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Increased Rate of Palmitate Oxidation in Adults Female: Comparison with Peri-pubertal Young Female Rats

Se Young Lee[1], Jong-Yeon Kim[1], Yong-Woon Kim[1], and So-Young Park[1,2]

[1]*Departments of Physiology,* [2]*Aging-associated Vascular Disease Research Center, College of Medicine, Yeungnam University, Daegu 705-7*
Korea

Although estrogen is known to play a role in fatty acid metabolism, it remains unclear whether fatty acid oxidation in mature female rats differs from fatty acid oxidation in peri-pubertal young rats. In this study, we measured fatty acid metabolism in the skeletal muscles and livers of 5 and 50 weeks old male and female rats. The rate of palmitate oxidation in the liver and gastrocnemius red in the 50-week-old female rats were elevated as compared to the 5-week-old females, whereas there were no differences in the male rats. The rate of palmitate oxidation in the gastrocnemius red was correlated inversely with intra-abdominal fat mass in the 5-week-old male and female rats, whereas the palmitate oxidation rate was positively correlated with fat mass in the liver and gastrocnemius red in the 50-week-old rats. HOMA-IR and plasma insulin levels were positively correlated with intra-abdominal fat mass in the pooled 50-week-old male and female rats, but this correlation was not apparent in 5-week-old rats. In summary, the rate of fatty acid oxidation measured in the middle-aged adult female rats was significantly higher than those measured in the peri-pubertal young female rats. This difference may be attributed to the influence of ovarian hormones.

Key Words: Female, Intra-abdominal fat mass, Palmitate oxidation rate, Skeletal muscle

INTRODUCTION

Fatty acid oxidation contributes to the overall energy needs of many tissues in post-absorptive state, and skeletal muscle acquires up to 90% of its energy from fatty acid oxidation (Dagenais et al, 1976; Colberg et al, 1995). Since the skeletal muscles constitute approximately 40 % of total body weight, the use of fat as a substrate in skeletal muscle is of central relevance to overall body fatness (Colberg et al, 1995; Kelley et al, 1999; Collins et al, 2001). Consistent with these observation, many earlier studies showed that the rate of fatty acid oxidation in the skeletal muscle tend to be lower in obese subjects (Kelley et al, 1999; Kim et al, 2000). Although both obese men and women have reduced the rate of fatty acid oxidation in fasting states (Kelley et al, 1999; Kim et al, 2000; Thyfault et al, 2004), several lines of evidence suggest that fatty acid metabolism is differentially regulated depending on gender.

Intramuscular triglyceride levels are found to be lower in females after exercise, whereas these levels are not altered appreciably in males by exercise (Steffensen et al, 2002). Several studies also reported that women to a greater degree rely on fatty acid oxidation during exercise than do men (Carter et al, 2001). Furthermore, peroxisome proliferative activated receptor alpha-deficient male mice, when treated with carnitine palmitoyltransferase 1 (CPT-1) inhibitor, which induces serious defects in fatty acid metabolism, result in 100 % mortality rate, whereas only 25% mortality is observed in the female subjects (Finck et al, 2005). These discrepancies between males and females may be the result of influence exerted by ovarian hormones. Whereas the effects of testosterone on lipid metabolism remain somewhat unclear, estrogen replacement has been demonstrated to enhance lipid utilization and oxidation in the heart and skeletal muscles (Hatta et al, 1988; Herrero et al, 2005). Ovariectomy, conversely, has been shown to reduce lipid metabolism-related gene expression in the skeletal muscles, along with an overall increase of body fat (Kamei et al, 2005). Therefore, it has been surmised that fatty acid metabolism in mature female rats under the influence of ovarian hormone would be different from fatty acid metabolism in peri-pubertal young rats.

In this study, we have assessed palmitate oxidation rates and the activity of carnitine palmitoyltransferase 1 (CPT-1), the rate-limiting enzyme for beta oxidation, in the skeletal muscles and livers of male and female rats at 5 and 50 weeks of age.

METHODS

Animals

Sprague-Dawley male and female rats employed in this

Corresponding to: So-Young Park, Department of Physiology, College of Medicine, Yeungnam University, 214-1, Daemyeong-dong, Daegu 705-717, Korea. (Tel) 82-53-620-4334, (Fax) 82-53-651-3651, (E-mail) sypark@medical.yu.ac.kr

ABBREVIATIONS: CPT-1, carnitine palmitoyltransferase 1; HOMA-IR, homeostasis model assessment of insulin resistance.

study were purchased from Jung-Ang Lab Animals (Seoul, Republic of Korea), and were housed in an animal unit at the College of Medicine, Yeungnam University, for at least 1 week prior to the commencement of the experiments. The rats were kept at 22°C on a 12-hour light-dark cycle, with *ad libitum* access to water and regular laboratory chow diet. Food was removed at 8 A.M., and the rats were anesthetized with sodium pentothal (45 mg/kg) at 11 A.M.. Blood was withdrawn from the aorta for plasma biochemistry. The perirenal and epdidymal fat masses and the gastrocnemius muscle mass were weighed. The liver, gastrocnemius red, and gastrocnemius white were collected and used in assay of palmitate oxidation rate and CPT-1 activity.

Oxidation rate of palmitate

Fresh samples obtained from the liver, gastrocnemius red, and gastrocnemius white were immediately placed into ice-cold SETH medium containing 250 mM sucrose, 1 mM EDTA, and 10 mM tris-HCl (pH 7.4). Twenty μl of SETH-medium was added per mg muscle as buffer. This mixture was then homogenized in a glass homogenization tube, using a motor-driven teflon pestle. In order to isolate mitochondria, the homogenate was centrifuged for 10 minutes at 700 g, after which the supernatant was further centrifuged for 10 minutes at 14,000 g. The pellet was suspended in one volume of SETH medium. This was to isolate intact mitochondria for metabolic studies (Scholte et al, 1997). The rate of fatty acid oxidation rates in the mitochondria were measured by the method developed by Kim et al, (Kim et al, 2000): The palmitate oxidation rate was measured by collecting and counting $^{14}CO_2$ generated during incubation. Thus 50 ml of mitochondrial homogenate was added to 150 ml of reaction mixture, containing the following (in mmole per liter): sucrose, 100; Tris HCl, 10; potassium phosphate, 5; potassium chloride, 80; magnesium chloride, 1; coenzyme A, 0.05; L-carnitine, 2; malate, 0.1; ATP, 2; dithiothreitol, 1; EDTA, 0.2; and bovine serum albumin, 0.3%; at pH of 7.4. They were preincubated for 15 minutes with a 95% O_2-5% CO_2 mixture at 30°C, and 0.2 mM palmitate-1-14C (0.5 mCi) was then used as a substrate. After 60 minutes of incubation at 30°C, 100 μl of 4N sulfuric acid was injected in order to terminate the reaction. CO_2 generated during the 60 minutes of incubation was trapped with 200 μl of 2 M sodium hydroxide, and the amount of trapped $^{14}CO_2$ was counted via liquid scintillation.

CPT-1 activity

CPT1 activity in the liver and skeletal muscle was measured by a slightly modified version of the method of McGarry et al, (McGarry et al, 1983) and Zierz (Zierz et al, 1987). In brief, 10 ml of the tissue homogenates, diluted 20-fold, was preincubated in a microcentrifuge tube for 10 minutes at 30°C. The reaction was initiated by the addition of 90 ml of reaction mixture (0.2 mM [^{3}H]-carnitine, 117 mM Tris-HCl, 0.28 mM reduced glutathione, 4.4 mM ATP, 4.4 mM $MgCl_2$, 16.7 mM KCl, 2.2 mM KCN, 40 mg/l rotenone, 0.1% BSA, and 50 mM palmitoyl CoA) to the preincubated tissue homogenates at 30°C for 10 minutes. The reaction was then quenched with 60 ml of 1.2 mM ice-cold HCl. The resultant [^{3}H]-palmitoylcarnitine was then extracted with water-saturated butanol, and assessed with liquid scintillation counting.

Analysis of plasma biochemicals

Plasma insulin, leptin (Linco, USA) and catecholamine (norepinephrine and epinephrine) (IBL, Germany) concentrations were determined by radioimmunoassay, and plasma glucose and triglyceride levels were assessed by enzymatic colorimetric methods (Sigma, USA).

Statistics

Values are expressed as means±SEM. Differences between two groups were assessed via student t-test. Simple correlation analysis was employed to determine the relationship existing between the variable factors. All statistical analyses were conducted using the SPSS system.

RESULTS

Body weight, percentage of fat mass, and muscle mass were found higher in male and female rats at 50 weeks, as compared to 5 weeks old. Although similar increase was also observed in the gastrocnemius muscle mass with age between the gender groups (233% and 262% in males and females, respectively), the increase in percentage of intra-abdominal fat mass was significantly more profound in the males than in the females (590% and 482%, respectively). Plasma insulin, leptin, and triglyceride levels were also increased in the 50-week-old rats of both groups. The observed increases in plasma triglyceride levels were similar between the groups, whereas plasma insulin and leptin levels were higher in the males than in the females (262% and 1542% in males and 151% and 526% in females, respectively) (Table 1). Palmitate oxidation rates in the liver and gastrocnemius red in the 50-week-old rats were clearly elevated as compared to the levels in the 5-week-old females, whereas no such difference was detected in the

Table 1. Changes of body weight, fat mass, and plasma biochemicals with age in rats

	Male		Female	
	5 weeks	50 weeks	5 weeks	50 weeks
Body weight (g)	197±10.6	773±19*	163±2.8	346±14*
Fat (epi+ retro, g)	2.7±0.3	50±3.3*	1.8±0.1	19±3.0*
% Fat	1.1±0.08	6.5±0.3*	1.1±0.1	5.3±0.6*
Gastrocnemius (g)	1.2±0.06	2.8±0.1*	0.8±0.02	2.1±0.4*
Insulin (ng/ml)	2.1±0.4	5.5±0.6*	4.3±0.6	6.5±0.6*
Glucose (mM)	9.4±0.27	9.4±0.31	10.7±0.42	10.3±0.32
Leptin (ng/ml)	1.4±0.3	21.6±0.7*	1.9±0.2	10.1±1.9*
Triglyceride (mM)	0.58±0.08	1.44±0.15*	0.65±0.05	1.35±0.06*

Result was presented as mean ± S.E. *p<0.05 vs corresponding 5 weeks.

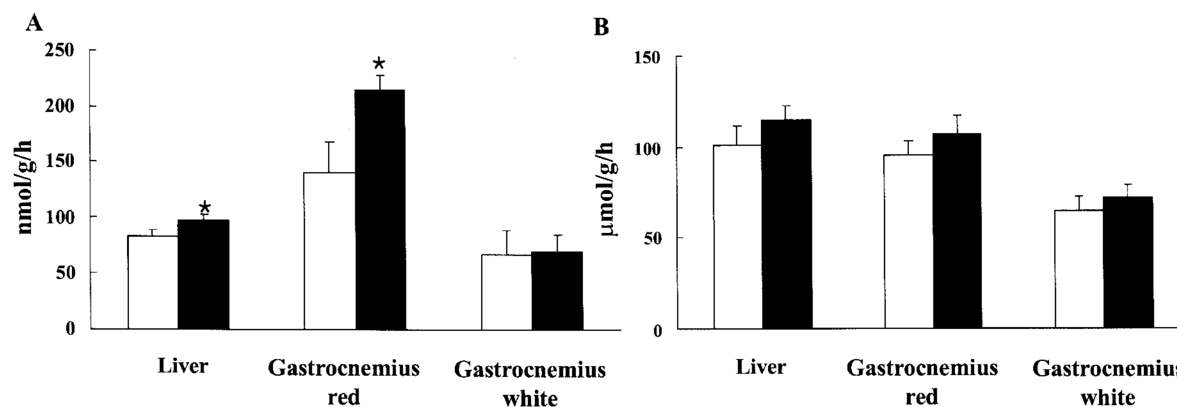

Fig. 1. Palmitate oxidation rate (A) and carnitine palmitoyltransferase I activity (B) in female rats. Open bars: 5 weeks old, filled bars: 50 weeks old. *$p<0.05$ vs 5 weeks old.

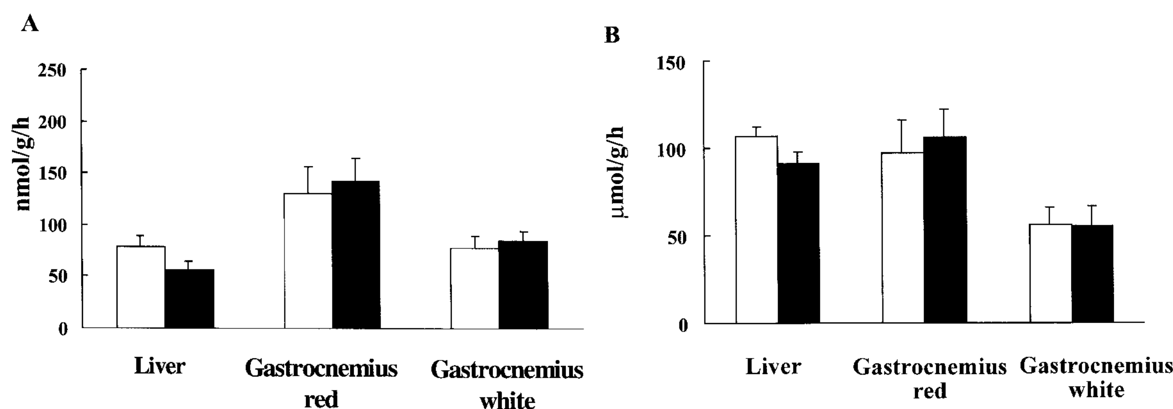

Fig. 2. Palmitate oxidation rate (A) and carnitine palmitoyltransferase I activity (B) in male rats. Open bars: 5 weeks old, filled bars: 50 weeks old.

Table 2. Correlation coefficients between palmitate oxidation rate and intra-abdominal fat mass in rats

		Palmitate oxidation rate			
		Male		Female	
		5 weeks	50 weeks	5 weeks	50 weeks
Liver	r	−0.45	0.78	−0.54	0.76
	p value	0.12	0.02	0.10	0.04
Gastrocnemius	r	−0.57	0.64	−0.62	0.65
red	p value	0.05	0.05	0.04	0.03
Gastrocnemius	r	−0.54	0.53	−0.48	0.52
white	p value	0.13	0.07	0.10	0.08

male rats. Palmitate oxidation rates in the gastrocnemius white did not differ with age in either the male or female subjects. CPT-1 activity in all tissues tested did not differ with age in either of the groups (Fig. 1 and 2). The correlation between the palmitate oxidation rate and the intra-abdominal fat mass was also evaluated. The palmitate oxidation rate in the gastrocnemius red was correlated inversely with intra-abdominal fat mass in the 5-week-old rats, regardless of gender ($p=0.05$ and $p=0.04$ in male and female, respectively). In the liver and gastrocnemius white, the palmitate oxidation rate was inversely correlated, however, it was not statistically significant (Table 2). On the other hand, the palmitate oxidation rate in the liver and gastrocnemius red of the 50-week-old rats was significantly positively correlated with fat mass in both gender

Table 3. Correlation coefficients between % fat mass and HOMA-IR or insulin in rats

		% Fat mass	
		5 weeks	50 weeks
HOMA-IR index	r	−0.28	0.59
	p value	0.43	0.05
Insulin	r	−0.25	0.52
	p value	0.49	0.05

HOMA-IR: homeostasis model assessment of insulin resistance.

groups (r=0.78 and 0.76 in males and 0.64 and 0.65 in females). Homeostasis Model Assessment Insulin Resistance (HOMA-IR) ($r=0.59$, $p<0.05$) and plasma insulin levels ($r=0.52$, $p<0.05$) were correlated positively with intra-abdominal fat mass in the 50-week-old rats in pooled male and female group, but such a correlation was not detected in the pooled 5-week-old rat group (Table 3).

DISCUSSION

Ovarian hormones are well established to exert metabolic effects, especially on lipid metabolism. The treatment of ovariectomized rats with estrogen stimulats fatty acid oxidation in the skeletal muscles and heart (Hatta et al, 1988; Grist et al, 2002). Furthermore, the treatment of male rats with estradiol has been shown to result in a significant increase in lipid availability during exercise (Kendrick et al, 1991). Therefore, we hypothesized that fatty acid oxidation in females might be different from that in males, and the present results indicated that the rate of palmitate oxidation in female rats were elevated at 50 weeks of age relative to the levels measured at 5 weeks of age, however, no such age-associated changes in palmitate oxidation rates were observed in the male rats. In previous studies, it has been demonstrated that puberty in Sprague-Dawley female rats begins at approximately 41 to 45 days of age, as evidenced by the initial estrous cycle (Muto et al, 2003; Tian et al, 2005). The young rats employed in our study were in the peripubertal period, whereas the 50-week-old rats were fully matured adults. Therefore, it appears that ovarian hormone may, indeed, be responsible for the observed elevations in palmitate oxidation rates in the 50-week-old female rats, relative to the values measured in the 5-week-old rats, which are not under the full influence of ovarian hormone. Previous studies have also shown that ovariectomies in female rats result in a reduction in the expression of lipid oxidative genes(Kamei et al, 2005), and estrogen replacement results in their recovery (Campbell et al, 2003) in the skeletal muscles. Since no differences of CPT-1 activity were observed in relation to gender and age in our study, it could be concluded that CPT-1 was not responsible for the observed elevations of the fatty acid oxidation rates in the middle-aged female rats. In contrast to the known effects of ovarian hormone, the effects of androgen on lipid metabolism remain to be thoroughly elucidated. Although some studies have demonstrated a reduction in fat mass mediated by testosterone (Schroeder et al, 2004), others have reported that testosterone reduces lipolysis in subcutaneous fat, and that it exerts no detectable effects on visceral fat (Dicker et al, 2004). Although acute changes in the circulating levels of ovarian hormone exert potent effects on substrate utilization in women, short-term testosterone treatment has been shown to exert no appreciable effects on substrate utilization in men (Braun et al, 2005). Furthermore, the administration of dehydroepiandrosterone treatment to women suffering from adrenal insufficiency has been shown to be not associated with measurable changes in lipid oxidation (Christiansen et al, 2005).

Earlier studies reported that changes in the rate of fatty acid oxidation are associated with aging, primarily in male subjects. Skeletal muscle fatty acid oxidation rates in the basal state have been shown to decline with age in rats (Tucker et al, 2002), whereas these rates increased with aging in rats in an insulin-stimulated state (Tucker et al, 2003). In humans in the basal state, no age-associated changes in the lipid oxidation rates have been observed to occur (Blaak et al, 1999), suggesting that there may be species differences inherent to the phenomenon of age-associated changes of lipid oxidation rate. In our study, no alterations in fatty acid oxidation rates were associated with aging in male rats in the basal state. We surmised that differences in the fasting time might have caused inconsistent results: The rats in our study were subjected to 3 hours of starvation, while rats in other studies were subjected to starvation overnight.

Fatty acid oxidation capacity is a crucial determining factor for overall body fatness. Reduced fatty acid oxidation has been demonstrated to increase body fatness in both rats (Dobbins et al, 2001) and humans (Kelley et al, 1999). Fat oxidation has been associated more closely with visceral fat mass than with subcutaneous fat mass (Deriaz et al, 2001). Since females tend to harbor relatively lower visceral fat mass than males (Kotani et al, 1994), the relationship between intra-abdominal fat mass and fatty acid oxidation rate can be expected to differ between males and females. The results of our study indicated that the correlation between palmitate oxidation rates and intra-abdominal fat mass did not differ between male and female rats, however, exhibited differences according to age. We were able to determine that fatty acid oxidation rates were correlated inversely with intra-abdominal fat mass in young rats. In the young rats, intra-abdominal fat mass was not related to insulin resistance, and this finding was corroborated by the lack of correlation between HOMA-IR and intra-abdominal fat mass. Therefore, we suggest that the reduced rate of fatty acid oxidation in the 5-week-old rats was responsible for the observed increases in intra-abdominal fat mass. Conversely, fatty acid oxidation was positively correlated with intra-abdominal fat mass in the 50-week-old rats. Insulin resistance is known to attenuate the inhibitory effects of insulin on fatty acid oxidation (Colberg et al, 1995). Since HOMA?IR and insulin levels were correlated positively with intra-abdominal fat mass, insulin resistance in middle-aged rats may be expected to result in the elevated rate of fatty acid oxidation rates in rats with higher intra-abdominal fat mass.

In summary, whereas fatty acid oxidation rates in middle-aged female rats were elevated in comparison to the rates measured in peri-pubertal female rats, no such age-associated variances were detected in male rats. Ovarian hormone is thought to be responsible, at least in part, for this difference.

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