Carnitine Stimulation of Glucose Oxidation in the Fatty Acid Perfused Isolated Working Rat Heart*

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The effects of L-carnitine on myocardial glycolysis, glucose oxidation, and palmitate oxidation were determined in isolated working rat hearts. Hearts were perfused under aerobic conditions with perfusate containing either 11 mM [2-3H/14C]glucose in the presence or absence of 1.2 mM palmitate or 11 mM glucose and 1.2 mM [1-14C]palmitate. Myocardial carnitine levels were elevated by perfusing hearts with 10 mM L-carnitine. A 60-min perfusion period resulted in significant increases in total myocardial carnitine from 4376 ± 211 to 949 ± 473 nmol/g dry weight. Glycolysis (measured as 3H2O production) was unchanged in carnitine-treated hearts perfused in the absence of fatty acids (4418 ± 300 versus 4547 ± 600 nmol glucose/g dry weight·min). If 1.2 mM palmitate was present in the perfusate, glycolysis decreased almost 2-fold compared with hearts perfused in the absence of fatty acids. In carnitine-treated hearts this drop in glycolysis did not occur (glycolytic rates were 2911 ± 231 to 4629 ± 460 nmol glucose/g dry weight·min, in control and carnitine-treated hearts, respectively. Compared with control hearts, glucose oxidation rates (measured as 14CO2 production from [U-14C]glucose) were unaltered in carnitine-treated hearts perfused in the absence of fatty acids (1819 ± 169 versus 2026 ± 171 nmol glucose/g dry weight·min, respectively). In the presence of 1.2 mM palmitate, glucose oxidation decreased dramatically in control hearts (11-fold). In carnitine-treated hearts, however, glucose oxidation was greater than that in control hearts under these conditions (158 ± 21 to 454 ± 85 nmol glucose/g dry weight·min, in control and carnitine-treated hearts, respectively). Palmitate oxidation rates (measured as 14CO2 production from [1-14C]palmitate) decreased in the carnitine-treated hearts from 728 ± 61 to 572 ± 111 nmol palmitate/g dry weight·min. This probably occurred secondary to an increase in overall ATP production from glucose oxidation (from 5.4 to 14.5% of steady state myocardial ATP production). The results reported in this study provide direct evidence that carnitine can stimulate glucose oxidation in the intact fatty acid perfused heart. This probably occurs secondary to facilitating the intramitochondrial transfer of acetyl groups from acetyl-CoA to acetyl-

nitine, thereby relieving inhibition of the pyruvate dehydrogenase complex.

Increasing myocardial carnitine (β-hydroxy-δ-trimethylaminobutyric acid) content can have a protective effect on the ischemic myocardium (1–4) and can improve heart function in cardiomyopathies associated with diabetes and carnitine deficiency (5–9). The mechanism by which carnitine exerts these effects has still not been completely delineated. Carnitine is a quaternary amine which translocates activated long-chain fatty acids across the mitochondrial matrix to the site of β-oxidation (10). Specific enzymes (carnitine palmitoyltransferase I and II) located on the matrix catalyze the reversible formation of carnitine esters of these long-chain fatty acids, to which the mitochondrial membrane is impermeable (11). By virtue of its role as a carrier, therefore, carnitine is essential for the oxidation of these substrates. Thus, the commonly proposed theories to explain the actions of carnitine in the diabetic or ischemic myocardium, by enhancing carnitine palmitoyltransferase I activity, was to increase fatty acid oxidation and prevent the accumulation of potentially toxic levels of long chain acyl-CoA.1 The accumulation of long chain acyl-CoA has been suggested to result in an inhibition of the mitochondrial ATP translocase (12).

In addition to this critical metabolic role, carnitine can exert other effects on the mitochondrial matrix. Carnitine can bind acetyl residues, where a specific short-chain carrier system (carnitine acetyltransferase, EC 2.3.1.7) is present and translocate them into the cytosol (13). On theoretical grounds, to bind acetyl residues and free up coenzyme A, carnitine should wield an essential part in the control of the intramitochondrial acetyl-CoA/CoA ratio. Recent interest has stemmed from this proposed role of carnitine as a modulator of the transmitochondrial acetyl-CoA/CoA ratio. In isolated mitochondrial preparations, carnitine lowers intramitochondrial acetyl CoA levels (14, 15). In heart mitochondria, carnitine increases CoA levels and reduces acetyl-CoA levels, resulting in a 10–20-fold decrease in the ratio of acetyl-CoA/CoA (14, 16). In human skeletal muscle mitochondria carnitine stimulates pyruvate oxidation, with a comparable increase in pyruvate dehydrogenase (EC 1.2.4.1.) activity (17). The change in the ratio of acetyl-CoA/CoA correlates with the efflux of acetyl carnitine from the mitochondria (14, 15), which is consistent with the suggestion that carnitine increasing the activity of the carnitine acetyltransferase present on mitochondrial membranes (17). The effectiveness of carnitine in stimulating glucose oxidation in intact muscle, however, has yet to be determined.

1 The abbreviation used is: CoA, coenzyme A.
Concentration is also similar to the concentration of fatty acids seen 3600) Physiograph. Palmitate can maximally inhibit glucose oxidation (25, 26). This 38330 monitored throughout the perfusion period using a Gould (model RS-159) pyruvate dehydrogenase complex. Products and substrates of 39210 occurs by slow Na+-dependent diffusion and a carrier-mediated trans-40211 port against a concentration gradient (21, 22). Therefore, in order to 41218 increase the use of glucose and concomitantly sup-43213 press the contribution of fatty acids as an energy substrate. If this indeed occurs, it would contradict the popular held notion that carnitine increases fatty acid oxidation.

In this study, we were interested in determining if carnitine-induced increases in the pyruvate dehydrogenase complex activity observed in isolated mitochondria also occurred in the intact working heart. To perform these experiments, we initially perfused hearts for a period of 60 min in the presence of 10 mM carnitine, since it has been demonstrated previously that carnitine is transported across the sarcolemmal membrane in a slow Na+-dependent manner (21, 22). Our results provide direct evidence that carnitine stimulates glucose oxidation in the intact fatty acid perfused working rat heart, with a parallel decrease in fatty acid oxidation.

**EXPERIMENTAL PROCEDURES**

**Materials**—L-Carnitine (β-hydroxy-β-trimethylaminobutyric acid, inner salt) was purchased from Sigma. [1-14C]Palmitate, D-[U-14C] glucose, and D-[2-14C] glucose 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 New England Nuclear Research Products. Dowex 1-X4 anion exchange resin (200-400 mesh chloride form) was obtained from Bio-Rad. ACS aqueous counting scintillant was purchased from Amersham Canada Ltd. (Oakville, Ontario). All other chemicals were reagent grade.

**Heart Perfusions**—Hearts from sodium pentobarbital anesthetized male Sprague-Dawley rats were excised, placed in ice-cold buffer, and quickly cannulated as described previously (23). Hearts were initially perfused with Krebs-Henseleit buffer containing 11 mM [2-3H/U-14C] glucose and 11 mM palmitate for 15 min in order to establish a steady state metabolic profile. Following this period, hearts were switched to the working mode and perfused for 90 min with buffer containing either 11 mM glucose or 11 mM glucose in the presence of 1.2 mM palmitate. All working heart buffer contained 3% bovine serum albumin and palmitate when used, was prepoured to albumin. The albumin used in these perfusions was routinely assayed for fatty acid content and was found to contain approximately 0.05 mM of fatty acids in a 3% albumin solution. We chose to use a concentration of 1.2 mM palmitate in the fatty acid perfused hearts, since we have previously demonstrated that this concentration of palmitate can maximally inhibit glucose oxidation (25, 26). This concentration is also similar to the concentration of fatty acids seen in diabetes (27), a condition in which carnitine treatment has been suggested to be beneficial.

Hearts were perfused at an 11.5 mm Hg left atrial filling pressure 80 mm Hg hydrostatic aortic afterload. Heart function, assessed by changes in heart rate and peak systolic pressure development, was monitored throughout the perfusion period using a Gould (model RS-3600) physiograph.

**L-Carnitine Loading in Hearts**—Carnitine uptake by the heart occurs by slow Na+-dependent diffusion and a carrier-mediated transport against a concentration gradient (21, 22). Therefore, in order to increase the total myocardial carnitine content, hearts were perfused in the working mode with buffer containing 11 mM glucose and 1.2 mM palmitate in the presence of carnitine for various time periods. We chose 30, 60, and 90 min of aerobic perfusion in which 10 mM carnitine was present in the buffer to determine the time course changes in heart carnitine content. Heart function was also assessed during these periods. Following these loading perfusions, hearts were switched to a 5-min Langendorff dripout, which has been demonstrated to remove any carnitine present in the ventricular cavities or extracellular space (21, 22). Thereafter, hearts were rapidly frozen with Wollan-Eberl's cryostat precooled to the temperature of liquid N2.

**Measurement of Glycolysis, Glucose Oxidation, and Palmitate Oxidation**—Glycolysis and glucose oxidation were measured simultaneously by perfusing hearts with Krebs-Henseleit buffer containing either 11 mM [2-3H/U-14C] glucose (specific activity of perfusate equaled 600,000 dpm/ml of H and 600,000 dpm/ml of 14C) or 11 mM [2-14C]glucose in the presence of 1.2 mM palmitate. To measure palmitate oxidation, hearts were perfused with 11 mM glucose and 1.2 mM [1-14C] palmitate (specific activity equalled 80,000 dpm/ml).

Steady state oxidative rates of glucose and palmitate were determined by measurement of 14CO2 as described previously (29, 30). After the 10-min retrograde dripout, hearts were perfused in a closed system that allowed quantitative collection of both gaseous and perfusate 14CO2. The 14CO2 liberated into the gaseous phase was trapped in a 1 M hyamine hydroxide solution in the air outlet line. Both gaseous and perfusate 14CO2 samples were taken at 15-min intervals during the initial and final 15 min of the 90-min aerobic perfusion. Perfusate samples were immediately stored under a volume of mineral oil to prevent liberation of 14CO2. The 14CO2 from the perfusate was subsequently extracted by injecting 1 ml of perfusate into sealed metabolic flasks containing 9 N H2SO4 and 400 μl of 1 M hyamine hydroxide in suspended centerwells. Flasks were then gently stirred for 1 h in order to release the perfusate 14CO2 present as [14C] bicarbonate. Centerwells were removed and counted in ACS scintil- liquid, using standard counting procedures. Glucose and palmitate oxidation rates were expressed as nanomoles of glucose or per gram oxidized per min/g dry weight.

To measure glycolysis, H2O was separated from [14C]glucose and [14C]glucose using columns containing Dowex 1-X4 anion exchange resin (200-400 mesh) suspended in 0.4 M potassium tetraborate (30). The Dowex in the columns was extensively washed with H2O prior to use. A 0.2-ml volume of perfusate was added to the column and eluted into scintillation vials with 0.8 ml of H2O. Following addition of ACS scintillant, the samples were subjected to standard double isotope counting procedures, with the windows set at 0-300 fm (3H) and 400-670 nm (14C). The Dowex columns were found to retain 98-99.6% of the total [14C]glucose and [14C]glucose present in the perfusate. The H2O (which passed through the column) was also counted for the small amount of [14C]glucose that passed through the column. This could be accomplished since an equal amount of [14C]glucose also passed through the column and could be used as an internal standard for the degree of [14C]glucose contamination in the H2O sample. The correction was also made for the degree of spillover into the H counting window, by measuring this degree of spillover using standards containing only [14C]glucose. Glycolytic rates were expressed as nanomoles of glucose metabolized per min/g dry weight.

**Perfusion Protocol**—A 90-min perfusion period was used to allow for a quantitative assessment of the effects of L-carnitine on glucose use. Both steady state glucose and palmitate oxidation and glycolytic rates were obtained during this period. For control values, samples were obtained at 5-min intervals during the first 15 min of perfusion. Immediately following this initial period, 10 mM L-carnitine was added to the recirculated perfusate, and the perfusion was then subsequently continued for a period of 80 min. Following the 60-80 period of carnitine loading, samples for the determination of glucose and palmitate oxidation and glycolytic flux were obtained at 5-15 min intervals throughout the remaining 15 min of the perfusion period.

A series of hearts was also perfused in the presence of 11 mM glucose and 1.2 mM palmitate for 15 min and subsequently frozen to obtain control levels of tissue metabolites.

**Tissue Analysis**—Frozen ventricular tissue was weighed and powdered in a mortar and pestle cooled to the temperature of liquid N2. A portion of the dried tissue was used to determine the dry-to-wet weight ratio. This ratio was calculated as weight of dry tissue with the wet tissue weight and the weight of the dried aortic tissue, total dry weight of the heart was determined. Extraction of ATP, long-chain acyl-CoA, and long-chain acylcarnitine was as described previously (23, 24).
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31). Measurement of ATP levels from perchloric acid extracts was determined with standard enzymatic assays (31). Extracted long-chain acyl-CoA was hydrolyzed and free CoA measured fluorometrically (31). Extracted long-chain acylcarnitine was also hydrolyzed and free carnitine measured radiometrically (32).

Statistical Analysis—Analysis of variance, followed by the Newman-Keuls test was used in the determination of statistical difference in groups containing three sample populations. The paired t test was used for statistical significant of group means. A value of p < 0.05 was considered as significant. All data are presented as mean ± S.E.

RESULTS

Effects of Carnitine Treatment Time on Total Heart Carnitine Content—We were first interested in determining the effects of perfusion time with carnitine on total intracellular myocardial carnitine accumulation. At all selected time periods, total myocardial carnitine content was increased significantly in hearts perfused with carnitine compared with the total myocardial carnitine content assayed in control hearts. Total myocardial carnitine in control hearts was 4376 ± 211 nmol/g dry weight. Following 30, 60, and 90 min of perfusion time with carnitine, respectively, total myocardial carnitine content increased to 8296 ± 335, 10,724 ± 710, and 12,239 ± 1191 nmol/g dry weight. This progressive increase in the accumulation of intracellular carnitine is consistent with the previous work of Vary and Neely (21, 22) in Langendorff hearts which suggests that myocardial uptake by the intact heart occurs by slow diffusion and a carrier mediated process.

Heart function in spontaneously beating hearts was monitored throughout the perfusions. Table I shows the heart rate, peak systolic pressure development, and the heart rate pressure product in hearts perfused with or without carnitine. There were no significant changes in heart function observed in carnitine-treated hearts perfused with glucose as substrate only. This also demonstrates that there is no deterioration of mechanical function during the long perfusion period. In the presence of palmitate, carnitine-treated hearts did have a small decrease in peak systolic pressure compared with non-carnitine-treated hearts perfused the fatty acid.

Myocardial Glucose Utilization and Palmitate Oxidation in Carnitine-treated Hearts—The effect of carnitine treatment on steady state glycolysis and glucose oxidation rates in hearts perfused in the absence of fatty acids is shown in Table II. Paralleling our earlier observations (26), glycolytic rates in control hearts were over two times higher than glucose oxidation rates, respectively. If ATP production in carnitine-treated hearts a slight decrease in the rate of palmitate oxidation was observed. When expressed in these terms, the increase in glycolysis and glucose oxidation remained, as did the decrease in palmitate oxidation.

Steady State Myocardial ATP Production—Steady state myocardial ATP production from exogenous rates of glycolysis, glucose, and palmitate oxidation is shown in Table III. As expected, if hearts are perfused with palmitate, the contribution of overall glucose utilization to ATP production is low. Both glycolysis and glucose oxidation contributed to 5.5 and 5.4% of steady state ATP production from exogenous substrate, respectively. If ATP production in carnitine-treated hearts is measured, the contribution to ATP production from glycolysis increased from 5.5 to 9.3%, whereas that of exogenous glucose increased from 5.4 to 14.5%.

An interesting observation seen in Table III is the contribution of exogenous palmitate to ATP production in carnitine-treated hearts dropped proportionally to the increase in ATP production from glucose use. The contribution of palmitate oxidation to ATP production dropped by 15%, whereas the combined increase in glycolytic and glucose oxidation ATP production increased by 15%. In addition, no major change in overall ATP production was observed. This observation is consistent with the close coupling of the glucose-fatty acid cycle (26) and a shift in acetyl-CoA production for the tricarboxylic acid cycle away from fatty acid β-oxidation,
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### TABLE II

**Effects of carnitine on overall steady state rates of glycolysis, glucose oxidation, and palmitate oxidation in isolated working hearts perfused in the presence and absence of palmitate**

Values are presented as mean ± S.E. for 6–10 hearts in each group. Hearts were perfused as described under "Experimental Procedures." L-Carnitine was added to the perfusate at the concentration of 10 mM for a period of 60 min. HR, heart rate; PSP, peak systolic pressure.

<table>
<thead>
<tr>
<th>Perfusion conditions</th>
<th>Glycolysis</th>
<th>Glucose oxidation</th>
<th>Palmitate oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol [P]glucose/g dry wt-min</td>
<td>nmol [14C]glucose/g dry wt-min</td>
<td>nmol [14C]palmitate/g dry wt-min</td>
<td></td>
</tr>
<tr>
<td>11 mM glucose +carnitine</td>
<td>4418 ± 300</td>
<td>1819 ± 169</td>
<td>728 ± 61</td>
</tr>
<tr>
<td>11 mM glucose, 1.2 mM palmitate +carnitine</td>
<td>4629 ± 460</td>
<td>454 ± 85*</td>
<td>527 ± 111</td>
</tr>
<tr>
<td>11 mM glucose +carnitine</td>
<td>45.3 ± 2.6</td>
<td>18.2 ± 1.4</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td>11 mM glucose, 1.2 mM palmitate +carnitine</td>
<td>25.6 ± 2.1</td>
<td>1.4 ± 0.2</td>
<td>4.6 ± 0.8*</td>
</tr>
<tr>
<td>+carnitine</td>
<td>44.9 ± 5.1</td>
<td>4.4 ± 0.8*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant compared with hearts perfused in the presence of palmitate.

### TABLE III

**Steady state myocardial ATP production in carnitine-treated hearts**

Values are presented as mean ± S.E. for 6–10 hearts for each group. Steady state ATP production values were determined from hearts perfused with 1.2 mM palmitate in Table II. ATP production was calculated by using values of 2 and 38 mol of ATP produced/mol of glucose utilized for glycolysis and glucose oxidation, respectively. For palmitate oxidation, a value 129 mol/ATP produced per mol palmitate oxidized was used.

<table>
<thead>
<tr>
<th>ATP source</th>
<th>No carnitine</th>
<th>Carnitine-treated</th>
<th>ATP production μmol/g dry wt-min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis</td>
<td>5.8 ± 0.5 (5.5%)</td>
<td>9.3 ± 0.9 (9.3%)</td>
<td>105.4</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td>5.7 ± 0.8 (5.4%)</td>
<td>16.4 ± 3.1 (14.3%)</td>
<td>99.4</td>
</tr>
<tr>
<td>Palmitate oxidation</td>
<td>92.9 ± 7.9 (89.1%)</td>
<td>73.8 ± 14.3 (74.4%)</td>
<td></td>
</tr>
<tr>
<td>Total ATP production</td>
<td>105.4</td>
<td>99.4</td>
<td></td>
</tr>
</tbody>
</table>

*Significant compared with hearts perfused in the absence of carnitine.

Effects of Carnitine Treatment on Myocardial Carnitine Esters, Long-chain Acyl-CoA, and ATP Levels—The redistribution of myocardial carnitine esters in carnitine-treated hearts perfused in the absence or presence of palmitate is shown in Table IV. In hearts perfused with 1.2 mM palmitate, an increase in all esters of carnitine was observed, including the short chain fraction which contains acetylcarnitine. Myocardial levels of long chain acylcarnitine were also elevated and significantly higher compared with control hearts perfused with palmitate. If glucose was used as sole substrate, the increase in total carnitine content was found mainly in the free carnitine fraction form. As expected in these hearts, long chain acylcarnitine levels were lower compared with hearts perfused with fatty acids. In carnitine-treated hearts perfused with palmitate, both free and short chain fractions contributed mainly to the total carnitine content.

The beneficial effects of carnitine on the heart in ischemia or in diabetes have often been postulated to occur secondary to a lowering of myocardial long chain acyl-CoA levels. Table IV also shows the effect of carnitine on myocardial long-chain acyl-CoA and ATP levels. In hearts perfused in the absence of fatty acids, carnitine-treated hearts had lower levels of long chain acyl-CoA compared with the control fatty acid perfused hearts and similar ATP levels. Following 60 min of carnitine loading in the presence of palmitate, a dramatic increase in the levels of long chain acyl-CoA was seen. This was accompanied by a significant reduction in myocardial ATP levels, compared with control hearts or to carnitine-treated hearts perfused in the absence of fatty acids. It is interesting to note, however, that under these perfusion conditions, mechanical function was greater in carnitine-treated hearts perfused with palmitate than in those perfused the absence of the fatty acid (Table I). It should be also mentioned that regardless of the effect of carnitine on long chain acyl-CoA and ATP, oxidative metabolism in these hearts was not significantly depressed.

**DISCUSSION**

It is well established that both glycolysis and glucose oxidation are inhibited by high levels of fatty acids (18, 20, 24, 26). According to Randle's classical glucose-fatty acid cycle, this occurs primarily through inhibition of both phosphofructokinase and the pyruvate dehydrogenase complex (18–20). In this study, we demonstrate in intact hearts that the effects of fatty acids are more dramatic on the pyruvate dehydrogenase complex than on phosphofructokinase. Although glucose oxidation and glycolysis are both reduced when hearts are perfused in the presence of 1.2 mM palmitate, the decrease in glucose oxidation is much more dramatic than the decrease in glycolysis. The main finding of this study, however, is the demonstration that increasing myocardial carnitine levels can stimulate glucose oxidation. Carnitine levels were elevated by perfusing hearts with L-carnitine for a period sufficient to allow for a significant increase in intracellular myocardial carnitine content, since the uptake of carnitine by the heart is a slow Na⁺-dependent facilitated process (21, 22). Another interesting observation is that fatty acid oxidation rates are not increased if carnitine levels are elevated. In fact, the increase in glucose oxidation was coupled with a concomitant decrease in the rate of palmitate oxidation, resulting in no change in overall ATP production. Although carnitine may be an important regulator of fatty acid oxidation if the muscle...
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TABLE IV
Myocardial carnitine esters, long chain acyl-CoA and ATP levels in carnitine-treated hearts perfused in the presence or absence of palmitate

| Values are presented as mean ± S.E. for 6-10 hearts in each group. Hearts were perfused as described under “Experimental Procedures.” L-Carnitine was added to the buffer at the concentration of 10 mM for 60 min. |
|---|---|---|---|---|---|---|
| Perfusion conditions | Carnitine esters | Long-chain acyl-CoA | ATP |
|  | Free | Short chain | Long chain | Total | nmol/g dry wt | μmol/g dry wt |
| 11 mM glucose, 1.2 mM palmitate | 2549 ± 319 | 1268 ± 177 | 463 ± 66 | 4276 ± 211 | 208.6 ± 15.3 | 22.5 ± 1.4 |
| 11 mM glucose, 1.2 mM palmitate + carnitine | 6149 ± 419 | 3068 ± 279 | 762 ± 123 | 9671 ± 404 | 327.1 ± 57.5 | 15.3 ± 0.9 |
| 11 mM glucose + carnitine | 7795 ± 330 | 1806 ± 256 | 178 ± 18 | 9496 ± 473 | 136.1 ± 15.2 | 19.2 ± 1.2 |

* Significant compared with hearts perfused in the absence of carnitine.

A role for carnitine in buffering the acetyl-CoA/CoA ratio in the mitochondria by forming acetylcarnitine has been proposed previously (94). It has recently been demonstrated by Lysiak et al. (14) in rat heart mitochondria that carnitine causes an increase in the levels of CoASH and a decrease in the levels of short-chain acyl-CoAs, in a manner that was dependent upon the substrate oxidized. In the presence of pyruvate, the largest effects on CoASH and acetyl-CoA were noted, with carnitine producing a 10-fold decrease in the ratio of acetyl-CoA/CoASH. This decrease in ratio of acetyl-CoA/CoASH correlated with the efflux of acetylcarnitine from heart mitochondria (14). If acetylcarnitine efflux is stimulated, the acetyl group of acetylcarnitine can be freed in the cytosol which would generate free carnitine movement back to the matrix available for accepting acetyl residues. One potential role for carnitine, therefore, is in buffering intramitochondrial acetyl residues, resulting in a decrease in intramitochondrial acetyl-CoA levels. In agreement with this concept, carnitine was found to stimulate [14C]CoA production from [1-14C]-pyruvate in human skeletal muscle mitochondria (17). This increase in [14C]CoA production was associated with a stimulation of acetylcarnitine synthesis as well as the pyruvate dehydrogenase complex activity. In the present study, we extend these previous observations by demonstrating that the action of carnitine observed in mitochondrial preparations also occurs in the intact heart. As a result, an additional physiological role of carnitine as a regulator of glucose oxidation should be considered, in addition to its classic role as an essential cofactor in fatty acid oxidation. This effect probably occurs secondary to a decrease in the intramitochondrial acetyl-CoA/CoA ratio, thereby relieving inhibition of the pyruvate dehydrogenase complex. The possibility that carnitine alters some other step in the tricarboxylic acid cycle cannot be completely excluded. A direct effect on the tricarboxylic acid cycle is unlikely, however, since this should cause a comparable increase in β-oxidation as well as pyruvate dehydrogenase complex activity. This is clearly not the case, since the increase in ATP production from glucose oxidation seen in carnitine treated hearts was accompanied by a parallel decrease in ATP production from fatty acid oxidation (Table III).

The myocardial pyruvate dehydrogenase complex is composed of three catalytic enzymes (pyruvate decarboxylase, dihydrolipoyl acetyltransferase, dihydrolipoyl dehydrogenase) and two regulatory enzymes (pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase) (18). In addition to the three catalytic components and two regulatory enzymes, pyruvate dehydrogenase complex also contains protein X. It has been suggested that this protein, which contains a lipoyl component, has an important role in the interactions of specific catalytic components of pyruvate dehydrogenase complex (19). The catalytic efficiency of myocardial pyruvate dehydrogenase complex is regulated by a phosphorylation-dephosphorylation mechanism of the α subunit of pyruvate decarboxylase. Pyruvate dehydrogenase kinase is the inhibiting and phosphorylating component of the pyruvate dehydrogenase complex. Acetyl-CoA, by stimulating this kinase, phosphorylates pyruvate dehydrogenase and decreases the proportion of the complex in the active dephosphorylated form. On the role of carnitine in the regulation of pyruvate dehydrogenase complex activity reported in mitochondrial preparations, various substrates were used to quantity the efflux and synthesis of acetylcarnitines (14, 15, 17, 24). In these studies, pyruvate was the prevailing substrate which demonstrated the greatest effects (14, 15, 17). It was of interest to note in spontaneously beating hearts that the carnitine-induced increase in glucose oxidation was dependent only on the presence of fatty acids. This substrate-specific effect was anticipated since in hearts perfused with glucose only, the tricarboxylic acid cycle demand for pyruvate-derived acetyl-CoA was markedly enhanced. It was not much of a surprise to notice in our study the lack of effect of carnitine on glucose
oxidation in hearts perfused in the absence of fatty acids, since the inhibitory effect of the acetyl-CoA/CoASH ratio on pyruvate dehydrogenase is already relieved. In the presence of a high concentration of fatty acids, however, the activity of pyruvate dehydrogenase complex is substantially reduced because of end product inhibition of acetyl residues on pyruvate dehydrogenase. In a normal physiological situation, β-oxidation of fatty acids are the major source for myocardial ATP production (26). Glucose can only contribute to approximately one-third of the total ATP production because fatty acids increase the acetyl-CoA/CoASH ratio (18). Hence, if any effects of carnitine on the intramitochondrial acetyl-CoA/CoASH ratio are to occur, this ratio must be elevated by the presence of fatty acids. This explains our results showing that the carnitine-induced increase in glucose oxidation occurs only when fatty acids are present in the perfusate.

A protective effect of carnitine against ischemic damage has been shown in a number of studies (1–4). Several other studies have also shown that carnitine supplementation can improve heart function in cardiomyopathies associated with diabetes and carnitine deficiency syndromes (5–9). Although the mechanisms by which this beneficial effect of carnitine occurs have not been yet delineated, it has been suggested that this natural occurring compound acts by stimulating fatty acid oxidation or by decreasing the levels of long chain acyl-CoA. Increases in the levels of long chain acyl-CoA can alter the function of critical membrane proteins, particularly the adenine nucleotide transporter located in the inner mitochondrial membrane (12). However, this has only been convincingly demonstrated in in vitro studies (35). In the intact heart, a consistent correlation between the accumulation of long chain acyl-CoA and myocardial ATP levels has not been found (6, 7, 29, 33, 36–38). Previous reports (5–7) have suggested that the addition of carnitine to fatty acid perfused aerobic hearts normalizes ATP levels by reducing long chain acyl-CoA levels. This effect was particularly evident in fatty acid perfused diabetic hearts (5–7), whereas in non-diabetic hearts carnitine decreased the levels of myocardial long chain acyl-CoA without exerting any effect on ATP levels (6, 7). It is suggested that carnitine acts by removing the long chain fatty acyl-CoA inhibition of the mitochondrial adenine nucleotide translocator. In the present study, we were able to dissociate this effect of carnitine on the mitochondrial adenine nucleotide translocator. By perfusing hearts in the presence of carnitine with a high concentration of palmitate, we actually observed a dramatic increase the levels of long chain acyl-CoA. Although this was accompanied by a decrease, albeit significant, in myocardial ATP levels, mechanical function was not altered in these palmitate-perfused hearts. In fact, when these hearts were perfused in the presence of carnitine, heart function was better compared with the glucose-perfused hearts which have shown higher myocardial ATP levels (Table IV). We are presently investigating whether this dissociative effect of carnitine also occurs in the diabetic and ischemic myocardium.

In summary, we demonstrate that carnitine significantly stimulates glucose oxidation in the fatty acid perfused intact rat heart. This effect was accompanied by a concomitant decrease in palmitate oxidation. We suggest that this probably occurs secondary to facilitating the intramitochondrial transfer of acetyl groups from acetyl-CoA to acetylcarnitine, thereby relieving inhibition of the pyruvate dehydrogenase complex. Our data is consistent with the earlier findings indicating that carnitine can stimulate the synthesis of ace-

carnitine as well as the activity of the pyruvate dehydrogenase complex observed in isolated mitochondria.

REFERENCES