J Mol Cell Cardiol 28, 825–833 (1996)

Young Investigator

Regulation of Fatty Acid Oxidation by Acetyl-CoA Generated from Glucose Utilization in Isolated Myocytes

Salah Abdel-aleem, Mohamed A. Nada,[1] Mohamed Sayed-Ahmed, Steven C. Hendrickson, James St Louis, Howard P. Walthall and James E. Lowe

Duke University Medical Center, Department of Surgery, Pathology and [1]Pediatrics, Durham, North Carolina, 27710, USA

(Received 18 April 1995, accepted in revised form 9 November 1995)

S. Abdel-aleem, M. A. Nada, M. Sayed-Ahmed, S. C. Hendrickson, J. St Louis, H. P. Walthall and J. E. Lowe. Regulation of Fatty Acid Oxidation by Acetyl-CoA Generated from Glucose Utilization in Isolated Myocytes. *Journal of Molecular and Cellular Cardiology* (1996) 28, 825–833. The regulation of fatty acid oxidation in isolated myocytes was examined by manipulating mitochondrial acetyl-CoA levels produced by carbohydrate and fatty acid oxidation. L-carnitine had no effect on the oxidation of [U-^{14}C]glucose, but stimulated oxidation of [1-^{14}C]palmitate in a concentration-dependent manner. L-carnitine (5 mM) increased palmitate oxidation by 37%. The phosphodiesterase inhibitor, enoximone (250 μM), also increased palmitate oxidation by 51%. Addition of L-carnitine to enoximone resulted in a two-fold increase of palmitate oxidation. Whereas, dichloroacetate (DCA, 1 mM), which stimulates PDH activity, decreased palmitate oxidation by 25%. Furthermore, the addition of DCA to myocytes preincubated with either L-carnitine or enoximone, had no effect on the carnitine-induced stimulation of palmitate, and reduced that of enoximone by 50%. Varied concentrations of DCA decreased the oxidation of palmitate and octanoate; but increased glucose oxidation in myocytes. The rate of efflux of acetylcarnitine was highest when pyruvate was present in the medium compared to efflux rates in presence of palmitate or palmitate plus glucose. Although the addition of L-carnitine plus enoximone resulted in a two-fold increase in palmitate oxidation, acetylcarnitine efflux was minimal under these conditions. Acetylcarnitine efflux was highest when pyruvate was present in the medium. These rates were dramatically decreased when myocytes were preincubated with enoximone, despite the stimulation of palmitate oxidation by this compound. These data suggest that: (1) fatty acid oxidation is influenced by acetyl-CoA produced from pyruvate metabolism; (2) L-carnitine may be specific for mitochondrial acetyl-CoA derived from pyruvate oxidation; and (3) it is probable that acetyl-CoA from β-oxidation of fatty acids is directly channeled into the citric acid cycle.

Key Words: L-carnitine; Pyruvate dehydrogenase; Acetyl-CoA; Fatty acid oxidation.

Introduction

Long-chain fatty acids represent the major substrate for energy production in the normal working heart (Neely and Morgan, 1974a). The relationship between fatty acids and glucose utilization, the glucose-fatty acid cycle, was proposed by Randle three decades ago (Randle *et al*., 1963, 1964). According to this hypothesis, glucose utilization in skeletal and cardiac muscle is inhibited by increasing cellular rates of fatty acid oxidation. It has been suggested that these effects may be mediated through the inhibition of the pyruvate dehydrogenase complex (PDH, EC 1. 2. 4. 1) by end products of fatty acid oxidation such as acetyl-CoA and NADH (Wieland, 1983; Behal *et al*., 1993). Also, an increase in β-oxidation leads to accumulation of citrate and subsequent inhibition of glycolysis and

Please address all correspondence to: Dr Salah Abdel-aleem, Duke University Medical Center, Box 3954, Durham, NC 27710, USA.

phosphofructokinase (Randle *et al.*, 1964b; Regen *et al.*, 1964). Recent studies with isolated rat heart mitochondria have suggested that the pool of acetyl-CoA derived from β-oxidation is different from that which is produced from carbohydrate oxidation (Lysiak *et al.*, 1986). The acetyl-CoA generated from carbohydrate is accessible to carnitine acetyl-transferase to form acetylcarnitine, whereas that produced by β-oxidation is more readily available to the Krebs cycle. If this is the case, the proposed regulation of PDH by fatty acid derived acetyl-CoA needs to be reevaluated, because this acetyl-CoA may be unavailable for PDH regulation. Furthermore, since these previous studies were performed with isolated mitochondria, which do not include the entire pathways of glucose and fatty acid metabolism, these findings need to be reevaluated in isolated tissue or cells in which all regulatory mechanisms are operative.

Likewise, mechanism of the reverse process, the decrease of fatty acid oxidation by increase of glucose utilization, is not fully understood. Recent evidence has been presented suggesting that depression of fatty acid oxidation induced by increasing glucose utilization is mediated through the increase of malonyl-CoA concentration in the heart (Awan and Saggerson, 1993; Saddik *et al.*, 1993). This compound is a potent inhibitor of carnitine palmitoyl-transferase I (CPT I, EC 2. 3. 1. 21) (McGarry *et al.*, 1978; Murthy and Pande, 1987). The sensitivity of CPT I inhibition to malonyl-CoA in cardiac tissues is much higher than that of liver (Cook, 1984). These studies have suggested that activation of PDH by insulin and dichloroacetate (DCA) causes an increase in the malonyl-CoA levels in the cytosol that may lead to inhibition of fatty acid oxidation. Malonyl-CoA is produced from acetyl-CoA by acetyl-CoA carboxylase (ACC, EC 6. 4. 1. 2). Cardiac and skeletal muscle tissues contain a specific isoform of this enzyme which is distinct from the liver enzyme (Bianchi, 1990). Another possible regulatory mechanism for fatty acid oxidation is inhibition of 3-ketoacyl-CoA thiolase (EC 2. 3. 1. 16) by acetyl-CoA produced from the PDH reaction. Kinetic studies with purified 3-ketoacyl-CoA thiolase isolated from pig heart tissue have revealed that it is inhibited by acetyl-CoA, $K_i = 4\ \mu M$ (Schulz, 1985).

The present study was designed to investigate the regulation of glucose and palmitate oxidation in isolated cardiac cells by the mitochondrial acetyl-CoA produced by carbohydrate and fatty acid metabolism. Manipulation of acetyl-CoA levels was achieved by using pharmacological agents such as L-carnitine, dichloroacetate (DCA), and enoximone. L-carnitine decreases acetyl-CoA level by increasing the efflux of mitochondrial acetylcarnitine (Lysiak *et al.*, 1986; Lysiak *et al.*, 1988). DCA increases acetyl-CoA levels by enhancing the PDH activity and stimulating the conversion of pyruvate into acetyl-CoA (Stacpoole, 1992). The phosphodiesterase inhibitor, enoximone, increases transport of fatty acids across the plasma membrane and acetyl-CoA production from fatty acid oxidation (Abdel-aleem and Frangakis, 1991; Abdel-aleem *et al.*, 1991).

Materials and Methods

Animals

Male Sprague–Dawley rats, weighing 200–250 g, were obtained from Charles River Laboratory (Raleigh, NC, USA). Rats were allowed free access to standard diet and water *ad libitum*. The animal protocol was approved by Duke University's Animal Use Committee. Animals were killed by decapitation.

Materials

[U-^{14}C]-D-glucose, [1-^{14}C]octanoate, and [1^{14}C]palmitate were purchased from New England Nuclear (Boston, MA, USA). Sigma was the source of bovine serum albumin (BSA, essentially fatty acid free). L-carnitine was a generous gift from Sigma-Tau Pharmaceuticals, Inc. (Gaithersburg, MD, USA). Joklik essential medium was purchased from Gibco Laboratories (NJ, USA). Collagenase type II was purchased from Worthington (NJ, USA).

Isolation of myocytes

Adult rat heart myocytes were isolated using the published method of Frangakis *et al.* (1980). Myocytes were isolated with Joklik essential medium containing 5.55 mM glucose, 25 mM $NaHCO_3$, 1.2 mM $MgCl_2$ and 0.5 mM $CaCl_2$. The viability of myocytes isolated by this procedure was 80–90% as determined by trypan blue exclusion.

Metabolic studies using myocytes

Myocytes (2 mg cell protein) suspended in 0.9 ml of Joklik medium, containing 25 mM $NaHCO_3$,

5.55 mM glucose, 1.2 mM $MgCl_2$, 0.5 mM $CaCl_2$ and 10 mM HEPES (pH 7.4), were placed in a 25-ml Erlenmeyer flask. Cells were preincubated with the desired concentrations of L-carnitine, DCA, and enoximone for 10 min at 37°C. To this cell suspension was added 0.1 ml of a single-labeled metabolic substrate yielding a final concentration of 0.2 mM [1-^{14}C]-palmitic acid (2.2×10^5 dpm), 0.2 mM [1-^{14}C]octanoate (2.1×10^5 dpm), and 5.55 mM [U-^{14}C]-D-glucose (2×10^5 dpm). The Erlenmeyer flask was then closed with a rubber septum containing a plastic center well. The incubation was continued during shaking at 37°C for 30 min. An injection of 0.4 ml of 1 M hyamine hydroxide was administered through the center well septum to absorb the released CO_2, and the reaction was terminated by injecting 0.4 ml of 7% perchloric acid through the center well into the incubation medium. The flasks were then shaken continuously for 2 h at 37°C, at which time the plastic center well was removed, placed into a scintillation vial containing 10 ml of Scinti Verse BD, and counted in a liquid scintillation counter. Control experiments with $NaH^{14}CO_3$ added to the cell suspension showed that the release of $^{14}CO_2$ was complete 1 h after the addition of perchloric acid. All results were corrected for non-specific release of $^{14}CO_2$ by subtracting blank counts from the counts after addition of radioactive substrate to the cell suspension.

Stock solutions of substrates were prepared by dissolving glucose or octanoate in a myocyte suspension buffer and by dissolving palmitic acid in a solution of defatted serum albumin in the cell suspension buffer. The molar ratio of palmitate to albumin was 2:1.

Analysis of acetylcarnitine by tandem mass spectrometry

Myocytes (2 mg cell protein) suspended in 0.9 ml of Joklik medium, containing 25 mM $NaHCO_3$, 5.55 mM glucose, 1.2 mM $MgCl_2$, and 0.5 mM $CaCl_2$ were preincubated with L-carnitine (5 mM), L-carnitine plus enoximone (0.25 mM), and DCA (1 mM) alone or in combination with other agents for 10 min at 37°C. Myocytes were then incubated for additional 30 min at 37°C after the addition of 0.1 ml of a metabolic substrate such as 0.2 mM palmitate, 5 mM pyruvate, and 5.55 mM glucose. At the end of this period, cell suspensions were centrifuged for 2 min. To 0.1-ml aliquots of the supernatant, 20 pmol of [$^2H_3$]acetylcarnitine was added as internal standard. The mixture was extracted with 0.8 ml methanol, centrifuged and clear supernatant was dried under nitrogen and immediately prepared for analysis of acetylcarnitine. The dried aliquots containing acetylcarnitine were incubated with 100 μl of 3 M HCl in n-methanol at 50°C for 15 min in a capped 1-ml glass vial. The esterifying agent was removed by evaporation under nitrogen and the derivativized sample was dissolved in 50 μl of methanol:glycerol (1:1; v/v) containing 1% octyl sodium sulfate (matrix). A QUATTRO tandem quadrupole mass spectrometer (Fisons-VG Instruments, Danvers, MA, USA) equipped with a liquid secondary ionization source and a cesium ion gun was used for the analysis of acetylcarnitine. This method of analysis is based on the detection of a common fragment ion of acetylcarnitine methyl esters produced by collision-induced dissociation as previously described by Millington *et al.* (1991). Approximately 2 μl of sample matrix were analysed and the data recorded and processed as previously described. The final spectra displayed the relative intensities of ions corresponding to the molecular weights of the individual acylcarnitine methyl esters. The concentrations of acetylcarnitine corresponding to m/z 218 were determined based on their intensities relative to the internal standard, labeled acetylcarnitine, which corresponds to m/z 221.

Statistical analysis

Statistical analysis were performed using the paired *t*-test and comparing groups to control or compare groups to each other. A $P<0.05$ was considered significant.

Results

Since L-carnitine has been shown to decrease acetyl-CoA levels in mitochondrial preparations, this experiment was performed to investigate if the changes induced by L-carnitine affect glucose and palmitate oxidation. Glucose and palmitate concentration in this experiment were 5.55 mM and 0.2 mM, respectively. These concentrations represent physiological or near physiological levels of these substrates. The rate of oxidation of these substrates was determined by measuring the rate of conversion of [1-^{14}C]palmitate, and [U-^{14}C]glucose into $^{14}CO_2$. As shown in Figure 1 L-carnitine significantly increased palmitate oxidation in a concentration-dependent manner. At a concentration of 5 mM, L-carnitine increased palmitate oxidation

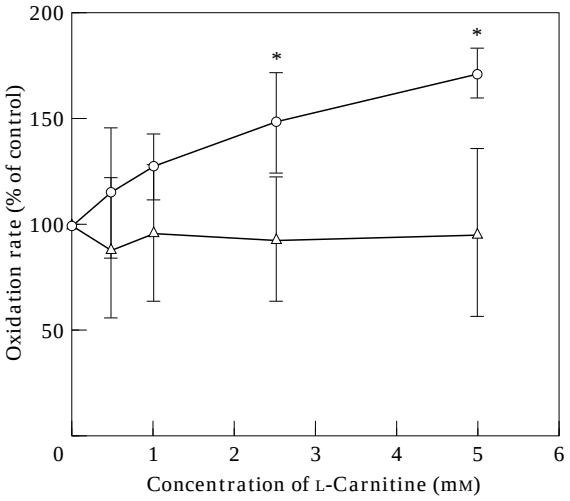

Figure 1 The effect of L-carnitine on the oxidation of [1-^{14}C]palmitate, and [U-^{14}C]-glucose in myocytes. Myocytes were preincubated without (control) or with carnitine for 10 min, then the oxidation of [1-^{14}C]palmitate (open circle) or [U-^{14}C]glucose (open triangle) were determined. The control values for the oxidation of palmitate and glucose were 2.06 ± 0.22 and 5.72 ± 1.72 (nmol/mg protein/30 min), respectively. Values are presented as the mean $\pm$ S.D. of at least three separate experiments. Asterisks indicate a $P<0.05$ of the effect of L-carnitine *v* the control.

Table 1 The regulation of palmitate oxidation by L-carnitine and dichloroacetate in myocytes

Addition	Rate of palmitate oxidation (nmol/mg protein/30 min)
None, control	2.33 ± 0.14
Carnitine (5 mM)	3.23 ± 0.45*
Enoximone (0.25 mM)	3.52 ± 0.17*
DCA (1 mM)	1.74 ± 0.18*
Carnitine (5 mM) + enoximone (0.25 mM)	4.76 ± 0.92*
DCA (1 mM) + carnitine (5 mM)	2.47 ± 0.49
DCA (1 mM) + enoximone (0.25 mM)	2.88 ± 0.11*
DCA (1 mM) + enoximone (0.25 mM) + carnitine (5 mM)	3.98 ± 0.76*

Cells were preincubated with L-carnitine, DCA, enoximone for 10 min. Reactions were initiated by addition of 0.2 mM palmitate. Values are presented as the mean $\pm$ S.D. of at least three separate experiments. * indicates a $P<0.05$ of various drug addition *v* the control.

by 60%. Glucose oxidation was unaffected by L-carnitine.

Table 1 shows effects of several pharmacological agents that regulate the acetyl-CoA produced from glucose and fatty acid metabolism on oxidation of palmitate. This experiment is designed to show whether the regulation of mitochondrial acetyl-CoA level exerts a corresponding effect on palmitate oxidation. Agents such as L-carnitine, DCA, and enoximone were used at single concentration which elicit significant effects on glucose and palmitate oxidation according to previous published studies in isolated mitochondria, myocytes, and isolated working heart preparation (8, 9, 21, 22). The effect of L-carnitine (5 mM), enoximone (0.25 mM), DCA (1 mM), L-carnitine (5 mM) plus enoximone (0.25 mM), DCA (1 mM) plus L-carnitine (5 mM), DCA (1 mM) plus enoximone (0.25), and DCA (1 mM) plus enoximone (0.25 mM) plus L-carnitine (5 mM), on palmitate oxidation was determined in isolated myocytes. Palmitate oxidation was significantly increased by 37% and 51% by L-carnitine, and enoximone, respectively. Palimitate oxidation was decreased 25% by DCA. Addition of L-carnitine plus enoximone caused almost a two-fold increase of palmitate oxidation. Although DCA significantly reduced enoximone-induced stimulation of palmitate oxidation (2.88 ± 0.11 *v* 3.52 ± 0.17), its reduction of carnitine-induced stimulation of palmitate oxidation was statistically insignificant (2.47 ± 0.49 *v* 3.23 ± 0.45). Also, the reduction of palmitate oxidation by DCA in the myocytes which preincubated with L-carnitine plus enoximone was not significant when compared to L-carnitine plus enoximone without DCA (3.98 ± 0.76 *v* 4.76 ± 0.92).

The rate of efflux of acetylcarnitine from isolated myocytes was studied under conditions which resulted in increasing or decreasing acetyl-CoA level produced from metabolism of glucose and fatty acids by the pharmacological agents described previously. These studies were designed to investigate whether acetylcarnitine efflux from myocytes is predominately produced by carbohydrate or fatty acid oxidation. Myocytes were preincubated with L-carnitine (5 mM) for 10 min, then cells were incubated with enoximone (0.25 mM), DCA (1 mM), or enoximone plus DCA for 5 min. At the end of this period, a metabolic substrate containing 0.2 mM palmitate, 5 mM pyruvate, or 5.55 mM glucose was added to cell suspensions and incubated for additional 30 min at 37°C in a shaking water bath. As shown in Table 2, the rate of efflux of acetylcarnitine was highest when pyruvate was present in the medium (237 ± 65.7 nmol/mg protein/30 min) compared to the efflux rate in presence of palmitate or palmitate plus glucose (38.92 ± 6.31 and 44.05 ± 3.1 nmol/mg protein/30 min). Although the addition of L-carnitine plus enoximone resulted

Table 2 The rate of efflux of acetylcarnitine in myocytes

Addition	Acetylcarnitine efflux rate (mmol/mg protein/30 min)
Palmitate (basal, no carnitine)	1.76 ± 0.06
Pyruvate (basal, no carnitine)	4.16 ± 0.54
Palmitate	38.92 ± 6.31
Palmitate + pyruvate	237.60 ± 65.70
Palmitate + pyruvate + enoximone	154.80 ± 8.80
Palmitate + pyruvate + enoximone + DCA	161.21 ± 14.60
Palmitate + pyruvate + DCA	247.52 ± 83.30
Palmitate + enoximone	26.73 ± 2.70
Palmitate + glucose	44.05 ± 3.1
Palmitate + glucose + enoximone	17.87 ± 2.67
Palmitate + glucose + DCA	41.91 ± 2.20

Cells were preincubated with L-carnitine (5 mM) for 10 min. For more experimental details see section under Materials and Methods. Values are presented as the mean ± S.D. of four separate experiments.

in a two-fold increase in palmitate oxidation (see Table 1), acetylcarnitine efflux was minimal under these conditions. The addition of enoximone to palmitate plus pyruvate incubations significantly decreased the acetylcarnitine efflux compared to incubations performed without enoximone. DCA had a negligible effect on acetylcarnitine efflux in presence of pyruvate and glucose. In absence of exogenous L-carnitine the rate of efflux of acetylcarnitine was minimal e.g., this rate was 1.76 ± 0.06 and 4.16 ± 0.54 when palmitate and pyruvate served as the metabolic substrate. The intracellular concentrations of acetylcarnitines were less than 1% of those exported to the exogenous medium (data are not shown).

To investigate the possible site of inhibition of fatty acids by DCA, its effect on palmitate and octanoate oxidation was studied in isolated myocytes. Octanoate was used in this experiment because, unlike palmitate, its oxidation is independent on CPT I activity. Figure 2 shows the effect of varied concentrations of DCA on palmitate and octanoate oxidation in myocytes. The pattern of inhibition of palmitate and octanoate oxidation by varied concentrations of DCA was similar. DCA (at 0.5 mM) did not significantly affect the oxidation of either palmitate or octanoate.

The effect of DCA on palmitate-induced stimulation by L-carnitine was studied in isolated myocytes to investigate if the effect of DCA occurs at all concentrations of L-carnitine. Myocytes were

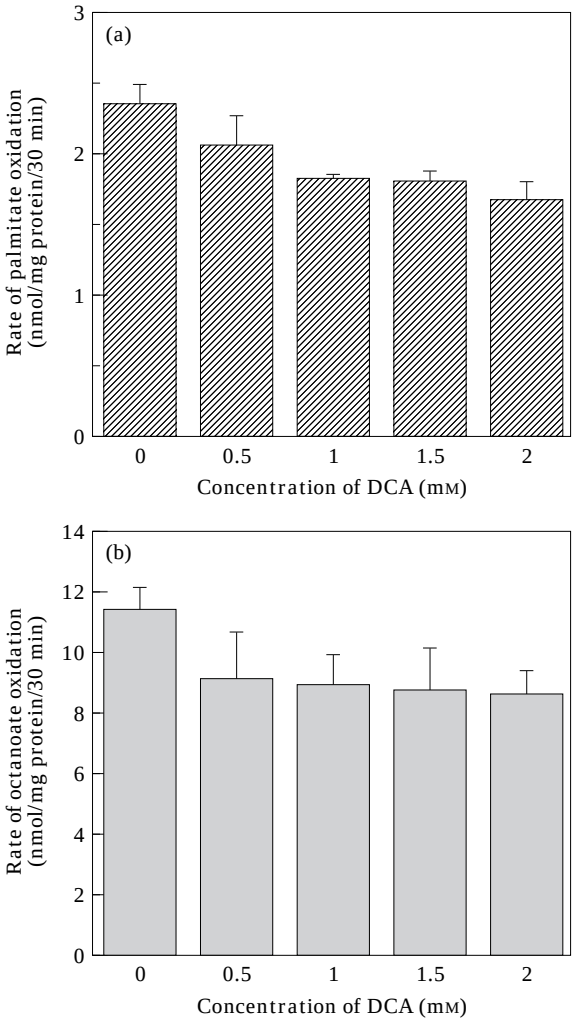

Figure 2 The effect of DCA on (a) palmitate, and (b) octanoate oxidation in myocytes. Myocytes were preincubated without (control) or with varied concentrations of DCA for 10 min, then the oxidation of palmitate and octanoate were determined. Values are the mean ± S.D. of four separate experiments. * indicates a $P<0.05$ of the effect of DCA *v* the control.

preincubated with varied concentrations of L-carnitine, then 1 mM of DCA was added to myocyte suspensions. Reactions were initiated by the addition of 0.2 mM [1-^{14}C]palmitate. As shown in Figure 3, DCA significantly decreased palmitate-induced stimulation by L-carnitine.

To investigate whether induced inhibition of fatty acid oxidation by DCA is accompanied by a corresponding increase of glucose utilization, the effect of DCA on glucose oxidation was studied in isolated myocytes. As shown in Figure 4, DCA increased glucose oxidation in a dose-dependent manner. DCA at a concentration of 2 mM increased glucose oxidation by 49%.

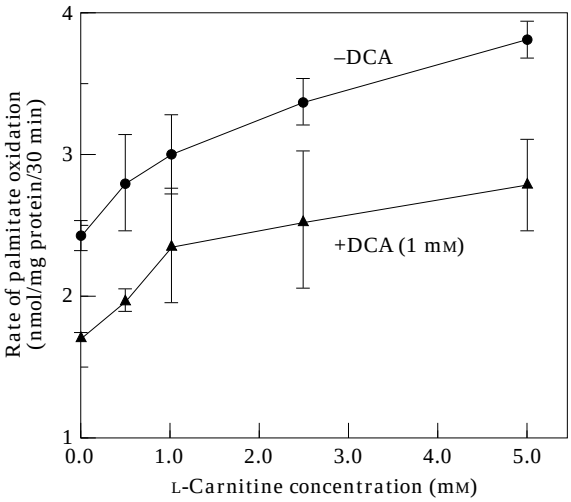

Figure 3 The effect of DCA on L-carnitine-induced stimulation of palmitate oxidation in myocytes. Cells were preincubated with varied concentrations of L-carnitine for 10 min, then 1 mM of DCA was added to the cell suspensions. Reactions were started by the addition of palmitate as described under Materials and Methods. Values are the mean ± S.D. of three separate experiments.

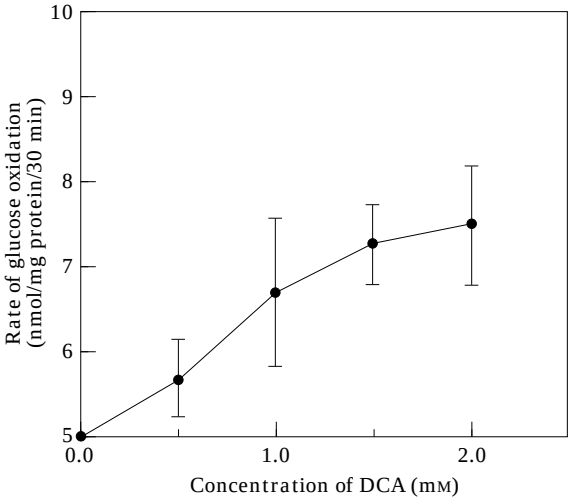

Figure 4 The effect of DCA on the oxidation of glucose in myocytes. Cells were preincubated with varied concentrations of DCA for 10 min. Reactions started by the addition of 5.55 mM of glucose. Values are the mean ± S.D. of three separate experiments.

Discussion

The regulation of fatty oxidation by L-carnitine and dichloroacetate

The present study was designed to examine the regulation of palmitate and glucose oxidation in isolated myocytes by regulating the mitochondrial

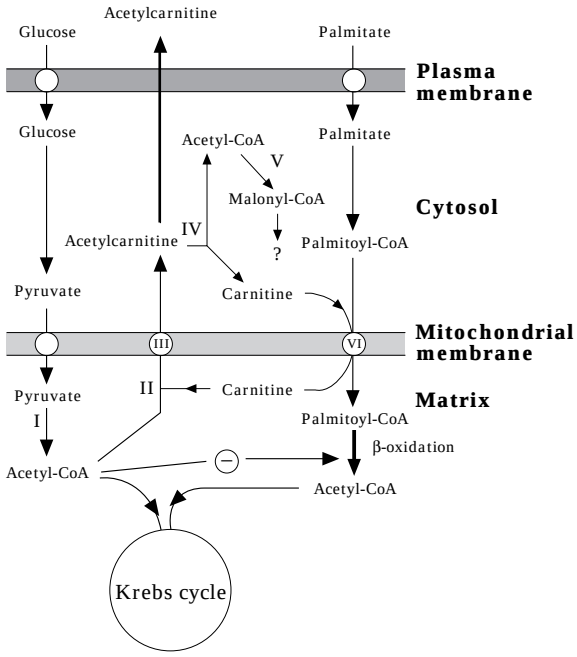

Figure 5 Proposed hypothesis for the regulation of glucose and fatty acid oxidation in isolated myocytes. I, Pyruvate dehydrogenase complex (PDH); II, acetylcarnitine transferase; III, carnitine acetylcarnitine translocase; IV, cytosolic acetylcarnitine transferase; V, acetyl-CoA carboxylase (ACC); VI, palmitoylcarnitine transferase I (CPT I), palmitoylcarnitine translocase, and palmitoylcarnitine transferase II (CPT II).

acetyl-CoA produced from carbohydrate and fatty acid metabolism. Regulation of mitochondrial acetyl-CoA levels by L-carnitine and DCA has been reported previously. L-carnitine decreases mitochondrial acetyl-CoA levels by increasing the efflux of acetylcarnitine generated from acetate produced by carbohydrate metabolism (Lysiak *et al.*, 1986; Uziel *et al.*, 1988). Conversely, DCA increases its level by activating the pyruvate dehydrogenase complex, PDH complex (Stacpoole, 1992). Therefore, stimulation of palmitate oxidation by L-carnitine and its inhibition by DCA in the present study suggests that palmitate oxidation may be regulated by the acetyl-CoA derived from pyruvate. Mitochondrial acetyl-CoA may depress oxidation of fatty acid by inactivating 3-ketoacyl-CoA thiolase, the final enzyme of the β-oxidation pathway. Acetyl-CoA has been shown to be a potent inhibitor of 3-ketoacyl-CoA thiolase purified from pig heart tissue (Schulz, 1985). The possibility that L-carnitine may increase palmitate oxidation by another mechanism such as increasing the mitochondrial uptake of palmitate can not be completely ruled out based on these experiments. However, the study by Wang

et al. has demonstrated several-fold stimulation of palmitoylcarnitine oxidation by L-carnitine during state 4 respiration in isolated rat heart mitochondria (Wang *et al*., 1991). Palmitoylcarnitine is a substrate whose oxidation is independent of the carnitine palmitoyltransferase system and dependent on mitochondrial β-oxidation system. Thus, its stimulation by L-carnitine suggests that this compound may regulate fatty acid oxidation at the β-oxidation level.

Another possibility, which has been proposed by Saddik *et al*. (1993), is that DCA decreases palmitate oxidation by increasing malonyl-CoA levels in the cytosol. Malonyl-CoA is produced from acetyl-CoA in the cytosol by acetyl-CoA carboxylase (ACC) in cardiac tissues (Wakil *et al*., 1958). Findings from the present study and Saddik *et al*.'s hypothesis agree on the regulation of fatty acid oxidation by acetyl-CoA derived from carbohydrate utilization. The present study suggests direct regulation of fatty acid oxidation by the acetyl-CoA. Whereas, the other hypothesis suggests its regulation by cytosolic malonyl-CoA derived from the acetyl-CoA generated from carbohydrate utilization. According to the hypothesis proposed by Saddik *et al*. (1993), L-carnitine should increase cytosolic concentrations of malonyl-CoA secondary to increased levels of acetylcarnitine, and thereby decrease fatty acid oxidation. The present study demonstrates an increase of palmitate oxidation by L-carnitine. In addition, DCA decreased the oxidation of octanoate, who's utilization is independent of CPT I activity. These results suggest that the regulation of palmitate oxidation by L-carnitine and DCA is not mediated by CPT I but rather through the β-oxidation system under these experimental conditions. These findings are also supported by inhibition of short chain fatty acid substrates such as 3-hydroxybutrate by DCA in cardiac tissues (McAllister *et al*., 1973). If the decrease of fatty acid oxidation by DCA is due to CPT I inhibition by malonyl-CoA, only long-chain fatty acid oxidation should be depressed. Probably the most supportive evidence for the proposed hypothesis comes from the study by Wang *et al*. (1991) where L-carnitine increased the oxidation palmitoylcarnitine in the isolated rat heart mitochondria. The oxidation of this substrate is also independent of the CPT I activity. This effect was associated with a corresponding decrease of mitochondrial acetyl-CoA and increase of free CoA-SH. The lack of inhibition of palmitate oxidation by malonyl-CoA may be due inaccessibility of CPT I to this compound. Cardiac CPT I is much more sensitive to malonyl-CoA inhibition than that of liver, K_i of this compound in the heart is lower by 10- and 100-fold than that of liver from fed and starved animals. However, the intracellular content of malonyl-CoA in cardiac tissue which is only 40% less than that of liver does not inhibit CPT I in the heart (McGarry *et al*., 1989). This suggests that increased levels of malonyl-CoA induced by DCA may not be accessible to the carnitine palmitoyltransferase (CPT I).

The specificity of mitochondrial L-carnitine

The additive stimulation of palmitate oxidation by L-carnitine plus enoximone suggests that these compounds exert stimulatory effects at different sites in the pathway of fatty acid oxidation. Indeed, enoximone has been shown to increase fatty acid oxidation by enhancing its cellular uptake (Abdel-aleem and Frangakis, 1991; Abdel-aleem *et al*., 1991). The stimulation of fatty acid oxidation by enoximone has been shown to be independent of its positive inotropic effect in the heart (Abdel-aleem and Frangakis, 1991). It should also be noted that the effect of enoximone on palmitate oxidation in mitochondria is different from that of myocytes. In isolated rat heart mitochondria, enoximone decreases palmitate oxidation by inactivating acyl-CoA synthetase (Abdel-aleem *et al*., 1992). Whereas, in isolated myocytes enoximone stimulates palmitate oxidation. Thus, the overall effect of enoximone in intact myocytes is stimulation of fatty acid oxidation probably by interacting with plasma membranes, thereby affecting fatty acid transport. L-carnitine may increase fatty acid oxidation by trapping mitochondrial acetyl-CoA generated from pyruvate, thus buffering its inhibitory effect on 3-ketoacyl-CoA thiolase. These data suggest that the increase or decrease of acetyl-CoA generated from pyruvate causes a corresponding decrease or increase of fatty acid oxidation in isolated myocytes.

The effect of L-carnitine on glucose oxidation should be examined carefully because this study was performed with [U-^{14}C]glucose where $^{14}CO_2$ is produced from the PDH reaction and the Krebs cycle. Broderick *et al*. (1992) have shown stimulation of glucose oxidation by L-carnitine in the perfused rat heart preparation. Recent studies from this laboratory have demonstrated stimulation of PDH complex, decrease of acetyl-CoA flux (generated from carbohydrates) through the Krebs cycle, and decrease of oxidative glucose metabolism by L-carnitine in myocytes (Abde-aleem *et al*., 1995). Discrepancies between these studies may be due to using different experimental conditions since Broderick's study was performed with isolated rat

heart preparation using [U-^{14}C]glucose in presence of 1.2 mM palmitate, while the other study was performed with isolated myocytes using [6-^{14}C]glucose in absence of exogenous fatty acids.

Measurement of the efflux of acetylcarnitine from isolated myocytes under conditions which caused stimulation of glucose and palmitate oxidation may provide further support for the proposed hypothesis by determining the source of acetylcarnitine accumulated in the medium. The rate of efflux is highest when pyruvate is present in the medium. This efflux is markedly decreased when enoximone plus L-carnitine is added to the medium and palmitate oxidation is concomitantly stimulated, despite a two-fold increase of palmitate oxidation. Rates of efflux of acetylcarnitine produced from pyruvate were decreased in presence of enoximone probably due to its inhibition of pyruvate flux through the PDH. It has been previously shown that enoximone decreases utilization of pyruvate in myocytes (Abdel-aleem and Frangakis, 1991). These findings suggest that acetyl-CoA produced by β-oxidation is inaccessible to carnitine acetyltransferase system. Whereas, acetyl-CoA produced from pyruvate is readily accessible to this system. Acetyl-CoA produced from β-oxidation probably is channeled directly to the citric acid cycle. This conclusion is supported by the demonstration of same effects on efflux of acetylcarnitine by L-carnitine when pyruvate and octanoate were used as metabolic substrates in the isolated rat heart mitochondria (Lysiak *et al.*, 1986).

The regulation of glucose and fatty acid oxidation in isolated cardiac cells

The regulation of glucose and fatty acid oxidation in isolated cardiac myocytes is illustrated in Figure 5. Inside the mitochondrial matrix, L-carnitine enhances efflux rates of acetyl-CoA generated from pyruvate by the PDH complex (reaction I). Reaction I is enhanced by L-carnitine, and DCA. Acetyl-CoA is converted into acetylcarnitine by carnitine acetyltransferase (reaction II). Acetylcarnitine is transported across the mitochondrial membrane into cytosol by carnitine: acetylcarnitine translocase (reaction III). The majority of this acetylcarnitine is released into the external medium. Acetylcarnitine that is not transported into the external medium is converted into acetyl-CoA and free carnitine in the cytosol by carnitine acetylcarnitine transferase. Cytosolic ACC may convert acetyl-CoA into malonyl-CoA. The role of this malonyl-CoA remains to be established, but our findings suggest that it is not accessible to CPT I.

These findings support our hypothesis that acetyl-CoA produced by carbohydrate utilization regulates the rate of fatty acid oxidation. Conditions that cause increase or decrease in the activity of the PDH complex will lead to a corresponding, increase or decrease of acetyl-CoA, and inhibition or stimulation of β-oxidation. On the other hand, increased utilization of fatty acids such as in starvation or diabetes may cause an increase of the oxidation of acetyl-CoA produced from β-oxidation, which may cause accumulation of the acetyl-CoA produced from pyruvate, thereby resulting in feedback inhibition of PDH complex and the oxidation of glucose.

In conclusion, these data show that: (1) the rate of fatty acid oxidation is influenced by the acetyl-CoA generated from pyruvate metabolism; (2) the mitochondrial carnitine or carnitine acetyltransferase may be specific for the acetyl-CoA which is generated from pyruvate oxidation, and (3) the acetyl-CoA produced from β-oxidation is probably directly channeled into the citric acid cycle.

Acknowledgement

Supported in part by the Harold and Linda Plemmons Cardiovascular Research Foundation.

References

ABDEL-ALEEM S, FRANGAKIS C, 1991. Stimulation of fatty acid oxidation by phosphodiesterase III inhibitors in rat myocytes. *J Cardiovasc Pharmacol* 18: 293–297.

ABDEL-ALEEM S, BADR M, FRANGAKIS C, 1991. Stimulation of polyunsaturated fatty acid oxidation by regulating its cellular uptake. *Life Sci* 49: 185–192.

ABDEL-ALEEM S, YOUSSEF J, BADR M, FRANGAKIS CJ, 1992. The inhibition of long-chain fatty acyl-CoA synthetase by enoximone in rat heart mitochondria. *J Cardiovasc Pharmacol* 19: 899–904.

ABDEL-ALEEM S, SAYED-AHMED M, HENDRICKSON S, ST LOUIS J, LOWE JE, 1995. Stimulation of non-oxidative glucose oxidation by L-carnitine in myocytes. *J Mol Cell Cardiol* 27: 2465–2472.

AWAN MM, SAGGERSON ED, 1993. Malonyl-CoA metabolism in cardiac myocytes and its relevance to the control of fatty acid metabolism. *Biochem J* 295: 61–66.

BEHAL RH, BUXTON DB, ROBERTSON JG, OLSON MS, 1993. Regulation of the pyruvate dehydrogenase multienzyme complex. *Annu Rev Nutr* 13: 497–520.

BIANCHI A, EVANS JL, IVERSON AJ, NORDLUND AC, WATTS TD, WITTERS LA, 1990. Identification of an isoenzymic form of acetyl-CoA *J Biol Chem* 265: 1502–1509.

BRODERICK TL, QUINNEY HA, LOPASCHUK GD, 1992. Carnitine stimulation of glucose oxidation in the fatty acid

perfused isolated working heart. *J Biol Chem* 267: 3758–3763.

COOK GA, 1984. Differences in the sensitivity of carnitine palmitoyltransferase to inhibition by malonyl-CoA are due to differences in K_i values. *J Biol Chem* 259: 12030–12033.

FRANGAKIS CJ, BAHL JJ, MCDANIEL H, BRESSLER R, 1980. Tolerance to physiological calcium by isolated myocytes from the adult rat heart: An improved cellular preparation. *Life Sci* 27: 815–825.

LYSIAK W, TOTH PP, SUELTER CH, BIEBER LL, 1986. Quantitation of the efflux of acylcarnitine from rat heart, brain, and liver mitochondria. *J Biol Chem* 261: 13698–13703.

LYSIAK W, LILLY K, DILISA F, TOTH PP, BIEBER LL, 1988. Quantitation of the effects of L-carnitine on the levels of acid-soluble short-chain acyl-CoA and CoASH in rat heart and liver mitochondria. *J Biol Chem* 263: 1151–1156.

MCALLISTER A, ALLISON SP, RANDLE PJ, 1973. Effects of dichloroacetate on the metabolism of glucose, pyruvate, acetate, 3-hydroxybutyrate and palmitate in rat diapragm and heart muscle in vitro and on extraction of glucose, lactate, pyruvate and free fatty acids by dog heart in vivo. *Biochem J* 134: 1067–1081.

MCGARRY JD, LEATHERMAN GF, FOSTER DW, 1978. Carnitine palmitoylfransferase I: The site of inhibition of hepatic fatty acid oxidation by malonyl-CoA. *J Biol Chem* 253: 4128–4136.

MCGARRY JD, WOELTJE KF, KUWAJIMA M, FOSTER DW, 1989. Regulation of ketogenesis and the renaissance of carnitine palmitoyl transferase. *Diabetes/Metabolism Rev* 5: 271–284.

MILLINGTON DS, KODO N, TERADA N, ROE D, CHASE DH, 1991. The analysis of diagnostic markers of genetic disorders in human blood and urine using tandem mass spectrometry with liquid secondary ion mass spectrometry. *Int J Mass Spectrometry Ion Proc* 111: 211–228.

MURTHY MSR, PANDE SV, 1987. Malonyl-CoA binding site and the overt carnitine palmitoyltransferase activity reside on the opposite sides of the outer mitochondrial membrane. *Proc Natl Acad Sci USA* 84: 378–382.

NEELY JR, MORGAN HE, 1974. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annu Rev Physiol* 36: 413–459.

RANDLE PJ, GARLAND PB, HALES CN, NEWSHOLME EA, 1963. The glucose-fatty acid cycle: its role in insulin sensitivity and the metabolic disturbance of diabetes mellitus. *Lancet* 1: 785–789.

RANDLE PJ, NEWSHOLME EA, GARLAND PB, 1964a. Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies, and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. *Biochem J* 93: 652–665.

RANDLE PJ, GARLAND PB, HALES CN, NEWSHOLME EA, DENTON RM, POGSON CI, 1964b. Interaction of metabolism and the physiological role of insulin. *Recent Progr Hormone Res* 22: 1–44.

REGEN DM, DAVIS WW, MORGAN HE, PARK CR, 1964. The regulation of hexokinase and phosphofructokinase activity in heart muscle. *J Biol Chem* 239: 43–49.

SADDIK M, GAMBLE J, WITTERS LA, LOPASCUK GD, 1993. Acetyl-CoA carboxylase regulation of fatty acid oxidation in the heart. *J Biol Chem* 268: 25836–25845.

SCHULZ H, 1985. In: Vance DE, and Vance JE (eds) *Biochemistry of Lipids and Membranes* Benjamin-Cummings, Menlo Park, CA.

STACPOOLE PW, 1992. Dichloroacetate. *Diabetes Care* 15: 785–791.

UZIEL G, GARAVAGLIA B, DI DONATO S, 1988. Carnitine stimulation of pyruvate dehydrogenase complex (PDHC) in isolated human skeletal muscle mitochondria. *Muscle & Nerve* 11: 720–724.

WAKIL S, TITCHENER EB, GIBSON DM, 1958. Evidence for the participation of biotin in the enzymic synthesis of fatty acids. *Biochim Biophys Acta* 29: 225–226.

WANG H-Y, BAXTER JR, CF, SCHULZ H, 1991. Regulation of fatty acid oxidation in rat heart mitochondria. *Arch Biochem Biophys* 289: 274–280.

WIELAND OH, 1983. The mammalian pyruvate dehydrogenase complex: structure and regulation. *Rev Physiol Biochem Pharmacol* 95: 123–170.