The Association of Acetyl-L-Carnitine With Glucose and Lipid Metabolism in Human Muscle In Vivo: The Effect of Hyperinsulinemia

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We examined whether hyperinsulinemia is associated with changes in the amount of L-carnitine and acetyl-L-carnitine in the muscle and whether the source of acetyl-coenzyme A (CoA) (glucose or free fatty acids [FFAs]) influences its further metabolism to acetyl-L-carnitine or through tricarboxylic acid in the skeletal muscle of man in vivo. Twelve healthy men (aged 45 ± 2 years; body mass index, 25.2 ± 1.0 kg/m^2) were studied using a 4-hour euglycemic-hyperinsulinemic clamp (1.5 mU/kg/min) and indirect calorimetry. Although the mean muscle free L-carnitine and acetyl-L-carnitine concentrations remained unchanged during hyperinsulinemia in the group as a whole, the individual changes in muscle free L-carnitine and acetyl-L-carnitine concentrations were inversely related ($r = -.72$, $P < .02$). The basal level of acetyl-L-carnitine was inversely related to the rate of lipid oxidation ($r = -.70$, $P < .02$). In a stepwise linear regression analysis, 77% of the variation in the change of acetyl-L-carnitine concentrations was explained by the basal muscle glycogen level (inversely) and nonoxidative glucose disposal rate (directly) during hyperinsulinemia ($P < .01$); by adding the final FFA concentration (inverse correlation) to the model, 88% of the variation was explained ($P < .001$). In conclusion, (1) hyperinsulinemia does not enhance skeletal muscle free L-carnitine or acetyl-L-carnitine concentrations in man, and (2) the acetyl group of acetyl-L-carnitine in human skeletal muscle in vivo is probably mostly derived from glucose and not through β-oxidation from fatty acids.

L-CARNITINE is an essential factor in the metabolism of fatty acids, branched-chain amino acids, and glucose.[1] A common feature of all of these metabolic fuels is that L-carnitine reversibly replaces coenzyme A (CoA) at some stage in their metabolism. L-Carnitine is necessary for the entry of long-chain fatty acids into the mitochondria,[2] and it is also important in the metabolism of other fatty acids. Its essential role in the metabolism of branched-chain amino acids is evident in disorders of branched-chain amino acid metabolism such as isovaleric acidemia.[3]

In glucose metabolism, L-carnitine reversibly replaces CoA of acetyl-CoA, thus forming acetyl-L-carnitine and free CoA. The ratio of CoA to acetyl-CoA regulates the activity of the pyruvate dehydrogenase complex (PDH).[4] Consequently, L-carnitine has been shown to stimulate PDH in human muscle in vitro[5] and in vivo.[6] Incubation of human muscle with L-carnitine resulted in an increase in acetyl-L-carnitine,[5] and a 4-week period of oral L-carnitine administration caused no change in muscle acetyl-L-carnitine content.[6] When PDH in the perfused rat heart is stimulated by dichloroacetate, the concentration of acetyl-L-carnitine is increased.[7] When the flux of glucose through PDH is increased by muscle work, acetyl-L-carnitine is increased and free L-carnitine is decreased in the muscle.[8,9] Thus, a portion of the glucose derived from glycogen is temporarily converted to acetyl-L-carnitine. Studies in vitro suggest that in the rat heart muscle acetyl-L-carnitine is derived mainly from pyruvate, whereas in the liver the situation is different.[10] Furthermore, primed L-carnitine infusion during hyperinsulinemia increased nonoxidative glucose disposal by 50%.[11] Pyruvate decreased and acetyl-L-carnitine increased in the plasma. Acetyl-L-carnitine in the muscle was not measured. However, because muscle is the main site of glucose disposal during hyperinsulinemia,[12] the carnitine-induced increase in insulin sensitivity probably occurred in the muscle. To our knowledge, no human studies in vivo have been reported on the interrelationship of muscle L-carnitine metabolism and acute hyperinsulinemia when no L-carnitine is administered.

Consequently, because acetyl-L-carnitine acts as a reservoir for acetyl groups in many situations when PDH is stimulated, the main aim of the study was to investigate if the concentration of acetyl-L-carnitine in muscle is increased after the increased availability of acetyl groups during hyperinsulinemia. In addition, we examined with indirect measures whether formation of acetyl-L-carnitine in vivo in human skeletal muscle is coupled to glucose-derived acetyl-CoA, as previously suggested in studies of rat heart muscle.[10]

SUBJECTS AND METHODS

Subjects

We examined 12 men with a mean age of 45 ± 2 years and a body mass index of 25.2 ± 1.0 kg/m^2. All subjects were healthy, and none were using any medication. They were asked to ingest at least 250 g carbohydrate per day and not to perform any intensive exercise for 2 days before the study. The purpose, nature, and possible risks of the study were explained to the subjects before their informed consent was obtained. The study protocol was approved by the Ethics Committee of Helsinki University Central Hospital.

Design

In each subject, we measured insulin-stimulated whole-body glucose disposal and glucose and lipid oxidation. A muscle biopsy from the vastus lateralis muscle was performed before and at the end of the 240-minute insulin infusion. From the muscle samples, we determined glycogen, free L-carnitine, and acetyl-L-carnitine concentrations. The serum insulin level was measured in the basal state and during insulin infusion. The serum free fatty acid (FFA) concentration was measured before the insulin infusion and at the end of hyperinsulinemia.

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Methods

Whole-body glucose disposal was determined during a 240-minute euglycemic hyperinsulinemia with the insulin clamp technique as previously described.[13,14] A primed continuous insulin infusion (9 pmol/kg/min, or 1.5 mU/kg/min) was used to achieve and maintain hyperinsulinemia. Blood glucose was determined from arterialized venous blood at 5- to 10-minute intervals, and the fasting level was maintained with a variable-rate 20% glucose infusion.[13,14] Insulin sensitivity was calculated from values measured during the last hour of insulin infusion. Oxidative glucose disposal and lipid oxidation were determined by indirect calorimetry (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland) in the basal state and at the end of insulin infusion (between 210 and 240 minutes).[14,15] Nonoxidative glucose disposal was calculated as the difference between total and oxidative glucose disposal.

Fifteen minutes before and at the end of the 240-minute insulin clamp study, a percutaneous muscle biopsy (100 to 150 mg) was performed with a Bergström needle under local anesthesia (1% lidocaine). The samples were obtained from the vastus lateralis from opposite sides before and after the study. The specimen was removed from the muscle with suction by a syringe attached to the needle, and the needle was immediately emptyed into liquid N_2. The muscle glycogen level was measured as previously described.[14] The samples were freeze-dried and dissected free from the connective tissue and blood, and the muscle was powdered. Three to 5 mg muscle powder was extracted with KOH and neutralized, glycogen was hydrolyzed with amyloglycosidase, and the liberated glucose was analyzed with the glucose oxidase method. The glycogen concentration is expressed as millimoles per kilogram of dry muscle. Preclamp muscle glycogen was obtained from 11 patients. Muscle free L-carnitine and acetyl-L-carnitine were analyzed as previously described.[6,16] Glucose in the plasma and glycogen were determined with a glucose oxidase method using the Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). The serum insulin level was measured radioimmunologically.[17] FFAs were determined fluorometrically.[18]

Statistical Analysis

In the statistical analysis of paired samples, Wilcoxon's signed-rank test was used, and Spearman's test was used for the correlation analysis. The proportion of variation in the dependent variables explained by variations in the independent variables was calculated by forward stepwise multiple linear regression analysis using the adjusted squared multiple R^2. P values less than .05 were considered significant. The results are given as the mean ± SEM.

RESULTS

Insulin infusion increased glucose oxidation from 7.8 ± 0.9 μmol/kg/min to 20.3 ± 0.8 μmol/kg/min ($P < .01$) and decreased lipid oxidation from 0.91 ± 0.08 mg/kg/min to 0.18 ± 0.05 mg/kg/min ($P < .01$). Nonoxidative glucose disposal (38.0 ± 5.2 μmol/kg/min) accounted for the majority of whole-body total glucose disposal (58.3 ± 5.6 μmol/kg/min) during insulin infusion. Serum FFA levels decreased from 599 ± 66 μmol/L to 98 ± 5 μmol/L at the end of insulin infusion ($P < .01$). The increase in muscle glycogen from 316 ± 35 mmol/kg dry muscle to 397 ± 50 mmol/kg dry muscle was of borderline significance ($P = .05$).

The concentration of free L-carnitine (24.0 ± 2.2 *v* 25.4 ± 2.3 nmol/mg noncollagen protein) and acetyl-L-carnitine (4.2 ± 0.9 *v* 3.8 ± 0.7 nmol/mg noncollagen protein) in the muscle remained unchanged during insulin infusion. Muscle free L-carnitine levels before and at the end of hyperinsulinemia were related ($r = .79$, $P < .01$), whereas there was no correlation between acetyl-L-carnitine levels in the basal and hyperinsulinemic states ($r = .06$). The level of acetyl-L-carnitine at the end of hyperinsulinemia was associated with the basal free L-carnitine ($r = .67$, $P < .05$). Changes in muscle free L-carnitine and acetyl-L-carnitine during hyperinsulinemia were inversely related (Fig 1). In the basal state, there was an inverse association between the level of acetyl-L-carnitine and the rate of lipid oxidation (Fig 2). The basal glycogen level was negatively associated with the change in acetyl-L-carnitine during hyperinsulinemia (Fig 3). Insulin-mediated glucose disposal was not related to free L-carnitine or acetyl-L-carnitine concentrations.

When calculated with forward stepwise linear regression analysis, basal glycogen alone accounted for 45% ($P < .05$) of the variation in the change in acetyl-L-carnitine during hyperinsulinemia. The fit of the model was improved by including nonoxidative glucose disposal (to 77%, $P < .01$) and by addition of the final FFA concentration (to 88%, $P < .001$). In the model, the association of the change in acetyl-L-carnitine with basal glycogen and FFA was inverse, whereas the relationship to nonoxidative glucose disposal was direct. The parameters best explaining the final acetyl-L-carnitine concentration were basal muscle glycogen content (inverse), FFA (inverse), oxidative glucose disposal at the end of hyperinsulinemia (direct), and age (inverse). Together, they accounted for 79% ($P < .01$) of the variation in acetyl-L-carnitine concentrations at the end of the study.

DISCUSSION

Although carnitine plays a key role in the metabolism of lipids, glucose, and branched-chain amino acids, surprisingly little is known about its relationship to insulin. In the current study, the metabolism of acetyl-L-carnitine and free L-carnitine were closely related. Their concentrations remained unchanged during insulin infusion in the group as a whole. At baseline, acetyl-L-carnitine was lowest in subjects in whom lipid oxidation was highest, suggesting that glucose is the main source of the acetyl group in acetyl-L-carnitine. This is in accordance with studies in the rat heart.[10] Insulin-stimulated glucose disposal did not increase acetyl-L-carnitine content in the muscle.

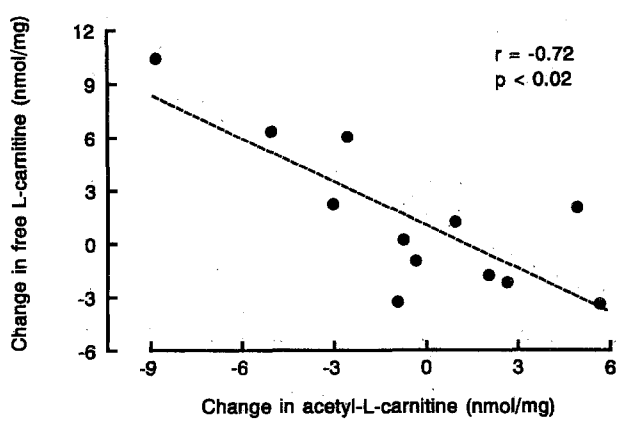

Fig 1. Relationship between changes in free carnitine and acetyl-L-carnitine during insulin infusion. Values are expressed as nmol/mg noncollagen protein.

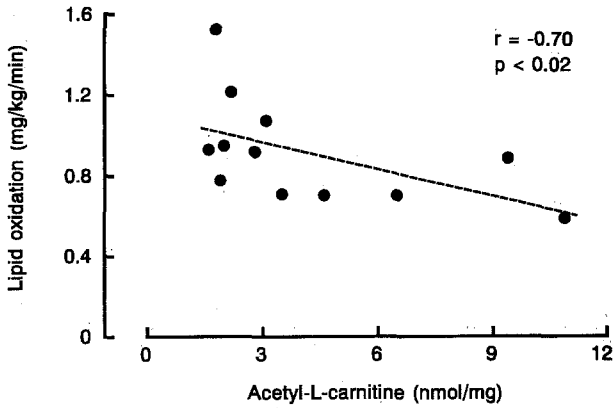

Fig 2. Correlation between the basal lipid oxidation rate and the level of muscle acetyl-L-carnitine (nmol/mg noncollagen protein) before insulin infusion.

Heart muscle, skeletal muscle, and kidney have the largest content of total carnitine to weight in rat tissues, whereas the plasma concentration is much lower.[19] Changes in the skeletal muscle carnitine concentration occur relatively slowly.[19] The slow turnover of carnitine in muscle is in keeping with our results demonstrating no change in the concentrations of free L-carnitine or acetyl-L-carnitine in muscle during the 4-hour hyperinsulinemia. However, it should be pointed out that acetyl-L-carnitine levels are steady-state values and do not constitute an end product per se. In our patients, skeletal muscle free L-carnitine content was sixfold greater than acetyl-L-carnitine content, in agreement with previous studies.[6] Our results suggest that the main exchange of carnitine occurs with acetyl-CoA. This is supported by the inverse association between changes in muscle free L-carnitine and acetyl-L-carnitine. Because concentrations do not reflect turnover, we do not know the impact of hyperinsulinemia on the turnover of L-carnitine or acetyl-L-carnitine.

Regarding the source of the acetyl group in acetyl-L-carnitine, the concentration of muscle acetyl-L-carnitine was inversely related to the basal lipid oxidation. In the basal state, our subjects were using fat as the main source of energy in muscle, as reflected by the high lipid oxidation rate. Most glucose oxidation in the basal state occurs in the brain. During insulin infusion, the fuel for oxidation in muscle shifts from fat to glucose, and skeletal muscle is the main site of glucose disposal during hyperinsulinemia.[12] In our study, the shift in fuel oxidation from fat to glucose during hyperinsulinemia is evident from the calorimetric results. Glucose and fat compete as the source of acetyl-CoA and energy for the tricarboxylic acid cycle. FFAs inhibit PDH[20] and acetyl-CoA carboxylase,[21] thus decreasing the formation of pyruvate-derived acetyl-CoA and malonyl-CoA. Insulin, on the contrary, stimulates these enzymes.[22,23] Through an increased malonyl CoA concentration, a high insulin concentration leads to a decreased access of fatty acids to mitochondria[24-26] and a decreased oxidation of fatty acyl–derived acetyl-CoA in the tricarboxylic acid cycle.

It is generally assumed that the origin of acetyl-CoA from fat or glucose has no effect on its further metabolism. However, Lysiak et al[10] have demonstrated in rat tissues that the fate of acetyl groups depends on the tissue and substrate in question. In rat heart, acetyl-CoA generated by β-oxidation was less accessible to carnitine acetyltransferase than acetyl-CoA derived from pyruvate. Similarly, data obtained in isolated rat heart myocytes suggest that acetyl-CoA from β-oxidation of fatty acids is channeled directly into the citric acid cycle.[27] Acetyl-CoA metabolism in skeletal muscle was not studied. Because lipid oxidation rates and serum FFA concentrations were high in our subjects in the basal state, they were probably oxidizing mainly fatty acid–derived acetyl-CoA in muscle. Thus, if acetyl-CoA derived from fatty acids would be accessible to acetyltransferase, one would expect a direct association between lipid oxidation and acetyl-L-carnitine. This inverse association between lipid oxidation and acetyl-L-carnitine in our subjects suggests that acetyl-CoA derived from β-oxidation in human skeletal muscle in vivo is not used for the formation of acetyl-L-carnitine, since a majority of β-oxidation–derived acetyl-CoA may enter the tricarboxylic acid cycle (Fig 4).

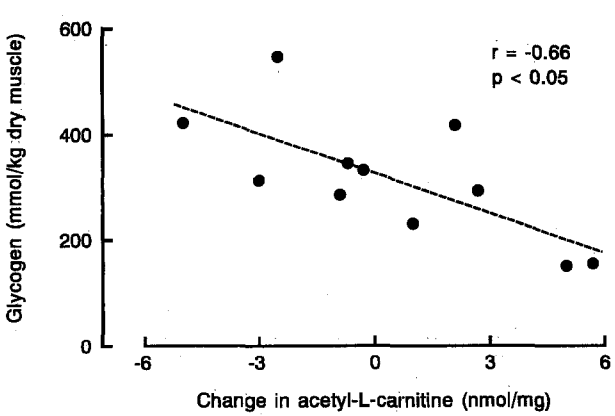

Fig 3. Relationship of the basal glycogen level to the change in acetyl-L-carnitine during hyperinsulinemia.

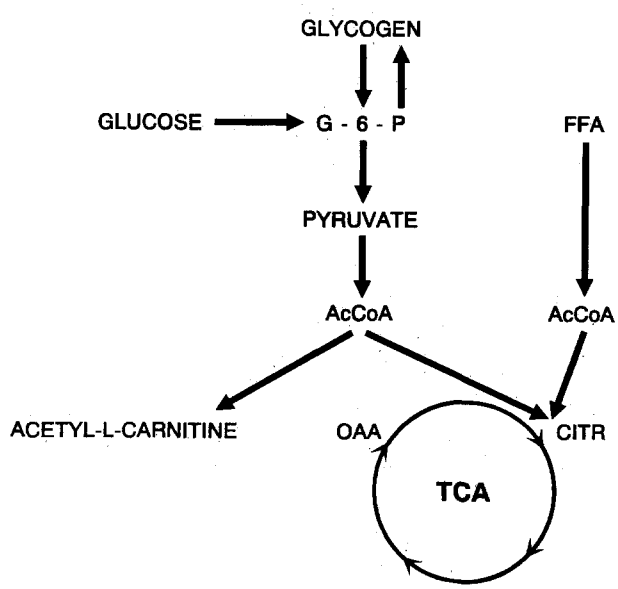

Fig 4. Possible mechanism for the relationship of acetyl-coA (AcCoA) and acetyl-L-carnitine in human muscle. Acetyl-L-carnitine is formed mainly from glucose-derived AcCoA, whereas AcCoA derived from FFAs through β-oxidation is more directly coupled to the tricarboxylic acid cycle (TCA). G-6-P, glucose-6-phosphate; OAA, oxaloacetate; CITR, citrate.

Furthermore, Kerner and Bieber[8] have demonstrated in rat skeletal muscle that a 48-hour fast is associated with increased total and free carnitine concentrations. However, the level of acetylcarnitine was decreased, perhaps due to reduced glucose-derived acetyl-CoA, which would be in agreement with the influence of the source of acetyl-CoA on its further metabolism. Our interpretation is supported by regression analysis of the variation in muscle acetylcarnitine content at the end of hyperinsulinemia. Both the final FFA concentration (inversely) and glucose oxidation rate (directly) increased the proportion of variation in acetyl-L-carnitine content explained by the model. During hyperinsulinemia, the majority of acetyl-CoA was derived from pyruvate, as seen from the calorimetric data. However, the concentration of acetyl-L-carnitine was not increased. This is in contrast to findings made during intense muscular contractions in humans,[9] when a rapid reduction of glycogen stores through pyruvate results in an increased concentration of acetyl-L-carnitine in muscle. In our study, insulin was responsible for the change in the source of acetyl groups in acetyl-CoA. This suggests that insulin may have effects on the turnover of acetyl-L-carnitine resulting in an unchanged level of acetyl-L-carnitine despite increased delivery of pyruvate-derived acetyl groups.

A previous study has demonstrated that insulin sensitivity, mainly nonoxidative glucose disposal, can be increased by a primed, continuous carnitine infusion during hyperinsulinemia.[11] In that study, acetylcarnitine in plasma was increased. We did not find any relationship between insulin sensitivity and the muscle acetylcarnitine level. Our results support previous studies showing that the level of muscle acetylcarnitine is stable[28] if the supply of glucose is not severely restricted.

Taken together, our results in human skeletal muscle in vivo suggest that muscle free L-carnitine or acetyl-L-carnitine concentrations do not change during hyperinsulinemia and insulin-stimulated glucose uptake. These data suggest that the acetyl group of acetylcarnitine is derived mainly from glucose, as previously reported for rat heart muscle in vitro. Acetyl-CoA derived from FFAs through β-oxidation is more directly coupled to the tricarboxylic acid cycle.

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REFERENCES

1. Bieber LL: Carnitine. Annu Rev Biochem 57:261-283, 1988
2. McGarry JD, Robles-Valdes C, Foster DW: Role of carnitine in hepatic ketogenesis. Proc Natl Acad Sci USA 72:4385-4388, 1975
3. Roe CR, Millington DS, Maltby DA, et al: L-Carnitine therapy in isovaleric acidemia. J Clin Invest 74:2290-2295, 1984
4. Williamson JR, Cooper RH: Regulation of the citric acid cycle in mammalian systems. FEBS Lett 117:K73-K85, 1980 (suppl)
5. Uziel G, Garavaglia B, Di Donato S: Carnitine stimulation of pyruvate dehydrogenase complex (PDHC) in isolated human skeletal muscle mitochondria. Muscle Nerve 11:720-724, 1988
6. Arenas J, Huertas R, Campos Y, et al: Effects of L-carnitine on the pyruvate dehydrogenase complex and the carnitine palmitoyl transferase activities in muscle of endurance athletes. FEBS Lett 341:91-93, 1994
7. McAllister A, Allison SP, Randle PJ: Effects of dichloroacetate on the metabolism of glucose, pyruvate, acetate, 3-hydroxybutyrate and palmitate in rat diaphragm 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, 1973
8. Kerner J, Bieber LL: The effect of electrical stimulation, fasting and anesthesia on the carnitine(s) and acyl-carnitines of rat white and red skeletal muscle fibres. Comp Biochem Physiol 75B:311-316, 1983
9. Harris RC, Foster CVL, Hultman E: Acetylcarnitine formation during intense muscular contraction in humans. J Appl Physiol 63:440-442, 1987
10. Lysiak W, Toth PP, Suelter CH, et al: Quantitation of the efflux of acylcarnitines from rat heart, brain and liver mitochondria. J Biol Chem 261:13698-13703, 1986
11. Ferrannini E, Buzzigoli G, Bevilacqua S, et al: Interaction of carnitine with insulin-stimulated glucose metabolism in humans. Am J Physiol 255:E946-E952, 1988
12. DeFronzo RA, Jacot E, Jequier E, et al: The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 30:1000-1007, 1981
13. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979
14. Ebeling P, Bourey R, Koranyi L, et al: Mechanism of enhanced insulin sensitivity in athletes: Increased blood flow, muscle glucose transport protein (GLUT-4) concentration and glycogen synthase activity. J Clin Invest 92:1623-1631, 1993
15. Ferrannini E: The theoretical bases of indirect calorimetry: A review. Metabolism 37:287-301, 1988
16. Arenas J, Ricoy JR, Encinas AR, et al: Carnitine in muscle, serum and urine of nonprofessional athletes: Effects of physical exercise, training and L-carnitine administration. Muscle Nerve 14:598-604, 1991
17. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol 33:732-738, 1971
18. Miles J, Glasscock R, Aikens J, et al: Microfluorometric method for the determination of free fatty acids in plasma. J Lipid Res 24:96-99, 1983
19. Brooks DE, McIntosh JEA: Turnover of carnitine by rat tissues. Biochem J 148:439-445, 1975
20. Erfle JD, Sauer F: The inhibitory effects of acyl-coenzyme A esters on the pyruvate and α-oxoglutarate dehydrogenase complexes. Biochim Biophys Acta 178:441-452, 1969
21. Thampy KG: Formation of malonyl coenzyme A in rat heart. Identification and purification of an isoenzyme of acetyl-coenzyme A carboxylase from rat heart. J Biol Chem 264:17631-17634, 1989
22. Feldhoff PW, Arnold J, Oesterling B, et al: Insulin-induced activation of pyruvate dehydrogenase complex in skeletal muscle of diabetic rats. Metabolism 42:615-623, 1993
23. Witters LA, Kemp BE: Insulin activation of acetyl-CoA carboxylase accompanied by inhibition of the 5′-AMP–activated protein kinase. J Biol Chem 267:2864-2867, 1992
24. McGarry JD, Leatherman GF, Foster DW: Carnitine palmitoyltransferase I. The site of inhibition of hepatic fatty acid oxidation by malonyl-CoA. J Biol Chem 253:4128-4136, 1978
25. Cook GA, Mynatt RL, Kashfi K: Yonetani-Theorell analysis of hepatic carnitine palmitoyltransferase-I inhibition indicates two distinct inhibitory binding sites. J Biol Chem 269:8803-8807, 1994
26. McGarry JD, Woeltje KF, Kuwajima M, et al: Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. Diabetes Metab Rev 5:271-284, 1989
27. Abdel-Aleem S, Nada MA, Sayed-Ahmed M, et al: Regulation of fatty acid oxidation by acetyl-CoA generated from glucose utilization in isolated myocytes. J Mol Cell Cardiol 28:825-833, 1996
28. Huertas R, Campos Y, Diaz E, et al: Respiratory chain enzymes in muscle of endurance athletes: Effect of L-carnitine. Biochem Biophys Res Commun 188:102-107, 1992