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Fuctional Relationship between Rate of Fatty Acid Oxidation and Carnitine Palmitoyl Transferase I Activity in Various Rat Tissues

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Lipids play many structural and metabolic roles, and dietary fat has great impact on metabolism and health. Fatty acid oxidation rate is dependent on tissue types. However there has been no report on the relationship between the rate of fatty acid oxidation and carnitine transport system in outer mitochondrial membrane of many tissues. In this study, the rate of fatty acid oxidation and carnitine palmitoyltransferase (CPT) I activity in the carnitine transport system were measured to understand the metabolic characteristics of fatty acid in various tissues. Palmitic acid oxidation rate and CPT I activity in various tissues were measured. Tissues were obtained from the white and red skeletal muscles, heart, liver, kidney and brain of rats. The highest lipid oxidation rate was demonstrated in the cardiac muscle, and the lowest oxidation rate was in brain. Red gastrocnemius muscle followed to the cardiac muscle. Lipid oxidation rates of kidney, white gastrocnemius muscle and liver were similar, ranging from 101 to 126 DPM/mg/hr. CPT I activity in the cardiac muscle was the highest, red gastrocnemius muscle followed by liver. Brain tissue showed the lowest CPT I activity as well as lipid oxidation rate, although the values were not significantly different from those of kidney and white gastrocnemius muscle. Therefore, lipid oxidation rate was highly ($p < 0.001$) related to CPT I activity. Lipid oxidation rate is variable, depending on tissue types, and is highly ($p < 0.001$) related to CPT I activity. CPT I activity may be a good marker to indicate lipid oxidation capacity in various tissues.

Key Words: Fat oxidation, CPT I

INTRODUCTION

Lipids play many structural and metabolic roles and dietary fat has great impact on metabolism and health. In many tissues, fatty acids are preferably oxidized to glucose under fed conditions, however, particularly more under caloric deficit or starvation, the purpose of such preference is to spare glucose for such tissues as brain and erythrocytes. Many of the details of the interplay between carbohydrate and lipid metabolisms in various tissues have amply been described. The conversion of glucose into fat is a process that occurs readily under the condition of optimal nutritional intake. With the exception of the glycerol moiety, fat cannot give rise to a net formation of glucose, because of the irreversible nature of the oxidative decarboxylation of pyruvate to acetyl-CoA. Certain tissues and cell types, including neurons and erythrocytes, are much more dependent on a continual supply of glucose than others. In the body, fatty acids are broken down to acetyl-CoA, which enters the citric acid cycle. Fatty acid oxidation begins with activation of the fatty acid in the both sides of mitochondria. Active fatty acids formed outside mitochondria cross the mitochondrial membrane by a process that requires carnitine. Carnitine palmitoyl transferase (CPT) I is believed to play a major role in the regulation of mitochondrial beta-oxidation of fatty acids in all human tissues (McGarry & Brown, 1997) and control a critical point in cellular lipid metabolism. It is the first element of a carnitine transport system that affects the net transport of long-chain acyl groups from the cytosol into the mitochondrial matrix, where they can be oxidized as a direct source of cellular energy or, in the liver, used for the synthesis of ketone bodies, and the second step is played by CPT II that converts fatty acyl carnitine to fatty acyl CoA in mitochondrial matrix for beta-oxidation. As an enzyme that catalyzes the rate-limiting step in fatty acid oxidation, CPT I is tightly regulated by its physiologic inhibitor, malonyl CoA, the first intermediate in fatty acid synthesis (Ruderman & Dean, 1998), but CPT II is not regulated by inhibitors such as malonyl CoA and not a limiting step for fatty acids oxidation (Fig. 1).

Fatty acid oxidation rate would be dependent on tissue types, indicating that each tissue has unique metabolic characteristics of fatty acid to be oxidized to yield ATP. It is now known that brain rarely uses fatty acid, but heart primarily uses fatty acid to produce ATP as an energy source. However, there has yet been no report on the relationship between fatty acid oxidation rate and CPT I

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ABBREVIATIONS: CPT I, carnitine palmitoyl transferase I; CPT II, carnitine palmitoyl transferase II.

activity of the carnitine transport system in many tissues. Therefore, fatty acid oxidation rate and CPT I activity were measured to understand the metabolic characteristics of fatty acid in different rat tissues.

METHODS

Experimental design & materials

White and red skeletal muscles, liver, kidney, and brain were used for the experiment from 5 specific-pathogen free Sprague-Dawley rats, weighing ~250 g. Rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg BW), and approximately 500 mg were then obtained immediately after sacrifice by bleeding from abdominal aorta.

Fatty acid oxidation rate and CPT I

Approximately 500 mg fresh tissue samples were immediately placed into 5 ml of a modified sucrose EDTA medium (SET) on ice, containing 250 mM sucrose, 1 mM EDTA, and 10 mM tris-HCl, pH 7.4 (Kim et al, 2000). Tissue samples were then minced thoroughly with scissors, and SET buffer was then added to a final 20-fold diluted (wt/vol) suspension. The minced samples were homogenized in a Potter-Elvehjem glass homogenizer at 10 passes across 30 sec at 1,200 rpm with a motor-driven teflon pestle. This method had been found to provide intact mitochondria for metabolic studies (Sholte et al, 1997).

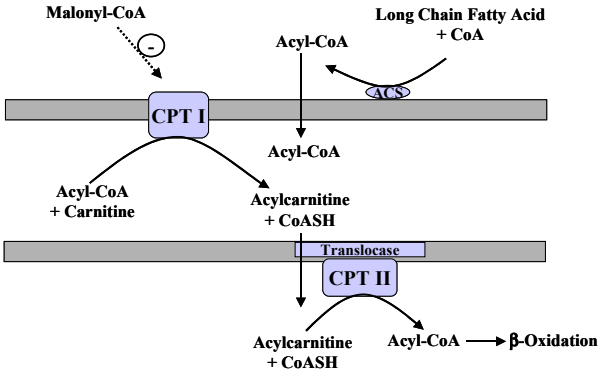

Fig. 1. Role of the carnitine palmitoyl transferase (CPT) I in fatty acid oxidation. FFA, long-chain free fatty acid; ACS: Acyl-CoA synthetase.

Fatty acid oxidation rates were determined in the fresh tissues homogenates, by the method of Kim et al. (2000): The oxidation rate of fatty acid was measured by counting the $^{14}CO_2$ produced from palmitic acid during incubation. Thus, 40μl of 20-fold diluted above homogenates were preincubated with 95% O_2-5% CO_2 mixture at 30°C for 15 min, and 160μl of reaction mixture (pH 7.4) was then added to the preincubated tissue homogenates. Final compositions of the incubation mixture were in mmole per liter: sucrose, 100; Tris HCl, 10; potassium phosphate, 5; potassium chloride, 80; magnesium chloride, 1; L-carnitine, 2; malate, 0.1; ATP, 2; coenzyme A, 0.05; dithiothreitol, 1; EDTA, 0.2; bovine serum albumin, 0.3%; and 0.2 mM palmitate-1-^{14}C (0.5μCi) as the substrate. After 60 min of incubation at 30°C, 100μl of 4N sulfuric acid were injected to stop the reaction. $^{14}CO_2$ produced during the 60 min incubation was trapped with 200μl of 2M sodium hydroxide. Trapped $^{14}CO_2$ was determined by liquid scintillation counting.

CPT I activity was measured using modification of the methods developed by McGarry et al. (1983) and Zierz & Engel (1987). This method measures the rate of palmitoylcarnitine formation from palmitoyl-CoA and carnitine. Briefly, 10 ul of 20-fold diluted tissues homogenates were preincubated for 20 min at 30°C in a microcentrifuge tube, and reactions were initiated by adding 90μl reaction mixture to the preincubated tissues homogenates for 10 min at 30°C. The reaction mixture (pH 7.4) included 117 mM Tris HCl, 0.28 mM reduced glutathione, 4.4 mM ATP, 4.4 mM MgCl2, 16.7 mM KCl, 2.2 mM KCN, 40 mg/l rotenone, 0.1% BSA, 50μM palmitoyl CoA, and 0.2 mM ^{3}H-carnitine (0.5μCi) as the substrate. The reaction was terminated with 60 ul of 1.2 mM ice-cold HCl, and ^{3}H-palmitoylcarnitine formed was extracted with water-saturated butanol and radioactivity was determined by liquid scintillation counting.

Table 1. Palmitate oxidation rate (DPM/mg/hr) of various tissues in rats

	WG	RG	Heart	Liver	Kidney	Brain
	112±37.8	263±33.5	662±44.0	101±26.0	126±20.3	9±1.0

Values are mean±S.D.

Statistical Analysis by ANOVA

	WG	RG	Heart	Liver	Kidney	Brain
WG	1.000	0.002	0.000	0.801	0.752	0.024
RG		1.000	0.000	0.001	0.004	0.000
Heart			1.000	0.000	0.000	0.000
Liver				1.000	0.571	0.041
Kidney					1.000	0.012
Brain						1.000

Numbers in boxes are p value. WG; white gastrocnemius muscle, RG; red gastrocnemius muscle.

Table 2. CPT I activity (DPM/mg/min) of various tissues in rats

	WG	RG	Heart	Liver	Kidney	Brain
	79±6.3	278±12.4	535±46.1	161±14.5	89±4.2	43±1.5

Values are mean±S.D.

Statistical Analysis

	WG	RG	Heart	Liver	Kidney	Brain
WG	1.000	0.00	0.000	0.011	0.795	0.200
RG		1.000	0.000	0.000	0.000	0.000
Heart			1.000	0.000	0.000	0.000
Liver				1.000	0.019	0.000
Kidney					1.000	0.127
Brain						1.000

Numbers in boxes are p value. WG; white gastrocnemius muscle, RG; red gastrocnemius muscle.

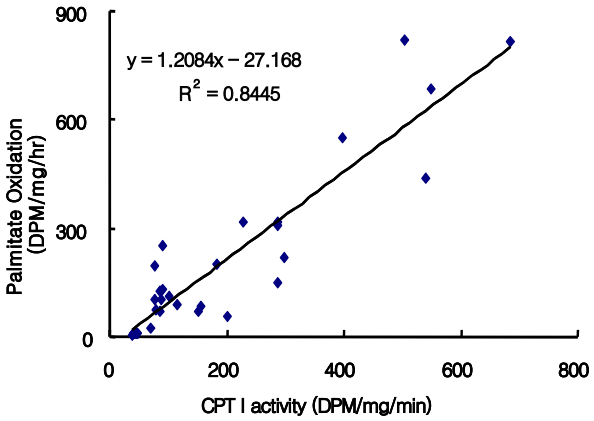

Fig. 2. Correlation between the fatty acid oxidation rate and the carnitine palmitoyl transferase (CPT) I activity of various tissues in rats. r^2=0.8445, $p<0.001$.

Statistics

Descriptive data, lipid oxidation rate, and CPT I activity in various tissues were compared with factorial analysis of variance by SPSS.

RESULTS

Lipid oxidation

As presented in Table 1, lipid oxidation rate (DPM/mg/hr) was the highest ($p<0.001$) in cardiac muscle (662±44.0), followed by red gastrocnemius muscle (263±33.5). Lipid oxidation rate of kidney (126±20.3), white gastrocnemius muscle (112±37.8), and liver (101±20.3) showed similar values. Brain tissue (9±1.0) showed hardly any lipid oxidation, as compared with other tissues.

Enzyme activity

As presented in Table 2, CPT I activity (DPM/mg/min) in the cardiac muscle (535±46.1) was the highest ($P<0.001$), followed by red gastrocnemius muscle (278±12.4) and liver (161±14.5). Brain tissue (43±1.5) showed the lowest CPT I activity as well as lipid oxidation rate, although there was no significant difference between those of kidney (89±4.2) and white gastrocnemius muscle (79±6.3).

Relationship between fatty acid oxidation and CPT I activity

When calculated by using SPSS, strong positive correlation was found between muscular fatty acid oxidation rate and CPT I activity (Fig. 2, r^2=0.8445, $p<0.001$).

DISCUSSION

At resting state, lipid oxidation contributes significantly to overall energy needs; up to 90% of the energy requirements of resting muscle are obtained from fatty acid oxidation (Dagenais et al, 1976; Bulow et al, 1988). Fatty acid oxidation rate was thought to be dependent on tissue types. Reduction in the lipid oxidation rate has, therefore, been postulated to promote positive fat balance and fat mass gain (Colberg, 1995; Astrup et al, 1997; Pagliassotti et al, 1997). In support of a persistent defect, ability to oxidize fatty acids has been reported to be reduced in the skeletal muscle of obese individuals both before and after weight loss (Kelley et al, 1999). The cellular mechanisms responsible for this decrease in muscular lipid oxidation in obese individuals are, however, unclear. Reduced activity of CPT, an enzyme which regulates the transport of long chain fatty acids across mitochondrial membranes, may be involved in the process (McGarry et al, 1983; Colberg et al, 1995; McGarry, 1995; Vusse & Reneman, 1996; McGarry & Brown, 1997; Kelley et al, 1999). The role, if any, of each of these alterations in muscular lipid oxidation has not yet been directly examined. Therefore, the results of the present study may provide some insights to the possible role of CPT I in lipid oxidation of the selected rat tissues. In conclusion, our results showed that lipid oxidation was varied greatly depending on different tissue types, which was closely related to CPT I activity. Therefore, CPT I ac-

tivity may be a good marker to understand the capacity of lipid oxidation in various tissues.

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