Regulation of Fatty Acid Utilization in Isolated Perfused Rat Hearts

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SUMMARY

Regulation of fatty acid utilization was studied in the isolated, perfused rat heart. The effects of increasing the concentration of perfusate fatty acid and the level of ventricular pressure development on the rates of fatty acid uptake and oxidation and on the tissue levels of CoA and carnitine derivatives were determined. In hearts that were developing low levels of ventricular pressure, the rate of palmitate uptake was proportional to its concentration in the perfusate as the concentration was raised from 0 to 0.6 mM bound to 3% albumin. The faster rate of uptake was associated with only a slight increase in the tissue content of long chain acyl-CoA, acylcarnitine, acetyl-CoA, and acetylcarnitine. Palmitate utilization appeared to be limited by the rate of fatty acid uptake or activation. As the concentration of palmitate was increased from 0.6 to 1.2 mM, the rate of uptake did not increase further; oxygen consumption and 14CO2 production from [U-14C]palmitate increased only slightly, and large amounts of acyl-CoA and acylcarnitine derivatives accumulated in the tissue. These observations indicated that the rate of fatty acid uptake at high levels of exogenous palmitate was limited by the rate of acetyl-CoA oxidation through the citric acid cycle. Fatty acid activation and transfer of the acyl unit from acyl-CoA to acylcarnitine may have been limited by high acetyl-CoA to CoA and acetylcarnitine to carnitine ratios. It was estimated that the concentration of CoA and carnitine decreased to below the level needed for optimal rates of fatty acid activation and oxidation. The mass-action ratio for the carnitine palmitoyltransferase was constant and independent of the exogenous fatty acid concentration. The mass-action ratio for the carnitine acetyltransferase was shifted toward acetyl-CoA formation as the level of palmitate was raised. A possible role of the carnitine acetyltransferase system in coupling the rate of flux through the citric acid cycle with fatty acid activation and acyl transfer is discussed.

Increased ventricular pressure development resulted in (a) a faster rate of oxidative phosphorylation as indicated by increased oxygen consumption (b) an acceleration of the citric acid cycle as indicated by a large increase in CO2 production, and (c) an increase in uptake and β-oxidation of palmitate. Acceleration of the citric acid cycle was associated with a decrease in the tissue content of fatty acids, acyl-CoA, acetyl-CoA, and acetylcarnitine and an increase in the levels of acylcarnitine, free CoA, and free carnitine. When high levels of exogenous palmitate were present, the increase in CoA and carnitine could have accelerated the rates of fatty acid activation and acyl transfer from acyl-CoA to acylcarnitine and thus increased the rate of palmitate uptake. With concentrations of palmitate below 0.6 mM, the changes in CoA and carnitine were probably too small to account for the increased fatty acid uptake. The tissue content of long chain acylcarnitine increased with acceleration of oxidative metabolism even though the levels of both acyl-CoA and acetyl-CoA decreased. The mass-action ratio for the carnitine palmitoyltransferase system shifted toward acylcarnitine formation. In contrast to palmitate, oxidation of octanoate was fast enough to maintain high levels of acetyl-CoA when fatty acid oxidation was accelerated by increased cardiac work. These results suggested that the rate of translocation of acyl units across the inner mitochondrial membrane limited the rate of long chain fatty acylcarnitine oxidation at high levels of ventricular pressure development.

The importance of fatty acids as substrates for energy metabolism in heart muscle is well established. From 60 to 90% of the total oxidative metabolism was accounted for by oxidation of fatty acids under a variety of conditions (1-3). Fatty acids were oxidized in preference to carbohydrate (4-7). Oxidation of fatty acids or ketone bodies inhibited the utilization of both extracellular glucose and tissue glycogen (8-11). This inhibition developed at the levels of glucose transport (6, 10), phosphofructokinase (12-14), glycogen phosphorylase (11), and pyruvate dehydrogenase (15). Although fatty acids represent an important and preferred substrate for energy metabolism in heart muscle, the mechanisms that regulate the utilization of this substrate are poorly understood. The rate of fatty acid uptake was shown to depend on the fatty acid to albumin molar ratio in the plasma (16-18). As this ratio was increased, fatty acid uptake and oxidation increased, and a larger fraction of the extracted fatty acid was recovered in tissue lipids (19). At any fatty acid to albumin ratio, however, the rate of uptake depended upon the metabolic state of the tissue. Uptake was increased by treating the tissue with epinephrine and was decreased by anoxia (2, 19, 20). The rate of uptake (6) and con-
version of [14C]palmitate to [14C]O2 (21) was accelerated by increased ventricular pressure development in isolated rat hearts.

The purpose of the present study was to identify the mechanisms that (a) increased fatty acid uptake as the concentration of exogenous palmitate was raised, (b) limited fatty acid oxidation at higher concentrations of exogenous palmitate, and (c) accelerated fatty acid uptake and oxidation in association with an increase in the rate of oxidative phosphorylation secondary to increased ventricular pressure development. To determine the rate-controlling steps of fatty acid utilization, the tissue levels of acyl-CoA and acylcarnitine derivatives, acyl-CoA and acylcarnitine, and CoA and carnitine were measured. Transient and steady state changes in the levels of these intermediates and in the rates of palmitate uptake and oxidation were determined after addition of palmitate to the perfusate and after increasing the level of ventricular pressure development.

**METHODS**

**Perfusion Technique**—Hearts from 200- to 250-g male rats were perfused by the Langendorff procedure as described earlier (22). Ventricular pressure development was increased by raising the aortic perfusion pressure. In this preparation, peak systolic ventricular pressure was from 10 to 40 mm Hg greater than the aortic perfusion pressure over the range of from 50 to 140 mm Hg aortic pressure. The animals were fasted overnight prior to use. The perfusate was Krebs-Henseleit bicarbonate buffer gassed with 95% O2-5% CO2. The buffer contained glucose (11 mM), bovine serum albumin (3%), and the concentrations of fatty acid indicated in the figures. The fatty acid was bound to albumin by solubilizing the free acid as the potassium salt and by injecting the salt mixture into the warm albumin medium. The albumin-fatty acid complex was dialyzed overnight against a large volume of buffer and filtered through a Millipore filter (8 µm) before use.

**Estimation of Rates of O2 Consumption, CO2 Production, and Uptake and β Oxidation of Palmitate**—Oxygen consumption was estimated by measuring the difference in arterial and venous PO2 and the rate of coronary flow. Coronary effluent was collected without exposure to air by cannulating the pulmonary artery and collecting the effluent under heptane. In the Langendorff preparation, the only fluid returned to the right atrium and ventricle was coronary effluent. PO2 was measured with a Clark electrode (Radiometer model PI-1812). 14CO2 production from [U-14C]palmitate was estimated in coronary effluent that was collected in the same way as for PO2 measurements. A sample of the perfusate was acidified, the 14CO2 released was collected in Hyamine, and the radioactivity was determined after addition of palmitate to the perfusate and after increasing the level of ventricular pressure development.

Steady state rates of fatty acid uptake were estimated by measuring the disappearance of fatty acids from the perfusate during 15 or 20 min of perfusion. The perfusate concentration of fatty acids was determined either by the microtitration method described by Noble (23) or by gas chromatography. For estimation of the transient rate of palmitate uptake, hearts were perfused with [U-14C]palmitate, and uptake was estimated from the decrease in radioactivity that occurred during the perfusions. The average rate of uptake during 20 min of perfusion was the same when uptake was determined as disappearance of titratable fatty acid or decrease in perfusate [U-14C]palmitate, indicating that exchange of labeled with unlabeled fatty acids in the tissue did not significantly influence the rate of uptake. Measuring the decrease in [U-14C]palmitate, however, allowed the transient rates of uptake to be followed over short time intervals.

The rate of β oxidation of exogenous [U-14C]palmitate was determined by measuring the appearance of label in acid-soluble intermediates (acyetyl-CoA, acetylcarboxylase, and acetyl-CoA and acetyl-carnitine) and in CO2. The amount of labeled in the acid-soluble intermediates was determined on 6% perchloric acid extracts of the tissue. The extract was washed twice with heptane (1:2 ml of extract). The heptane phase was separated by mild centrifugation and was removed by aspiration. By this method more than 95% of key citric acid cycle intermediates that were added to the unwashed extract were recovered in the aqueous phase. The amount of label that appeared in the perfusate in compounds other than CO2 or palmitate was less than 5% of the total label in the acid-soluble fraction.

**Estimation of Tissue Levels of Metabolic Intermediates**—At the end of perfusion, hearts used for estimates of metabolic intermediates were frozen with a Wollenberg clamp maintained at the temperature of liquid nitrogen, powdered in a persulfur mortar, and extracted with cold 6% perchloric acid. The extract was neutralized with KOH and used for the analysis of acetyl-CoA, acetylcarboxylase, free CoA, and free carnitine. The perchloric acid precipitate was washed with 0.6% perchloric acid and used for the analysis of long chain acyl CoA and acyl carnitine derivatives.

The samples were assayed for acetyl-CoA, acetylcarboxylase, and CoA within 1 hour after making the extract. The assays were performed in a Zeiss fluorometer. Acetyl-CoA was assayed by the citrate synthase method as described by Herrera and Freinkel (24). CoA was assayed by the a-ketoglutarate dehydrogenase method of Garland et al. (25), and carnitine was determined by combing the CoA assay with a carnitine acetyltransferase and citrate synthase method of Pearson et al. (26). Long chain acyl-CoA and acetylcarboxylase were assayed as free CoA and carnitine after alkaline hydrolysis of the washed perchloric acid precipitate obtained from the tissue homogenates (27). Long chain acyl-CoA was hydrolyzed at pH 11 to 12 for 15 min at 55° in the presence of 10 mM dithiothreitol. Long chain acyl-carnitine was hydrolyzed at pH 12.5 to 13.0 for 2 hours at 70°. These methods are specific for CoA and carnitine derivatives with chain lengths greater than 10 carbons.

**Estimation of Tissue Free Fatty Acids**—A sample of frozen tissue powder was homogenized with cold CHCl3:CH3OH (2:1). After centrifugation at 10,000 × g for 15 min, the supernatant was flash-evaporated and then desiccated to complete dryness. The residue was dissolved in CHCl3 and run through a small silicic acid column (0.5 g of silicic acid per column) to remove phospholipids. The columns were washed several times with CHCl3, and the combined washes for each sample were dried in a water bath at 60° under a stream of N2. The residue was dissolved in 0.2 ml of CHCl3:CH3OH (2:1), and the free fatty acids were separated from other lipid components by thin layer chromatography. The fatty acids were eluted with CHCl3 and methylated with BF3 (28). The methyl esters were quantitated with a Hewlett-Packard model 120 gas chromatograph (DEGS 20 column). C16, C18, C18:1, and C18:2 comprised approximately 90% of the total tissue fatty acids. The other minor peaks were not used in calculating tissue fatty acid. Pentadecanoate (C15) was added to the initial homogenate to act as a carrier and internal standard.

To determine the amount of tissue fatty acids that were present in the vascular space, 14C-albumin was added to the buffer and its distribution in the tissue was determined. A
small portion of the powdered tissue was weighed and the protein was dissolved in formic acid. A sample of the solution (0.2 ml) was added to 10 ml of dioctane scintillator, neutralized with NH₄OH, and counted for β⁻ in a liquid scintillation counter. The perfusate concentration of β⁻-albumin was determined by precipitating the albumin with 6% perchloric acid, dissolving the protein in formic acid, and counting an aliquot of the solution. The size of the albumin space in milligrams per g of tissue was calculated as:

\[ \frac{\text{β⁻ cpm per g of tissue}}{\text{β⁻ cpm per ml of perfusate}} \]

The concentration of perfusate fatty acid was determined by the Dole (29) extraction procedure. The upper heptane phase was removed and dried, and fatty acids were measured by gas chromatography as described above. The amount of fatty acid present in the vascular space in micromoles per g of dry tissue was calculated as micromoles of fatty acid per ml of perfusate \times milliliters of albumin space per g of tissue. With these measurements, the levels of tissue fatty acids were corrected for those bound to albumin in the vascular space.

RESULTS

Effects of Palmitate Concentration and Ventricular Pressure Development on Rate of Fatty Acid Uptake, O₂ Consumption, and ¹⁴CO₂ Production from [U⁻¹⁴C]Palmitate—The rate of palmitate uptake increased as its concentration in the perfusate was raised at both low and high levels of ventricular pressure development (Fig. 1). The increase in uptake with concentration was not linear, however, and the rate leveled off when the concentration of palmitate was about 0.0 and 0.9 mM in hearts developing 30 and 100 mm Hg ventricular pressure, respectively. Raising the pressure significantly increased the rate of uptake at all palmitate concentrations studied. These results indicated that the rate of fatty acid utilization was a function of its concentration only when the exogenous concentration was low. At high concentrations, the rate of uptake was limited by intracellular processes. An increase in ventricular pressure accelerated the rate of uptake and caused the process to become saturated at a higher concentration of exogenous palmitate.

The effect of increased pressure on palmitate uptake was associated with a faster rate of oxidative phosphorylation as indicated by a large increase in oxygen consumption. Fig. 2 shows the transient and steady state changes in oxygen consumption that resulted from increasing ventricular pressure from 60 to 120 mm Hg in hearts perfused with buffer containing glucose (11 mM) and either 0, 0.4, or 1.2 mM palmitate. A rapid increase in oxygen consumption occurred as ventricular pressure development was raised. The rate of oxygen consumption was somewhat higher at both levels of pressure development when palmitate was included in the perfusate.

Oxidation of fatty acids, as determined by ¹⁴CO₂ production from [U⁻¹⁴C]palmitate, was somewhat faster at 1.2 than at 0.4 mM palmitate when the level of ventricular pressure was maintained at 60 mm Hg (Fig. 3). The rate of ¹⁴CO₂ production increased rapidly during the first few minutes after addition of palmitate to the perfusate. The rate of production leveled off in hearts perfused with 0.4 mM palmitate but continued to increase slowly with 1.2 mM. Raising the level of pressure development at either zero time or after 6 min of perfusion with palmitate resulted in a large increase in CO₂ production from the fatty acid. At this higher level of ventricular pressure, ¹⁴CO₂ production was the same with both concentrations of palmitate, indicating that the rates of oxidation of acetyl units produced from exogenous palmitate were the same at both 0.4 and 1.2 mM palmitate. These data indicated that the rate of flux of labeled acetyl units through the citric acid cycle was somewhat faster at 1.2 than at 0.4 mM palmitate. The level of pressure development was increased 3- to 4-fold.

Effects of Increased Ventricular Pressure and Exogenous Palmitate

![Fig. 1. Effects of perfusate palmitate and ventricular pressure development on the rate of palmitate uptake. The rate of palmitate uptake by hearts perfused at 60 (--), and 100 (---) mM Hg ventricular pressure development was estimated by measuring the disappearance of palmitate from the perfusate during 20 min of perfusion. Thirty milliliters of buffer containing glucose (11 mM), albumin (8%), and the concentration of palmitate shown in the figure were recirculated through the heart for the 20-min perfusion period. Each value represents the mean ± S.E.M. for six hearts.](http://www.jbc.org/)
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of pressure development, reIargc amounts of acetyl-CoA was raised (Fig. 4). Over the range of 0 to 0.5 mm palmitatc, only a small accumulation of acetyl-CoA occurred, whereas the increase in countaining glucose (11 mm), albumin (3%), and the concentration of palmitate shown in the figure. Ventricular pressure development was adjusted to either 60 (---) or 120 (- - -) mm Hg and perfu-

sion was continued for 6 min. Each value represents the mean ± S.E.M. for 6 to 10 determinations.

rate on Tissue Content of CoA, Carnitine, and Their Aoyl Deriva-

tives after 6 Min of Perfusion—The major product of fatty acid oxidation in heart muscle is acetoyl-CoA. Since the rates of ketogenesis and lipogenesis are very low, the predominant fate of acetoyl-CoA is oxidation through the citric acid cycle or transfer of the acetyl unit to carnitine. Therefore, the tissue content of CoA, carnitine, and their acyl derivatives was determined in order to localize the steps in fatty acid oxidation that limited palmitate uptake at high concentrations and that accelerated uptake with increased ventricular pressure development. The levels of these intermediates were measured after 6 min of perfusion with palmitate. Other studies demonstrated that maximum increases in the tissue content of acetoyl-CoA and citric acid cycle intermediates occurred within 6 min after introducing palmitate in the perfusate (15).

At 60 mm Hg ventricular pressure, both acetoyl-CoA and acetoylarnitine, accumulated as the concentration of palmitate was raised (Fig. 4). Over the range of 0 to 0.5 mm palmitate, only a small accumulation of acetoyl-CoA occurred, whereas the increase in acetoylarnitine was more pronounced. The ratio of acetoylarnitine to acetoyl-CoA was about 15:1. As the concent-

centration of palmitate was raised above 0.5 mm at the low level of pressure development, relatively large amounts of acetoyl-CoA accumulated in the tissue. In this case, the acetoylarnitine to acetoyl-CoA ratio decreased to about 10:1. The tissue content of CoA and carnitine decreased as their acetyl derivatives accumulated. The increase in the level of acetyl derivatives and the decrease in CoA and carnitine were associated with a leveling off in the rate of palmitate uptake, suggesting that the rate of acetoyl-CoA oxidation through the citric acid cycle limited the rate of fatty acid oxidation.

Increasing ventricular pressure from 60 to 120 mm Hg pre-

vented a large accumulation of acetoyl-CoA (Fig. 4). The levels of acetoylarnitine were also significantly lower at the higher pressure. This decrease in acetoyl units associated with an increase in 14CO2 production indicated that the rate of oxida-
tion of palmitate was not sufficient to maintain high levels of acetoyl-CoA. The faster rate of acetoyl-CoA oxidation resulted in higher levels of CoA when the concent-

of palmitate was above 0.6 mm; but, at lower palmitate concentrations, the level of CoA was significantly reduced. The levels of carnitine were higher with increased pressure at palmitate concentrations greater than 0.3 mm.

In hearts that were developing 60 mm Hg ventricular pressure, the levels of both long chain acyl-CoA and acetoylarnitine derivat-

es increased as the concentration of palmitate was raised from 0 to 1.2 mm (Fig. 4). The levels of these intermediates did not increase further as palmitate was raised above 1.2 mm (data not shown). Acyl-CoA increased by only 60% while the level of acetoylarnitine increased by about 300%. Raising the concent-

ration of palmitate from 0 to 0.3 mm increased the levels of these intermediates only slightly but caused the largest increase in palmitate uptake. The largest rise in acetoylarnitine occurred at the palmitate concentrations where acetoyl-CoA also accumulated. Increasing the level of cardiac work reduced acyl-CoA slightly, but produced a 30% increase in acetoylarnitine. This increase in acetoylarnitine associated with a faster rate of fatty acid oxidation and decreases in both long and short chain CoA derivat-

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levels of long chain acyl derivatives increased rapidly and accumulated in the perfusate. The increase in acylcarnitine was larger and reached a maximum within about 2 min after introducing palmitate, while the increase in acyl-CoA at all time periods studied. Acyl-CoA appeared to reach a steady state after 10 min. The level of acyl-CoA decreased rapidly between 4 and 6 min in hearts that were perfused with palmitate as substrate but not with octanoate, indicating that production of acetyl-CoA from octanoate, but not from palmitate, was fast enough to keep pace with the increased rate of flux through the citric acid cycle. These results suggested that carnitine-dependent processes limited acetyl-CoA production from palmitate when the rate of fatty acid oxidation was accelerated.

**Transient Effects of Increased Ventricular Pressure Development and Concentration of Palmitate on Tissue Levels of Acyl-CoA and Acylcarnitine Derivatives—**Transient changes in the tissue content of CoA and carnitine and their acyl derivatives after addition of either 0.4 or 1.2 mM palmitate to the perfusate and after increasing ventricular pressure development are shown in Fig. 5. The levels of long chain acyl derivatives increased rapidly and reached a maximum within about 2 min after introducing palmitate into the perfusate. The increase in acylcarnitine was larger than the increase in acyl-CoA at all time periods studied. Acylcarnitine decreased between 4 and 10 min of perfusion and appeared to reach a steady state after 10 min. The level of acyl-CoA decreased rapidly between 4 and 6 min in hearts that were perfused with 1.2 mM palmitate and then continued to decrease at a slower rate. With 0.4 mM palmitate, the levels of both intermediates decreased after 2 min of perfusion.

The tissue content of acetyl-CoA and acylcarnitine increased rapidly after addition of 1.2 mM palmitate, reached a maximum within 4 to 6 min, and then slowly declined. The levels of both CoA and carnitine decreased to a minimum level within 2 min. With 0.4 mM palmitate, the acyl derivatives increased to a maximum within 2 min and then declined. At this low concentration of fatty acid, acetyl-CoA increased by about 80% in comparison to a 5-fold increase with 1.2 mM palmitate. The level of acetylcarnitine, on the other hand, increased to the same extent within 2 min with either 0.4 or 1.2 mM palmitate, but the level declined more rapidly at the lower concentration of fatty acid. With 0.4 mM palmitate, the early rise in acetylcarnitine was much larger in magnitude than was the rise in acetyl-CoA. The levels of both CoA and carnitine reached a minimum within 2 min after adding 0.4 mM palmitate and then slowly increased as the level of their acetyl and acyl derivatives declined. With low levels of palmitate, excess acetyl units were preferentially stored as the carnitine derivative in both the 6-min (Fig. 4) and

| Substrate added | Peak systolic pressure | Substrate uptake | Acetyl-CoA |
|----------------|------------------------|-----------------|-----------|
|                | Initial | Final | mM | Hz | µmoles/g/hr | µmoles/g dry |           |
| Palmitate      | 1.8     | 1.7   | 0.0 | 55 | ± 7 | 374 ± 24 | 200 ± 25 |
| Octanoate      | 1.5     | 1.3   | 120 | 98 | ± 14 | 326 ± 35 | 383 ± 25 |
| Octanoate      | 5.0     | 4.3   | 120 | 90 | ± 14 | 378 ± 20 | 371 ± 38 |

**Fig. 5.** Effects of perfusion time, ventricular pressure development, and perfusate palmitate concentration on the tissue levels of acyl-CoA, acylcarnitine, CoA, and carnitine. Perfusion with buffer containing glucose (11 mM) and either 0.4 mM palmitate (○, △) or 1.2 mM palmitate (●, △) was started at zero time on the figure. The hearts had received a 10-min preliminary perfusion with buffer containing 11 mM glucose as the only substrate. The level of ventricular pressure development was either main mained at 60 mm Hg (---) or increased to 120 mm Hg (---) after 6 min of perfusion with palmitate. Each point represents the mean of 6 to 10 determinations. The changes in carnitine with increased ventricular pressure in hearts perfused with 0.4 mM palmitate were not statistically significant except at 1 min after the pressure change.  

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**Effects of pressure development and substrate on tissue levels of acyl-CoA in the isolated rat heart**

Hearts were perfused for 10 min with Krebs-Henseleit bicarbonate buffer containing glucose (11 mM) before switching to perfusion with buffer containing glucose and the concentration of fatty acid shown in the table. Perfusion with the fatty acids was continued for an additional 15 min. The rates of fatty acid disappearance from the perfusate were measured over the 15-min period and the tissue levels of acetyl-CoA were measured in hearts that were quick frozen at the end of 15 min.
in the transient studies (Fig. 5). At the high level of palmitate, proportionally more of the acetyl units accumulated as the CoA derivative. The acetylcarnitine to acetyl-CoA ratio was about 0.1:1 at 0.4 and 1.2 mM palmitate, respectively, throughout the perfusion.

Increasing the level of ventricular pressure development produced a rapid decrease in the tissue content of the acetyl derivatives and of the long chain CoA derivatives, whereas the level of long chain acylcarnitine increased (Fig. 5). With 1.2 mM palmitate, raising the level of ventricular pressure resulted in higher levels of both CoA and carnitine. With the lower concentration of palmitate, there was only a transient increase in carnitine and the levels of CoA decreased.

Effects of Palmitate and Ventricular Pressure Development on Fraction of Total CoA That Was Present as CoA, Acetyl-CoA, and Long Chain Acyl-CoA—In the experiments illustrated in Figs. 4 and 5, the tissue content of CoA decreased when fatty acid oxidation was accelerated at low levels of exogenous palmitate. These changes in CoA could not be completely accounted for by a parallel increase in the acetyl and acyl derivatives. The tissue content of CoA was therefore dependent on the levels of acetyl-CoA, long chain acyl-CoA, and some other acid-soluble CoA derivative. The amount of total CoA present as acetyl-CoA, acyl-CoA, and CoA is shown in Fig. 6. The sum of these three metabolites increased as the concentration of palmitate was raised and decreased as the ventricular pressure was raised from 60 to 120 mm Hg. These results indicated that the level of some other acid-soluble CoA derivative increased rapidly as the pressure was raised at both 0.4 and 1.2 mM palmitate. As reported elsewhere (32), changes in the level of succinyl-CoA under the conditions used in the present experiments could account for the observed changes in this acid-soluble CoA. This increase in the acid-soluble CoA derivative with increased pressure resulted in lower levels of CoA when exogenous palmitate was less than 0.8 mM (Figs. 4 and 5). With higher concentrations of palmitate, the decrease in acetyl-CoA more than compensated for the rise in the other derivative and CoA accumulated in the tissue.

Effects of Perfusate Palmitate and Increased Ventricular Pressure Development on Tissue Content of Free Fatty Acids—In order to further characterize the effects of increased pressure on palmitate uptake, the tissue level of free fatty acids was estimated. At 60 mm Hg, the tissue content of fatty acids increased 2- to 3-fold after addition of 0.4 mM palmitate to the perfusate (upper panel, Fig. 7). Raising the level of cardiac work greatly reduced the tissue content of fatty acids within 2 min. This effect was even more pronounced after 10 min. The data presented in the upper panel of Fig. 6 represent the sum of all C16, C18, C18:1, and C18:2 fatty acids and do not distinguish between intracellular fatty acids and those bound to proteins in the interstitial space. Since palmitate was the only exogenous fatty acid provided, changes in the tissue content of fatty acids other than palmitate may be a better indicator of the changes that occurred in the intracellular pool. At 60 mm Hg, the tissue content of non-palmitate fatty acids was only slightly higher when 0.4 mM palmitate was added.

![Figure 6](http://www.jbc.org/)

**Fig. 6.** Effects of perfusion time, palmitate concentration, and ventricular pressure development on the fraction of total CoA that was present as the sum of CoA, acetyl-CoA, and long chain acyl-CoA. Hearts were perfused as described in Fig. 5 with 0.4 mM palmitate at 60 (●) or 120 (▲) mm Hg ventricular pressure development. The open circles represent values for hearts perfused at 60 mm Hg without any exogenous substrate. The upper panel represents the sum of all of the tissue C16, C18, C18:1, and C18:2 fatty acid, while the lower panel represents fatty acids other than palmitate. The data presented in Fig. 7 with 0.4 mM palmitate at 60 (●) or 120 (▲) mm Hg ventricular pressure. The tissue content of each intermediate was determined from the same hearts. Each point represents the average of determinations from four hearts.

![Figure 7](http://www.jbc.org/)

**Fig. 7.** Effects of perfusion time and ventricular pressure development on the tissue content of fatty acids in hearts perfused with low levels of palmitate. Hearts were perfused as described in Fig. 5 with 0.4 mM palmitate at 60 (●) or 120 (▲) mm Hg ventricular pressure development. The open circles represent values for hearts perfused at 60 mm Hg without any exogenous substrate. The upper panel represents the sum of all of the tissue C16, C18, C18:1, and C18:2 fatty acid, while the lower panel represents fatty acids other than palmitate. The data presented in the figure were corrected for fatty acids in the albumin space. Each value represents the mean ± S.E.M. for four to six samples which contained two hearts per sample. FFA, free fatty acids.
to the perfusate (lower panel, Fig. 7). The tissue content of these fatty acids was virtually depleted within 2 min after raising the level of cardiac work, indicating that non-palmitate fatty acids were readily available as substrates for oxidative metabolism. These data suggested that increased palmitate uptake resulted from a faster rate of fatty acid removal from the intracellular space. Lower levels of intracellular fatty acids would establish a more favorable concentration gradient for their diffusion into the cells. The decrease in intracellular fatty acids, associated with increased levels of acylcarnitine (Fig. 5), also suggested that increased ventricular pressure accelerated the rate of either the fatty acid activating enzymes or acyl transfer from acyl-CoA to acylcarnitine.

Transient Changes in Rates of Fatty Acid Uptake and β Oxidation upon Addition of Palmitate to Perfusion and after Increasing Ventricular Pressure Development—In Fig. 1, palmitate uptake was measured as disappearance of the fatty acid from the perfusate over a 20-min perfusion period which only allowed the average rate of uptake to be estimated. From the transient changes in the tissue levels of CoA and carnitine intermediates (Fig. 5), it appeared likely that the rate of uptake would be more rapid during the first several minutes of perfusion. For this reason, the rates of palmitate uptake and β oxidation were determined over shorter time intervals in the presence of a high fatty acid to albumin ratio.

At 60 mm Hg, the rate of uptake was maximum within 2 min after addition of palmitate, and uptake exceeded the rate of β oxidation (Fig. 8). As a result, acyl-CoA and acylcarnitine accumulated in the tissue during the early perfusion times. The rate of β oxidation was rapid during the first 2 min, decreased between 2 and 6 min as acetyl-CoA accumulated, and then slowly increased over the next 10 min of perfusion as the level of acetyl-CoA decreased (Fig. 5). The minimum rate of β oxidation at 6 min corresponded to the maximum acetyl-CoA to CoA ratio in the tissue (Fig. 5). The rate of palmitate uptake, on the other hand, continued to decrease between 2 and 15 min and then began to increase, suggesting that the rate of uptake responded slowly to changes in the rate of β oxidation.

Increasing the ventricular pressure from 60 to 120 mm Hg resulted in a maximum increase in β oxidation within 1 min. The rate of palmitate uptake, however, did not increase significantly until 4 min after the increase in pressure, again suggesting that changes in the rate of uptake lagged behind corresponding changes in the rate of β oxidation.

Effects of Increased Perfusate Palmitate and Ventricular Pressure on Mass-Action Ratios of Carnitine Palmitoyltransferases and Carnitine Acetyltransferases—Since the ratio of products to substrates for both the carnitine palmitoyl- and acetyltransferase systems remained relatively constant under a wide variety of conditions, it has been suggested that these enzyme systems operate in the tissue near their equilibrium positions (33, 34). Therefore, the slope of the curve that results from a plot of acyl-CoA to CoA versus acylcarnitine to carnitine should be linear. As illustrated in Fig. 9, the relationships between these ratios for the long chain derivatives were relatively linear for each level of ventricular pressure, even though the individual ratios varied by as much as 10-fold as the fatty acid concentration was raised. The slope of the curve, however, was reduced by about 40% when cardiac work was raised. Since the slope of these curves is related to the equilibrium position or mass-action ratio by the equation given in Fig. 9, these results suggested that the mass-action ratio for the carnitine palmitoyltransferase system remained relatively constant over a wide range of fatty acid concentrations, but that the ratio was shifted toward formation of acylcarnitine with increased cardiac work. This effect was especially pronounced at the higher concentration of palmitate. Similar results were obtained when those ratios were calculated from the transient data (Fig. 10), indicating that the mass-action ratios were linear with perfusion time as well as with fatty acid concentration.

The curves for the carnitine palmitoyltransferase system intersected the Y axis at an acyl-CoA to CoA ratio greater than zero (0.20 to 0.24), whereas the curves for the carnitine acetyltransferase system passed through the origin. This suggested that a portion of the long chain acyl-CoA that was measured in the whole tissue was not equilibrated with acylcarnitine. The nature of this "nonequilibrated" pool of acyl-CoA is not known, but it may represent a protein-bound fraction that was not readily available as substrate for the transferase enzymes.

At 60 mm Hg, the slope of the curve relating acetyl-CoA to CoA and acetylcarnitine to carnitine was not linear (Fig. 9). The slope increased when the concentration of palmitate was raised from 0 to 1.2 mM, suggesting that the mass action ratio for
**DISCUSSION**

The rate of fatty acid utilization by most tissues is largely dependent on its concentration in the plasma (16–18, 35). The correlation between concentration and rate of uptake is especially evident in adipose tissue and liver where a major fate of fatty acids is storage as neutral lipids. The rate of uptake by heart muscle was also concentration-dependent (3, 18), but oxidation, rather than storage as complex lipids, was the more prominent fate of fatty acids (11, 15, 18). Increasing the fatty acid to albumin ratio resulted in higher tissue levels of fatty acids, acyl-CoA, and acylcarnitine derivatives and lower levels of free CoA and carnitine (15).

The overall rate of fatty acid utilization by heart muscle should be determined primarily by the supply of exogenous fatty acid and by the energy demands of the tissue. At a constant rate of energy utilization, increased supply of fatty acids would be expected to have a limited ability to accelerate fatty acid uptake. The upper limit would be reached when the supply of fatty acids exceeds the capacity of the cells to bind the fatty acids and to convert acyl units to CO₂ complex lipids, or to metabolic intermediates. Binding of fatty acids and conversion of acyl units to metabolic intermediates could have only a small, transient effect, with the major determinant of uptake being oxidation to CO₂.

In the present study, the rate of fatty acid utilization was limited by either the rate of uptake or activation when the exogenous concentration of palmitate was low. As the concentration was raised from 0 to about 0.4 mM, fatty acid uptake increased proportionately, but the tissue content of acylcarnitine, acyl-CoA, and acetylcarnitine remained relatively unchanged, and
the levels of free CoA and carnitine remained high. In hearts developing low levels of ventricular pressure, the capacity of the cells to oxidize fatty acids and to convert acyl units to complex lipids was saturated at concentrations greater than about 0.6 mM. As the level of exogenous palmitate was raised from 0.6 to 1.2 mM, large amounts of acyl-CoA and acylcarnitine derivatives accumulated in the tissue, but no further increase in uptake was observed. The rate of palmitate oxidation at high concentrations was limited by the rate of acetyl-CoA oxidation through the citric acid cycle. This conclusion was based on the observations that when perfusate palmitate was raised from 0.4 to 1.2 mM the rates of oxygen consumption and \( ^{14} \text{CO}_2 \) production from [\( U-{ }^{14} \text{C} \)]palmitate were increased only slightly, while the level of acetyl-CoA increased 5-fold. Flux through the citric acid cycle has been shown to be geared to the rate of oxidative phosphorylation (36). This coupling is thought to occur through feedback control of the cycle by changes in the levels of high energy phosphate and NADH. Since oxygen consumption was maintained at a constant rate in the present study by controlling the level of ventricular pressure development, the rate of flux through the citric acid cycle would be expected to remain fairly constant as the concentration of palmitate was raised.

After addition of 1.2 mM palmitate to the perfusate, the rates of uptake and \( \beta \) oxidation were fastest during the first 2 min, and they exceeded the rate of \( ^{14} \text{CO}_2 \) production from palmitate. As a result, acetyl-CoA and acylcarnitine accumulated in the tissue. Fatty acid uptake was about twice as fast as \( \beta \) oxidation, and both long chain acyl-CoA and acylcarnitine accumulated. Associated with the high levels of tissue acetyl-CoA and acylcarnitine and low levels of CoA and carnitine, the rates of both \( \beta \) oxidation and palmitate uptake decreased. The rate of uptake may have been limited by a decreased supply of CoA and carnitine for fatty acid activation and acyl transfer.

Interpretation of the data for whole tissue contents of CoA derivatives is complicated by the fact that these derivatives exist in at least two pools, and that the carnitine palmitoly- and acetyltransferase systems consist of at least two enzymes arranged in series (see model in Fig. 11). The changes that were observed in the whole tissue content of CoA derivatives may have occurred primarily in one compartment and could, therefore, greatly influence interpretation of the results. In heart muscle, fatty acids are activated on the sarcoplasmic reticulum and the outer mitochondrial membrane (41). The inner mitochondrial membrane appears to be impermeable to carnitine and CoA derivatives as well as to free carnitine and CoA (42, 43). Carnitine and its derivatives are thought to be located exclusively outside the inner membrane. CoA and its acyl derivatives, however, are compartmentalized in both the cytosol and mitochondrial matrix.

Oxidation of long chain fatty acids by heart mitochondria is completely carnitine-dependent (30, 31), and translocation of the fatty acyl unit across the inner mitochondrial membrane requires prior transfer from acyl-CoA to acylcarnitine. This transfer is catalyzed by a carnitine palmitoyltransferase (Enzyme I, Fig. 11) which appears to be located on the outer aspect of the inner mitochondrial membrane (44). Acyl transfer occurs across the inner membrane and transfer from extramitochondrial acylcarnitine to matrix CoA is catalyzed by a second carnitine palmitoyltransferase (Enzyme II, Fig. 11). This enzyme is more tightly associated with the inner membrane. Intramitochondrial acetyl-CoA produced by \( \beta \) oxidation is then further oxidized through the citric acid cycle or the acetyl unit is transferred to acetylcarnitine in the extramitochondrial spaces by a carnitine acetyltransferase (Enzyme B, Fig. 11). In the cytosol, acetyl units may be transferred from acylcarnitine to cytosolic CoA by another carnitine acetyltransferase (Enzyme A, Fig. 11).

In the present study, the increase in both acetylcoenzyme A and acetyl-CoA as the concentration of palmitate was raised to above 0.6 mM indicates that the level of acetyl-CoA increased in both the mitochondrial matrix and cytosol. Therefore, the level of CoA probably decreased in both spaces. The intracellular volume of heart muscle is approximately 2.5 ml per g of dry tissue (6, 11). Assuming equal distribution of CoA between cytosolic and matrix spaces, the concentration of this metabolite decreased from 0.11 to 0.03 mM as the level of palmitate was raised from 0 to 1.2 mM in hearts developing 60 mm Hg ventricular pressure. This decrease in CoA could have limited the rate of fatty acid activation which would therefore account for the leveling off in the rate of palmitate uptake at concentrations above 0.6 mM. Fritz et al. (39) demonstrated that oxidation of palmitate by whole heart homogenates was stimulated 3-fold by an increase in CoA from 0.01 to 0.1 mM at a constant level of carnitine. The levels of CoA estimated in the present study fall within this concentration range. The \( K_m \) for CoA of the microsomal fatty acid activating enzymes in liver was 0.03 to 0.05 mM (45, 46). Preliminary data from this laboratory indicated that the apparent \( K_m \) of the heart enzymes was between 0.03 and 0.04 mM. Therefore, the level of cytosolic CoA in the intact heart may have decreased to a value near its \( K_m \) for fatty acid activation as the concentration of palmitate was raised.

The mitochondrial matrix space can be estimated to be about 0.4 ml per g dry weight (47). When this space was subtracted from the intracellular volume, the concentration of carnitine, which is exclusively cytosolic, was calculated to have decreased from 1.8 to 0.6 mM as the concentration of palmitate was raised from 0 to 1.2 mM. Pande (48) demonstrated that 1.5 mM carnitine produced a maximum rate of acyl-CoA oxidation by isolated rat heart mitochondria. In the present study, the concentration

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**Fig. 11. Model of compartmentalization of CoA and carnitine derivatives between cytosol and mitochondrial matrix.** This figure illustrates the two transferase systems for translocation of fatty acyl units across the inner mitochondrial membrane. The model was first proposed by Fritz and Yue (37) and later modified by Yates and Garland (38). It includes the observation that carnitine (Cn) and its long chain (FACn) and acetyl (AcCn) derivatives are located exclusively in the extramitochondrial space, whereas CoA and its long chain (FACoA) and acetyl (AcCoA) derivatives exist in at least two pools (cytosolic and mitochondrial matrix). I and II represent the outer and inner carnitine palmitoyltransferases, respectively. The carnitine acetyltransferase system is believed to be composed of an outer (A) and inner (B) enzyme similar to the long chain transferase system (39, 40). FFA, free fatty acid; Cit, citrate; succ CoA, succinic CoA; \( \beta \)-ox, \( \beta \) oxidation.
of carnitine was below this level at fatty acid concentrations greater than 0.4 mM. Therefore, accumulation of acetyl-CoA and acetylcarnitine and the reduced levels of CoA and carnitine secondary to saturation of the citric acid cycle were probably responsible for limiting the rate of fatty acid utilization at high levels of palmitate. Any change in the rates of oxidative phosphorylation and flux through the citric acid cycle that would increase the tissue content of CoA and carnitine should have an effect on the rate of fatty acid uptake.

In the present study, the rate of oxidative phosphorylation was increased by raising the level of mechanical work that was performed by the heart. Since the tissue levels of ATP and creatinine phosphate have been shown not to change under the conditions imposed in this study (11), oxygen consumption was measured as an index of the rate of oxidative phosphorylation. The rate of oxygen consumption was increased more than 2-fold by raising the level of ventricular pressure from 60 to 120 mm Hg. The small increase in oxygen consumption when palmitate was present probably reflected the lower P:O ratio that results from oxidation of fatty acid as compared to oxidation of glucose. The theoretical ATP yield per oxygen consumed is 3.2 for complete oxidation of glucose compared to 2.5 for palmitate. Oxidation of long chain fatty acids has been shown to inhibit glucose utilization in heart muscle, with oxidation of fatty acids accounting for as much as 90% of the oxygen consumed (11). Therefore, under identical rates of ATP production, oxygen consumption would be expected to increase by about 13% with a shift from glucose to palmitate oxidation.

Associated with the increase in oxidative phosphorylation, flux through the citric acid cycle was accelerated as demonstrated by a 3- to 4-fold increase in the rate of 14CO2 production from [U-14C]palmitate. When palmitate was present at concentrations greater than 0.6 mM, the tissue levels of both acetyl-CoA and acetylcarnitine were greatly reduced, and the levels of CoA and carnitine were increased. In hearts that were perfused with 1.2 mM palmitate, the rate of β oxidation was rapidly stimulated, and the tissue levels of long chain acyl-CoA decreased to a minimum within 1 min following the increase in oxygen consumption. Uptake of palmitate was accelerated, but this change was not evident until about 4 min. This slower response of uptake as compared to β oxidation corresponded to a slow rise in tissue CoA and carnitine. The increase in palmitate uptake could have resulted from higher levels of CoA and carnitine and, consequently, faster rates of fatty acid activation and acyl transfer to carnitine. The tissue levels of long chain acyl carnitine increased in association with the rise in free carnitine. Therefore, coupling of fatty acid uptake to oxidative phosphorylation may occur through changes in the levels of CoA and carnitine secondary to control of the citric acid cycle and oxidation of acetyl-CoA and acetylcarnitine.

These results suggest that the carnitine acyltransferase system plays an important role in coupling the rate of fatty acid uptake to the activity of the citric acid cycle. This may explain why the activity of this enzyme system is higher in tissues that are predominantly oxidative (49). By transferring acetyl units that are formed in the mitochondrial matrix to cytosolic CoA and carnitine, the carnitine acyltransferases may integrate the rates of oxidation of acetyl units through the citric acid cycle and the rate of activation of fatty acids in the cytosol. With low levels of exogenous palmitate, acetyl units accumulated as acetyl-carnitine while acetyl-CoA levels remained very low. However, the mass-action ratio for the transferase enzymes shifted toward acetyl-CoA formation as the level of exogenous palmitate was increased. These results suggest that some intracellular effector that was dependent on the exogenous fatty acid concentration shifted the mass-action ratio for the carnitine acyltransferases toward acetyl-CoA formation. Higher tissue levels of long chain acyl-CoA, a competitive inhibitor of these enzymes with respect to acetylcarnitine and carnitine (50), may have accounted for this effect. The result of this shift would be to limit the rate of fatty acid activation and to aid in the inhibition of β oxidation at high levels of palmitate by increasing the acetyl-CoA to CoA ratio.

In the presence of low levels of palmitate, changes in the tissue content of CoA and carnitine did not appear to be of sufficient magnitude to account for the increase in fatty acid uptake. With 0.4 mM palmitate, fatty acid activation and/or acyl transfer from acyl-CoA to carnitine may have been stimulated by other mechanisms. This was suggested by the large reduction in tissue fatty acids and the rise in acylcarnitine that were associated with a faster rate of palmitate uptake and oxidation. Under this condition, the tissue content of CoA decreased and there was only a small, transient increase in carnitine. There is evidence that fatty acid activating enzymes in the liver may be controlled by factors other than availability of substrates (51). The decrease that was observed in whole tissue CoA, however, may not reflect the direction of changes in the cytosolic content of this metabolite. Most of the decrease may have occurred within the mitochondrial matrix since a parallel increase in succinyl CoA has been reported to occur under similar conditions (32). Therefore, it is possible that the level of CoA in the cytosolic compartment did increase enough with increased ventricular pressure to account for the stimulation of fatty acid activation.

At low rates of oxygen consumption, fatty acid utilization appeared to be limited by the rate of uptake or activation at low exogenous concentrations and by flux through the citric acid cycle when high levels of palmitate were present. When oxygen consumption was increased by raising ventricular pressure, the rates of the citric acid cycle and fatty acid uptake were accelerated and the limiting step for fatty acid utilization was shifted to oxidation of acylcarnitine. The tissue content of acylcarnitine increased even though the rate of β oxidation was accelerated and the levels of acyl-CoA and acetylcarnitine both decreased. These results indicated that either translocation of acyl units across the inner mitochondrial membrane or β oxidation had a limited capacity to produce acetyl-CoA. When flux through the citric acid cycle was accelerated, the capacity to produce acetyl-CoA was not great enough to maintain high tissue levels of this intermediate. Since oxidation of octanoate, which may bypass the carnitine-dependent translocation step (31, 31), maintained high levels of acetyl-CoA as the rate of fatty acid utilization was more than doubled, the capacity of β oxidation did not appear to be exceeded by raising the level of cardiac work. If the capacity of acyl translocation (Enzyme II in Fig. 11) became limiting for acetyl-CoA production at the high level of pressure, a decrease in the mass-action ratio for the carnitine palmityltransferase system would be expected. From the plot of the ratio of acyl-CoA to CoA versus acetylcarnitine to carnitine, the mass-action ratio for this enzyme system did appear to decrease with acceleration of palmitate oxidation. Since this plot defines the mass-action ratio in the direction of acyl-CoA production, this apparent decrease in ratio could have resulted from (a) a simulation of the outer transferase by factors other than availability of substrates which would have increased acetylcarnitine production, or (b) a decrease in the mass-action ratio for the inner transferase (Fig. 11). A stimulation of the outer transferase seems unlikely since
it did not appear to be rate-limiting under any of the conditions studied. The observed decrease in the mass-action ratio for this enzyme system, therefore, was probably due to a decrease in the ratio for the inner transferase. Such a decrease in the ratio of products to substrates for the inner enzyme would be expected if translocation of acyl units restricted oxidation of acylcarnitine at higher rates of oxidative phosphorylation.

Pande (48) suggested that the rate of acylcarnitine oxidation by isolated heart mitochondria was limited by the capacity of the $\beta$ oxidation system. Bremer (52), however, presented evidence that the total capacity of the $\beta$ oxidation system was too large to be rate-limiting. The results from the present study suggest that, at high rates of oxidative phosphorylation, the capacity to translocate acyl units across the inner mitochondrial membrane limited oxidation of long chain acylcarnitine.

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