetyl coenzyme A and succinyl coenzyme A levels in hearts frozen at the end of the high work (Table 6). This would give an indication as to whether acetyl coenzyme A supply for the tricarboxylic acid cycle and the level of tricarboxylic cycle intermediates were affected by the lower rates of fatty acid oxidation. Acetyl coenzyme A levels in hypertrophied hearts were significantly lower than in control hearts. Because the majority of acetyl coenzyme A is in the mitochondria it suggests that the supply of acetyl coenzyme A for the tricarboxylic cycle from fatty acid β-oxidation may be limiting in the hypertrophied hearts. Propionyl L-carnitine treatment prevented the decrease in acetyl coenzyme A and succinyl coenzyme A from occurring. This increase in acetyl coenzyme A and succinyl Coenzyme A levels occurred even though rates of fatty acid oxidation were not increased in hypertrophied hearts from propionyl L-carnitine-treated rats.

Previous studies have suggested that increasing tissue carnitine content can stimulate the transfer of acetyl coenzyme A from the mitochondrial to the cytoplasmic compartment (Lysiak et al., 1988; Broderick et al., 1992). Since increased cytoplasmic acetyl coenzyme A has the potential to stimulate acetyl coenzyme A carboxylase (Saddik et al., 1993; Lopaschuk et al., 1994), we also measured malonyl coenzyme A levels (the product of acetyl coenzyme A carboxylase, which is a potent inhibitor of fatty acid oxidation). Malonyl coenzyme A levels were decreased in hypertrophied hearts compared to control (which is expected if myocardial acetyl coenzyme A levels are decreased). Of interest is that propionyl L-carnitine treatment resulted in a dramatic increase in malonyl coenzyme A levels in the hypertrophied hearts. Surprisingly, a similar effect of propionyl L-carnitine on malonyl coenzyme A levels was not observed in control hearts.

Table 5
Effects of long term propionyl L-carnitine treatment (60 mg·kg^{-1}·day^{-1} orally) on oxygen consumption rates in isolated working hearts from control and aortic-banded rats perfused at moderate and high workloads

Perfusion condition	Oxygen consumption (μmol·g dry weight^{-1}·min^{-1})	
	Moderate workload	High workload
Control		
Vehicle ($n = 14$)	65±6	70±8
Propionyl L-carnitine ($n = 15$)	62±4	72±5
Hypertrophy		
Vehicle ($n = 15$)	50±4	59±6
Propionyl L-carnitine ($n = 19$)	57±5	66±5

Values are the means±S.E.M. of the number of hearts shown in parentheses. Oxygen consumption was measured in hearts perfused at a 11.5 mm Hg left atrial preload and 80 mm Hg afterload (Moderate workload) or at a 11.5 mm Hg and in which the aortic afterload line was clamped (High workload).

Table 6
Effects of propionyl L-carnitine treatment on myocardial content of acetyl coenzyme A, succinyl coenzyme A, and malonyl coenzyme A in control and hypertrophied isolated working rat hearts frozen at the end of a high workload

Perfusion Condition	Acetyl coenzyme A	Succinyl coenzyme A	Malonyl coenzyme A
Control			
Vehicle	19.4±2.8	9.0±2.5	51.4±9.02
Propionyl L-carnitine	17.4±2.1	33.4±4.7	33.9±8.5
Hypertrophy			
Vehicle	8.7±1.2 [a]	15.0±1.7 [a]	22.6±4.0 [a]
Propionyl L-carnitine	25.3±6.5 [b]	49.7±5.4 [a,b]	100.1±8.8 [a,b]

Values represent the means±S.E.M. (nmol·g dry weight^{-1}). (n = 7–8). [a] Significantly different from comparable vehicle group. [b] Significantly different form comparable control group.

4. Discussion

The efficacy of propionyl L-carnitine in improving contractile function in hypertrophied hearts has been shown in this study, the preceding study, and in a number of earlier studies (Micheletti et al., 1994a,b; Motterlini et al., 1992; Yang et al., 1992; Bartels et al., 1992). It was originally thought that this beneficial effect was due to increasing carnitine levels in the hypertrophied myocardium, overcoming the documented depression of fatty acid oxidation seen in these hearts (El Alaoui-Talibi et al., 1992; Allard et al., 1994). However, recent evidence by Micheletti et al. (1994b) has shown that the beneficial effects of propionyl L-carnitine occur independent of its effect on restoring tissue carnitine. In our study we demonstrate that increasing tissue carnitine levels with propionyl L-carnitine treatment does not normalize fatty acid oxidation rates in hypertrophied hearts. In fact, fatty

acid oxidation rates actually decreased further if hypertrophied hearts were perfused at high work, despite the fact that propionyl L-carnitine treatment improved mechanical function in the hypertrophied hearts. Since cardiac function improved despite a decrease in rates of overall ATP production it appears that the primary effect of propionyl L-carnitine in the hypertrophied heart is to increase cardiac efficiency. Both cardiac work per ATP produced and cardiac work per oxygen consumed were increased in propionyl L-carnitine-treated hypertrophied hearts subjected to high workloads.

The fact that carnitine levels are decreased in the hypertrophied heart was first demonstrated by Reibel et al. (1983) and has since been confirmed by a number of studies. Even in the mild hypertrophy model used in this study, we also observed a significant decrease in tissue carnitine levels. El Alaoui-Talibi et al. (1992) have suggested that these low levels of carnitine may limit the entry of long chain fatty acids into the mitochondria due to a decrease in carnitine palmitoyltransferase 1 activity. This has been suggested to lead to a decrease in acetyl coenzyme A supply from β-oxidation, possibly limiting tricarboxylic acid cycle activity. As shown in Table 5 we did observe a decrease in acetyl coenzyme A levels in the untreated hypertrophied hearts compared to control hearts, as well as a decrease in the levels of the tricarboxylic acid cycle intermediate, succinyl coenzyme A. The possibility of limited carbon substrate, specifically from long-chain fatty acids such as palmitate is supported by the recent work of Cheikh et al. (1994). These authors demonstrated that in severely hypertrophied hearts due to volume overload a loss of respiratory control occurred, and appeared to be due to a limitation in the availability of appropriate oxidizable substrate. A loss of the relationship between the cytoplasmic adenylate system and myocardial oxygen consumption supports the hypothesis that the hypertrophied heart may be limited in mitochondrial NADH, especially during high work periods. This loss of respiratory control could be prevented by perfusion of hearts with octanoate, a short chain fatty acid which can bypass carnitine palmitoyltransferase 1 when taken by the mitochondria. Our own data do not suggest a limitation of β-oxidation of long chain fatty acids per se, because an increase in the rates of fatty acid oxidation occurred in the hypertrophied hearts when external work was increased. However, decreased rates of fatty acid β-oxidation and lower levels of acetyl coenzyme A and succinyl coenzyme A in hypertrophied hearts compared to control hearts do suggest that a limitation of carbon substrate supply to the tricarboxylic acid cycle at moderate work may occur.

The original objective of this study was to prevent the decrease in fatty acid oxidation from occurring in the hypertrophied heart by increasing tissue carnitine levels with propionyl L-carnitine. It was assumed that by stimulating carnitine palmitoyltransferase 1 this would normalize fatty acid utilization by the heart. Theoretically, this would increase the availability of mitochondrial NADH driving mitochondrial respiration and ATP production (Cheikh et al., 1994), resulting in an improvement of heart function. However, following long-term propionyl L-carnitine treatment, neither control nor hypertrophied hearts exhibited any acceleration in the rates of fatty acid oxidation. Instead, during the high work period the propionyl L-carnitine-treated hypertrophied hearts actually exhibited depressed rates of fatty acid oxidation. This could not be explained by an increase in the contribution of lactate and glucose oxidation as a source of acetyl coenzyme A for the tricarboxylic acid cycle. As a result, our data suggest that the translation of the ATP into mechanical work (i.e. cardiac efficiency), is improved in the hypertrophied heart, and not the actual production of ATP as suggested by Cheikh et al. (1994). This is supported by the observation that ATP and creatine-P levels remained high even though ATP production and oxygen consumption did not increase even though workload increased. The reason for the improvement in efficiency is not clear. At both moderate and high workloads the cardiac efficiency was slightly lower in hypertrophied hearts compared to control hearts. This may be due to a greater use of ATP for non-contractile purposes. A number of abnormalities in Ca^{2+} modulation occur in the hypertrophied heart (Perreault et al., 1993). A recent study by Hata et al. (1994) in normal dog hearts has demonstrated that Ca^{2+} accumulation in the heart can dramatically decrease cardiac efficiency, and that a greater amount of ATP produced is directed towards basal metabolism as opposed to contractile function. It is possible that the marked increase in cardiac efficiency in the propionyl L-carnitine-treated hypertrophied hearts occurs secondary to improving some of the abnormalities in Ca^{2+} modulation in the heart. Whether the chronic propionyl L-carnitine treatment used in this protocol is altering Ca^{2+} handling in excitation-contraction coupling was not determined in this study. Another possibility is that propionyl L-carnitine is improving the actual efficiency of the contractile proteins to perform mechanical work. Chronic propionyl L-carnitine treatment will correct the prolongation of isometric contraction time course in papillary muscles obtained from aortic-banded rats, as well the delayed isometric relaxation rate and reduction in shortening velocity (Micheletti et al., 1994b). Whether this increases the efficiency of contractile function is not clear. It is also not clear how chronic propionyl L-carnitine treatment would reverse these mechanical alterations seen in the hypertrophied heart. To date, the relationship between chronic alterations in

metabolism and excitation-contraction coupling remains poorly understood. Acute addition of propionyl L-carnitine to isolated hypertrophied rat hearts will result in a dramatic stimulation of glucose oxidation (Schönekess et al., unpublished observations). As a result propionyl L-carnitine treatment of aortic-banded rats has the potential to produce a better coupling between myocardial glycolysis and glucose oxidation in vivo. Glycolysis uncoupled from glucose oxidation is a significant source of H^+ production in the heart. A better coupling would decrease H^+ production derived from hydrolysis of ATP derived from glycolysis uncoupled from glucose oxidation (Lopaschuk et al., 1993). A chronic decrease in H^+ production in hearts could potentially prevent some of the changes in contractile proteins that occur in the hypertrophied hearts, as well as increase cardiac efficiency. While we did not observe an increase in glucose oxidation in isolated hearts from aortic banded rats treated with propionyl L-carnitine, it should be noted that propionyl L-carnitine was not present in the perfusion buffer of the isolated perfused hearts.

The increased cardiac efficiency in the propionyl L-carnitine-treated hypertrophied hearts resulted in a normalization of acetyl coenzyme A and succinyl coenzyme A levels even though fatty acid β-oxidation was not normalized at either moderate or high workloads. In fact, at high workload propionyl L-carnitine resulted in an actual decrease in fatty acid oxidation. The reason for this decrease in fatty acid oxidation may involve a decrease in carnitine palmitoyltransferase 1 activity secondary to an increase in malonyl coenzyme A. We have recently demonstrated that increasing intramitochondrial acetyl coenzyme A levels results in an increase in cytoplasmic malonyl coenzyme A production (Saddik et al., 1993). The short chain carnitine carrier system in the mitochondria may function to transport acetyl groups from intramitochondrial acetyl coenzyme A to cytoplasmic coenzyme A. Increasing intracellular carnitine levels stimulates the carnitine acetyltransferase and carnitine acetyltranslocase pathway (Lysiak et al., 1988) which result in an efflux of acetylcarnitine from the mitochondria. Transfer of the acetyl groups from acetylcarnitine back onto coenzyme A should then result in a rise in cytoplasmic acetyl coenzyme A. We suggest that this activates cytoplasmic acetyl coenzyme A carboxylase which produces malonyl coenzyme A, a potent inhibitor of carnitine palmitoyltransferase 1. As shown in Table 5, propionyl L-carnitine treatment resulted in a dramatic increase in malonyl coenzyme A levels in the hypertrophied hearts. The increase in myocardial carnitine content following propionyl L-carnitine treatment may lead to increased activity of this pathway (Broderick et al., 1992), thereby shuttling acetyl groups out of the mitochondria into the cytoplasm. This increase in malonyl coenzyme A, and the further decrease in fatty acid oxidation would only be expected to occur if intramitochondrial acetyl coenzyme A levels were sufficient to maintain adequate tricarboxylic acid cycle activity. This is clearly the case in the propionyl L-carnitine-treated hypertrophied hearts, again suggesting that the primary effect of propionyl L-carnitine is to increase cardiac efficiency. Although malonyl coenzyme A levels were markedly elevated in propionyl L-carnitine-treated hypertrophied hearts, a similar increase was not seen in propionyl L-carnitine-treated control hearts. The reasons for this are not clear from this study.

This study clearly shows that chronic propionyl L-carnitine treatment increases contractile function in the hypertrophied heart. While inotropes such as digoxin, dobutamine, and epinephrine are also capable of increasing contractile function, their effects are accompanied by an increase in energy demand and oxygen consumption. Propionyl L-carnitine differs from classical inotropes in that the improvement in hypertrophied heart function following propionyl L-carnitine treatment is not associated with an increase in energy demand. Unlike other inotropes which increase both contractile function and ATP demand, propionyl L-carnitine improves the efficiency of translating ATP production into contractile function. This improvement of cardiac efficiency has obvious potential clinical benefit if energy metabolism is limiting due to a decrease in cardiac oxygen supply. Optimizing myocardial energy metabolism with agents such as propionyl L-carnitine may provide a novel approach to increasing inotropy in heart disease.

In summary, we have found that hypertrophied hearts from aortic-banded animals show decreased levels of carnitine, depressed rates of fatty acid oxidation and a decrease in mechanical function. Chronic propionyl L-carnitine treatment increased myocardial carnitine as well as mechanical function, but this was not linked to an increase in fatty acid oxidation rates. Rather, the primary effect of propionyl L-carnitine was to improve the amount of cardiac work performed per ATP produced in the hypertrophied hearts. The biochemical mechanisms responsible for this increase in cardiac efficiency remain to be determined.

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