Different sites of inhibition of carnitine palmitoyltransferase by malonyl-CoA, and by acetyl-CoA and CoA, in human skeletal muscle

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The inhibition of carnitine palmitoyltransferase (CPT, EC 2.3.1.21) by malonyl-CoA, acetyl-CoA and free CoA was studied in sonicated skeletal-muscle homogenates from normal human subjects and from five patients with a mutant CPT [Zierz & Engel (1985) *Eur. J. Biochem.* **149**, 207–214]. (1) Malonyl-CoA, acetyl-CoA and CoA were competitive inhibitors of CPT with palmitoyl-CoA. (2) Acetyl-CoA and CoA inhibited normal and mutant CPT to the same degree, whereas malonyl-CoA inhibited mutant CPT more than normal CPT. (3) Triton X-100 abolished the inhibition of normal CPT by malonyl-CoA, but not by acetyl-CoA or CoA. Triton X-100 by itself caused loss of activity of the mutant CPT. (4) In the concentration range 0.1–0.4 mM, the inhibitory effects of any two of the three inhibitors were synergistic. (5) The inhibitory constants (K_i) for acetyl-CoA and CoA were close to 45 μ M. The K_i for malonyl-CoA was 200-fold lower, or 0.22 μ M. Addition of 40 μ M-acetyl-CoA or CoA resulted in a 3-fold increase in the K_i for acetyl-CoA. Addition of 20 μ M-CoA resulted in a 3-fold increase in the K_i for acetyl-CoA. (6) The findings indicate that acetyl-CoA and CoA can inhibit CPT at the catalytic site or a nearby site which is different from that at which malonyl-CoA inhibits CPT. (7) The fact that small changes in the concentration of acetyl-CoA and CoA can antagonize the inhibitory effect of malonyl-CoA suggests that these compounds could modulate the inhibition of CPT by malonyl-CoA.

INTRODUCTION

CPT catalyses the transfer of long-chain fatty acids through the mitochondrial inner membrane. It is generally assumed that there are two pools of CPT associated with this membrane: CPT I on the outer, and CPT II on the inner, surface of the membrane [1,2]. Malonyl-CoA is a potent inhibitor of CPT in liver, heart, skeletal muscle and other tissues [3–6]. In liver, but not in heart, the malonyl-CoA-sensitivity of CPT is influenced by the nutritional state of the animal [7–9], suggesting that different mechanisms can control CPT activity in different tissues.

The mechanism by which malonyl-CoA interacts with CPT is not completely understood. Inhibition by malonyl-CoA requires membrane association of the enzyme [5], and the binding site for malonyl-CoA appears to be distinct from the catalytic site of the enzyme, or even from the enzyme itself [10,11]. Malonyl-CoA binding is thiol-group-dependent [12,13] and the inhibition is competitive with long-chain acyl-CoAs [3,7,9].

Further studies of the nature of malonyl-CoA inhibition revealed that structurally related compounds, such as methylmalonyl-CoA, succinyl-CoA, acetyl-CoA and CoA, also inhibited CPT in liver, heart and skeletal muscle [14,15]. These compounds displaced malonyl-CoA from the mitochondrial membrane in a competitive manner, and it was concluded that malonyl-CoA and the related CoA esters interacted at the same binding site [14]. Subsequent studies revealed both low- and high-affinity binding sites for malonyl-CoA on mitochondrial

membranes derived from liver and brain [6,16,17]. In addition, it has been suggested that the high-affinity malonyl-CoA site may be exclusive for this inhibitor, whereas the low-affinity malonyl-CoA site may be a second allosteric site on CPT which also binds acyl-CoA [18].

Recently, a mutant CPT was identified in human skeletal muscle [19]. The mutant enzyme shows abnormal substrate/product inhibition, higher than normal inhibition by malonyl-CoA and, unlike the normal enzyme, loses nearly all of its catalytic activity in the presence of 0.4% Triton X-100 [19]. The present study shows that acetyl-CoA and CoA inhibit normal and mutant CPT in human muscle to the same extent. Furthermore, 0.4% Triton X-100 abolishes the sensitivity of the normal enzyme to inhibition by malonyl-CoA, but not by acetyl-CoA or CoA. This indicates that acetyl-CoA and CoA can inhibit CPT at a site different from that at which malonyl-CoA inhibits CPT.

MATERIALS AND METHODS

Clinical material

The muscle specimens for this study were obtained from human subjects who had muscle biopsies for diagnosis of muscular symptoms. These subjects were deemed to be 'normal controls' if they were ultimately found to have no muscle disease by combined clinical, electromyographic and histological criteria. Specimens from 23 normal controls were used in this study. Muscle specimens were also studied from five cases of CPT

Abbreviation used: CPT, carnitine palmitoyltransferase (EC 2.3.1.21).

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deficiency. The mutant enzyme in these cases of CPT deficiency has been previously characterized [19]. Tissues were frozen immediately at -150°C and then stored under liquid N_2 for subsequent biochemical studies.

Chemicals

DL-[methyl- ^{14}C]Carnitine was obtained from Amersham (Arlington Heights, IL, U.S.A.). Palmitoyl-CoA, malonyl-CoA, acetyl-CoA and CoA were obtained from P-L Biochemicals (Milwaukee, WI, U.S.A.). Fatty acid-free bovine serum albumin was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals were reagent grade and were obtained from commercial sources.

Assay of CPT

Frozen tissues were homogenized with 19 vol. of a solution containing 50 mM-Tris buffer (pH 7.2), 100 mM-KCl, 5 mM- MgSO_4 , 1 mM-EDTA and 1 mM-ATP. Since the tissues had been initially frozen, it could be expected that most mitochondria would be disrupted on thawing, so that the CPT activity associated with outer and inner surfaces of the mitochondrial inner membrane would have access to substrate in the enzyme assays. To ensure that all mitochondria were disrupted, the homogenates were additionally sonicated for 2×30 s at 6 W (Ultrasonic Cell Disruptor; Kontes, Vineland, NJ, U.S.A.). Non-Collagen protein was determined by the methods of Lilienthal *et al.* [20] and Lowry *et al.* [21]. CPT activity was measured in the sonicated homogenates by using the forward assay optimized for human skeletal muscle [19]. This measures the rate of palmitoylcarnitine formation when palmitoyl-CoA and labelled carnitine are incubated with the homogenate [22]. The standard reaction system contained 0.1 M-Tris buffer (pH 7.6), 2 mM-KCN, 0.1% fatty acid-free bovine serum albumin, 1 mM-dithiothreitol, 0.08 mM-palmitoyl-CoA, 0.1 ml of a 5% (w/v) muscle homogenate and 5 mM-DL-carnitine ($0.04 \mu\text{Ci}$ of DL-[methyl- ^{14}C]carnitine/ μmol) in a final volume of 1.0 ml. The reaction was started with 0.1 ml of a 5% muscle homogenate and incubated for 20 min at 30°C . The reaction was stopped and the product [^{14}C]palmitoylcarnitine was extracted by the method of Solberg [23] with 2-methylpropan-1-ol and $(\text{NH}_4)_2\text{SO}_4$ -saturated water, and 0.5 ml of the organic phase was assayed for radioactivity in a liquid-scintillation counter. The inhibitors malonyl-CoA, acetyl-CoA or CoA were added to the reaction mixture immediately before the reaction was started with homogenate, as indicated in the Results section.

In some experiments Triton X-100 was added to sonicated homogenates to give a final concentration of 0.4% (v/v), and the homogenate was kept on ice for 15 min. CPT was then measured in the whole homogenate. The final detergent concentration in the assay was 0.04% (v/v).

The K_i values for malonyl-CoA, acetyl-CoA and CoA were determined by the graphical method of Dixon [24], by using inhibitor concentrations from 0.05 to 1.5 μM for malonyl-CoA and from 5 to 120 μM for acetyl-CoA and CoA in the presence of 0.02 mM-, 0.03 mM- and 0.08 mM-palmitoyl-CoA. The influence of acetyl-CoA or CoA on the K_i of malonyl-CoA was tested by the same protocol but in the presence of 40 μM -acetyl-CoA or

-CoA, and the influence of CoA on the K_i of acetyl-CoA was tested in the presence of 20 μM -CoA.

Since CoA is a product of the forward assay, we also tested the linearity of the forward reaction with time and protein in the presence of 0.4 mM-CoA and 80 μM -palmitoyl-CoA, or in the presence of 80 μM -CoA and 20 μM -palmitoyl-CoA. In either instance, the reaction remained linear with time up to 20 min in the presence of 5 mg wet wt. of muscle.

RESULTS

Inhibition of CPT activity

As reported previously, in the forward assay the activities of the normal and mutant CPT were not significantly different [19]. Malonyl-CoA, acetyl-CoA and CoA inhibited both normal and mutant CPT. The malonyl-CoA inhibition reached a plateau between concentrations of 0.1 and 0.2 mM, but, for all concentrations of malonyl-CoA that were tested, the mutant CPT showed lower activity than the normal CPT. At 0.2 mM-malonyl-CoA, 39% of the initial activity of normal CPT, but only 14% of the initial activity of the mutant CPT, remained ($P < 0.01$) (Fig. 1).

Concentrations of up to 0.4 mM-acetyl-CoA or -CoA inhibited CPT activity progressively, and there was no significant difference between the normal and the mutant enzyme. However, the inhibitory effects of either acetyl-CoA or CoA on either the normal or the mutant enzyme were much less than that of malonyl-CoA. At 0.4 mM-acetyl-CoA, 68% and 75% of the normal and mutant enzyme activity remained respectively (Fig. 1). At the same CoA concentration 67% and 72% of the

Fig. 1. Inhibition of normal and of mutant CPT

CPT activity was measured in the forward assay as described in the Materials and methods section in sonicated homogenates of normal muscles (▲), in muscles of patients with CPT 'deficiency' (●), and in homogenates of normal muscles preincubated with 0.4% Triton X-100 (△). Malonyl-CoA, acetyl-CoA and CoA were present in the assay as indicated. Values are expressed as percentage of initial CPT activity without inhibitor. Symbols and vertical lines represent means $\pm$ s.d. for three patients and four normal subjects. Except for the effect of Triton X-100 on the malonyl-CoA-sensitivity, the data in the left panel were previously published [19].

Table 1. Inhibition of CPT by CoA, acetyl-CoA and malonyl-CoA

CPT was measured in the forward assay in sonicated homogenates of normal human muscles as described in the Materials and methods section. CoA, acetyl-CoA and malonyl-CoA were present in the assay as indicated either alone or in combination. Values are expressed as percentages of the initial CPT activity determined without inhibitor, and are means $\pm$ s.d. for the numbers of subjects shown in parentheses. Values connected by braces are significantly different, indicating synergistic inhibition.

Inhibitor(s) (mM)	CPT activity (%)	<i>P</i>	
CoA (0.1)	82.5 $\pm$ 6.1 (6)	< 0.05	< 0.005
CoA (0.2)	74.8 $\pm$ 4.8 (8)		
CoA (0.4)	68.8 $\pm$ 3.5 (4)		
Acetyl-CoA (0.1)	86.2 $\pm$ 4.9 (19)		
Acetyl-CoA (0.1) + CoA (0.1)	68.5 $\pm$ 1.9 (4)		
Acetyl-CoA (0.2)	74.6 $\pm$ 7.4 (8)		
Acetyl-CoA (0.2) + CoA (0.2)	56.6 $\pm$ 2.2 (4)	< 0.01	
Acetyl-CoA (0.4)	68.2 $\pm$ 5.3 (3)		
Malonyl-CoA (0.1)	41.4 $\pm$ 3.9 (5)	< 0.05	< 0.05
Malonyl-CoA (0.1) + CoA (0.1)	35.5 $\pm$ 5.4 (5)		
Malonyl-CoA (0.1) + acetyl-CoA (0.1)	35.0 $\pm$ 3.5 (5)		
Malonyl-CoA (0.2)	39.2 $\pm$ 4.4 (6)		
Malonyl-CoA (0.2) + CoA (0.2)	30.9 $\pm$ 5.8 (6)		
Malonyl-CoA (0.2) + acetyl-CoA (0.2)	32.7 $\pm$ 4.1 (6)		
Malonyl-CoA (0.4)	38.8 $\pm$ 2.7 (4)		

activity remained in the normal and mutant enzyme respectively (Fig. 1).

The inhibition by acetyl-CoA was augmented by the addition of CoA, or vice versa (Table 1). The combined inhibitory effect of 0.2 mM-CoA plus 0.2 mM-Acetyl-CoA was significantly greater than that of either 0.4 mM-CoA or 0.4 mM-acetyl-CoA (Table 1). The inhibition by 0.1 mM- or 0.2 mM-malonyl-CoA was also significantly increased when either 0.1 mM- or 0.2 mM-CoA or -acetyl-CoA was present (Table 1). These three inhibitors thus act in a synergistic manner, and not as mutually exclusive inhibitors [25].

Effect of Triton X-100

The effect of Triton X-100 on the inhibition of normal CPT by malonyl-CoA, acetyl-CoA and CoA was investigated. Parallel studies could not be performed with mutant CPT, because the detergent by itself abolished nearly all of the mutant enzyme's activity [19]. A concentration of 0.4% Triton X-100 was sufficient to solubilize CPT from the mitochondrial membrane [17]. Preincubation of homogenates with 0.4% Triton X-100 resulted in a nearly complete loss of sensitivity of CPT to inhibition by malonyl-CoA, but did not change the sensitivity of the enzyme to inhibition by acetyl-CoA or CoA (Fig. 1).

Inhibitory constants

The mean K_i values for malonyl-CoA of normal and mutant CPT were previously shown to be 0.22 μ M and 0.15 μ M respectively [19]. Since these K_i values were not significantly different, and since acetyl-CoA and CoA did not cause abnormal inhibition of the mutant enzyme (Fig. 1), Dixon plots for acetyl-CoA, CoA, malonyl-CoA and a combination of these inhibitors were obtained only

Table 2. Inhibitory constants (K_i) for CPT in human muscle

CPT was measured in the forward assay in sonicated homogenates of normal human muscle as described in the Materials and methods section at 20 μ M-, 30 μ M- and 80 μ M-palmitoyl-CoA and in the presence of different inhibitors. K_i values were determined from Dixon plots [24]. Under all conditions the lines of the Dixon plots were linear. The values are means $\pm$ s.d., for the numbers of subjects given in parentheses. The K_i for malonyl-CoA was significantly increased by addition of acetyl-CoA ($P < 0.001$) or CoA ($P < 0.005$). The K_i for acetyl-CoA was significantly increased by addition of CoA ($P < 0.001$).

Inhibitor	K_i (μ M)
Malonyl-CoA	0.22 $\pm$ 0.04 (4)
Malonyl-CoA in the presence of 40 μ M-acetyl-CoA	0.67 $\pm$ 0.10 (3)
Malonyl-CoA in the presence of 40 μ M-CoA	0.68 $\pm$ 0.19 (3)
Acetyl-CoA	47.8 $\pm$ 7.1 (3)
Acetyl-CoA in the presence of 20 μ M-CoA	144.0 $\pm$ 11.0 (3)
CoA	44.6 $\pm$ 5.3 (3)

for the normal CPT. The presence of 40 μ M-acetyl-CoA or 40 μ M-CoA increased the K_i value for malonyl-CoA 3-fold, from 0.22 μ M to 0.67 μ M and 0.68 μ M respectively (Table 2). The K_i values for acetyl-CoA and CoA were 47.8 μ M and 44.6 μ M respectively, about 200-fold higher than for malonyl-CoA. The presence of 20 μ M-CoA increased the K_i value for acetyl-CoA about 3-fold, to 144.0 μ M.

DISCUSSION

Does malonyl-CoA inhibit CPT at the same site as CoA and acetyl-CoA?

The CPT of normal human muscle is inhibited by malonyl-CoA, acetyl-CoA and CoA, but on a molar basis acetyl-CoA and CoA are much less potent inhibitors than malonyl-CoA is. Similar results were previously obtained in intact rat skeletal-muscle mitochondria, and it was concluded that the three inhibitors acted on the same site on the mitochondrial membrane [14]. However, the fact that in the presence of 0.4% Triton X-100 sensitivity to inhibition by acetyl-CoA and CoA is preserved, whereas sensitivity to inhibition by malonyl-CoA is lost, indicates that acetyl-CoA and CoA do not act through the same site as malonyl-CoA. Inhibition of CPT by CoA in the presence of Triton X-100 was previously noted in rat skeletal muscle and liver [14,15], but this was attributed to mass action, since CoA is a product in the forward assay. This, however, is unlikely, because we found that the CPT reaction was linear with time and protein even under unfavourable equilibrium conditions when 0.02 mM-palmitoyl-CoA and 0.08 mM-CoA were present in the forward reaction system. Furthermore, Triton X-100 also did not affect the inhibitory effects of acetyl-CoA, which is not a product of the reaction. It is also conceivable that the inhibitory effect of acetyl-CoA stems from the hydrolytic liberation of free CoA during the assay. This is unlikely for two reasons: (1) equal amounts of acetyl-CoA plus CoA had a significantly stronger inhibitory effect than twice the concentration of free CoA (Table 1); (2) the addition of 20 μ M-CoA increased the K_i for acetyl-CoA from 47.8 μ M to 144 μ M, and this shift is much higher than can be inferred from the Dixon plot for CoA.

The experiments with mutant CPT provide additional evidence that the mechanism of CPT inhibition by malonyl-CoA is different from that by acetyl-CoA and CoA: for every concentration tested, the inhibition by malonyl-CoA was significantly higher than of normal CPT, but the inhibition by acetyl-CoA and CoA was the same as that of the normal CPT (Fig. 1).

The sites at which CoA and acetyl-CoA act on CPT

The present and previous [10,11,17,18] studies suggest that malonyl-CoA inhibits CPT at a site distinct from the active site of the enzyme. Furthermore, the present study demonstrates that acetyl-CoA and CoA inhibit CPT at a site different from the malonyl-CoA site. The simplest explanation of these results is that acetyl-CoA and CoA inhibit CPT by competing with palmitoyl-CoA at the active site. Since acetyl-CoA and CoA are both synergistic and therefore mutually non-exclusive inhibitors [25], they could be competing with palmitoyl-CoA for different portions of the substrate binding site (e.g. the site for binding the acyl moiety and the site for binding the CoA part of palmitoyl-CoA). However, the possibility remains that CoA and/or acetyl-CoA also could be allosteric competitive inhibitors of CPT at a second, non-catalytic, binding site. Recent studies on rat liver mitochondria also support this idea, since palmitoyl-CoA could displace malonyl-CoA from its low-affinity binding site which was distinct from the catalytic site [18]. In both cases the binding of one inhibitor affects the binding of the other and also the binding of malonyl-

CoA. Regardless of which of these possibilities is correct, it is clear that the site(s) at which CoA and acetyl-CoA inhibit the enzyme (a) cannot be dissociated from the active site by detergent solubilization and (b) are not altered in the mutant CPT.

Although malonyl-CoA inhibits CPT at a site different from that at which CoA and acetyl-CoA inhibit CPT, both CoA and acetyl-CoA have an effect at the malonyl-CoA site of CPT. The kinetic data obtained in the present study indicate that CoA and acetyl-CoA antagonize the inhibitory effects of malonyl-CoA by increasing the K_i for malonyl-CoA. This effect is consistent with the displacement of malonyl-CoA by CoA and acetyl-CoA from the mitochondrial membrane [14]. 2-Bromopalmitoyl-CoA, like CoA and acetyl-CoA, also has a dual effect on CPT: a direct inhibitory effect at a site distinct from the malonyl-CoA site, and displacement of malonyl-CoA from its binding site [26].

Inhibition of CPT I versus CPT II

In the present study, CPT activity and its inhibition by malonyl-CoA, acetyl-CoA and CoA were measured in sonicated homogenates of frozen human muscle. Therefore total enzyme activity was measured in all experiments even without the addition of Triton X-100. The use of frozen muscles was dictated by the fact that specimens from patients with the CPT mutation were only available in the frozen state. A previous study showed that CoA and acetyl-CoA inhibit CPT in intact rat liver, heart and skeletal-muscle mitochondria [14], and another study demonstrated that acetyl-CoA inhibits CPT in disrupted rat liver mitochondria [15]. On the basis of the present and previous studies one cannot conclude whether CoA and acetyl-CoA inhibit only CPT I or also CPT II in muscle or liver.

Physiological significance

Malonyl-CoA exerts a regulatory effect on long-chain fatty acid oxidation in liver by inhibition mitochondrial CPT [27]. Malonyl-CoA may also interact with CPT in heart and skeletal muscle because in these tissues (a) CPT is sensitive to malonyl-CoA, (b) significant amounts of malonyl-CoA are present, and (c) malonyl-CoA decreases with starvation [5]. In fed rats the malonyl-CoA concentration in skeletal muscle is 2.1 μ M, and this value decreases to 0.4 μ M after 48 h starvation [5]. If the concentration of malonyl-CoA in human muscle is comparable with that in rat muscle, then the K_i of 0.22 μ M for malonyl-CoA in human muscle would allow regulation of CPT by malonyl-CoA in human muscle.

The present study also shows that slight changes in the concentrations of acetyl-CoA or CoA can modulate the malonyl-CoA inhibition of CPT by influencing the K_i value of malonyl-CoA. These data imply that an increase in acetyl-CoA in muscle when fatty acid metabolism is stressed would further stimulate fatty acid oxidation by increasing the K_i of malonyl-CoA. At the present time there are only limited data on the cytosolic and intramitochondrial concentrations of CoA and acetyl-CoA under different physiological states in skeletal muscle, and the data on changes of acetyl-CoA and free CoA with starvation are controversial. According to one study, starvation induces a nearly 2-fold increase of acetyl-CoA in rat muscle (from 2.4 to 4.3 μ M) [28], but this was not confirmed by another study [29]. No

significant changes in free CoA concentration were found with starvation in rat skeletal muscle [28,29].

Finally, the present study also shows that high concentrations (0.2–0.4 mM) of CoA or acetyl-CoA have a direct and synergistic inhibitory effect on CPT activity in muscle.

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REFERENCES

1. Bremer, J. (1983) *Physiol. Rev.* **63**, 1420–1480
2. Bieber, L. L. & Farrell, S. (1983) *Enzymes* 3rd Ed. **16**, 627–644
3. McGarry, J. D., Leatherman, G. F. & Foster, D. W. (1978) *J. Biol. Chem.* **253**, 4128–4136
4. Saggerson, E. D. & Carpenter, C. A. (1981) *FEBS Lett.* **129**, 229–232
5. McGarry, J. D., Mills, S. E., Long, C. S. & Foster, D. W. (1983) *Biochem. J.* **214**, 21–28
6. Bird, M. I., Munday, L. A., Saggerson, E. D. & Clark, J. B. (1985) *Biochem. J.* **226**, 323–330
7. Cook, G. A. (1984) *J. Biol. Chem.* **259**, 12030–12033
8. Paulson, D. J., Ward, K. M. & Shug, A. L. (1984) *FEBS Lett.* **176**, 381–384
9. Saggerson, E. D. & Carpenter, C. A. (1981) *FEBS Lett.* **132**, 166–168
10. Mills, S. E., Foster, D. W. & McGarry, J. D. (1984) *Biochem. J.* **219**, 601–608
11. Bremer, J., Woldegiorgis, G., Schalinske, U. & Shrago, E. (1985) *Biochim. Biophys. Acta* **833**, 9–16
12. Saggerson, E. D. & Carpenter, C. A. (1982) *FEBS Lett.* **137**, 124–128
13. Zammit, V. A. (1983) *Biochem. J.* **214**, 1027–1030
14. Mills, S. E., Foster, D. W. & McGarry, J. D. (1983) *Biochem. J.* **214**, 83–91
15. McCormick, K., Notar-Francesco, V. J. & Sriwatanakul, K. (1983) *Biochem. J.* **216**, 499–502
16. Zammit, V. A., Corstorphine, C. G. & Gray, S. R. (1984) *Biochem. J.* **222**, 335–342
17. Bird, M. I. & Saggerson, E. D. (1984) *Biochem. J.* **222**, 639–647
18. Grantham, B. D. & Zammit, V. A. (1986) *Biochem. J.* **233**, 589–593
19. Zierz, S. & Engel, A. G. (1985) *Eur. J. Biochem.* **149**, 207–214
20. Lilenthal, J. L., Zierler, K. L., Folk, B. P., Buka, R. & Riley, M. J. (1950) *J. Biol. Chem.* **182**, 501–508
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
22. Norum, K. R. (1965) *Biochim. Biophys. Acta* **99**, 511–522
23. Solberg, H. E. (1972) *Biochim. Biophys. Acta* **280**, 422–433
24. Dixon, M. (1953) *Biochem. J.* **55**, 170–171
25. Segel, I. H. (1975) *Enzyme Kinetics*, pp. 465–504, John Wiley and Sons, New York
26. Edwards, M. R., Bird, M. I. & Saggerson, E. D. (1985) *Biochem. J.* **230**, 169–179
27. McGarry, J. D. & Foster, D. W. (1980) *Annu. Rev. Biochem.* **49**, 395–420
28. Goodman, M. N., Berger, M. & Ruderman, N. B. (1974) *Diabetes* **23**, 881–888
29. Carroll, J. E., Villadiego, A. & Morse, D. P. (1983) *Muscle Nerve* **6**, 367–373

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