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Effects of exercise on adiponectin and adiponectin receptor levels in rats

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Abstract

Adiponectin reportedly reduces insulin-resistance. Exercise has also been shown to lessen insulin-resistance, though it is not known whether exercise increases levels of adiponectin and/or its receptors or whether its effects are dependent on exercise intensity and/or frequency. Catecholamine levels have been shown to increase during exercise and to fluctuate based on exercise intensity and duration. In light of this information, we examined the effects of exercise on catecholamine, adiponectin, and adiponectin receptor levels in rats. Our data showed that blood adiponectin levels increased by 150% in animals that exercised at a rate of 30 m/min for 60 min 2 days per week, but not 5 days, per week; no such increase was observed in rats that exercised at a rate of 25 m/min for 30 min. The effects of exercise on adiponectin receptor mRNA were variable, with adiponectin receptor 1 (AdipoR1) levels in muscle increasing up to 4 times while adiponectin receptor 2 (AdipoR2) levels in liver fell to below half in response to exercise at a rate of 25 m/min for 30 min 5 days per week. We also observed that urinary epinephrine levels and plasma lipids were elevated by exercise at a rate of 25 m/min for 30 min 2 days per week. Exercise frequency at a rate of 25 m/min for 30 min correlated with AdipoR1 and AdipoR2 mRNA expression in the muscle and liver, respectively ($r=0.640$, $p<0.05$ and $r=-0.808$, $p<0.0005$, respectively). Urinary epinephrine levels correlated with AdipoR2 mRNA expression in liver tissues ($r=-0.664$, $p<0.05$) in rats that exercised at a rate of 25 m/min for 30 min. Thus, exercise may regulate adiponectin receptor mRNA expression in tissues, which might cause increases in glucose uptake and fatty acid oxidation in the muscle. The effect of exercise on adiponectin levels depends on the specific conditions of the exercise.

Keywords: Adiponectin; Adiponectin receptor; Catecholamine; Epinephrine; Exercise

Introduction

Adiponectin is a protein hormone produced and secreted exclusively by adipocytes that regulates the metabolism of lipids and glucose, and which has anti-inflammatory properties. It has been reported to play a role in the development of cardiovascular disease, type 2 diabetes, and obesity, and its levels were shown to be suppressed in insulin-resistant and obese animals and humans (Okamoto et al., 2001; Arita et al., 1999; Hotta et al., 2000). Two types of adiponectin receptors have been identified i.e., adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2), which are primarily expressed in muscle and liver cells, respectively (Yamauchi et al., 2003). Adiponectin levels were reported to rise in response to weight loss and glitazone therapy, but not after chronic exercise training (Hulver et al., 2002; Boudou et al., 2003; Yatagai et al., 2003). On the other hand, Kriketos et al. reported that one week of exercise training increased circulating adiponectin levels and improved insulin sensitivity (Kriketos et al., 2004). Jurimae et al. reported that adiponectin levels were modulated in response to a single cycle of maximal exercise in highly trained male rowers (Jurimae et al., 2005), though others failed to find such an effect in healthy subjects (Ferguson et al., 2004). Yokoyama et al. reported that aerobic exercise did not alter plasma adiponectin levels in overweight, insulin-resistant, nondiabetic individuals (Yokoyama et al., 2004), similar to findings reported by Marcell et al. who failed to find an effect of moderate to intense exercise on adiponectin levels or insulin sensitivity in type 2 diabetics (Marcell et al., 2005). Recently, it has been reported that adiponectin levels are independently related to physical exercise (Tsukinoki et al., 2005). Thus, the

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effect of exercise on adiponectin levels is still unclear, though the data perhaps suggest that the more intense the exercise the more likely it is to influence adiponectin levels. The mechanisms by which adiponectin levels are regulated during exercise are unknown.

It is well established that catecholamine levels increase during exercise in direct proportion to its duration and intensity (Erdem et al., 2002). Exercise performed at an intensity that is above the anaerobic threshold (such as bicycle ergometer) leads to a marked increase in plasma catecholamine levels, which accurately reflect physiological stress and sympathetic nervous system activity. Reduced nocturnal urinary excretion of catecholamines has been suggested to be a sign of overtraining syndrome and reflects a lower degree of intrinsic sympathetic activity in these individuals. Athletes with overtraining syndrome also exhibit lower maximal plasma catecholamines after exhaustive exercise (Lehmann et al., 1992). The plasma catecholamines are promising peripheral candidates for mediating increases in cholesterol due to stress. They perform a myriad of physiological functions, several of which are relevant for lipid metabolism. Perhaps most significantly, they stimulate adipocytes to release free fatty acid (FFAs) into the blood stream. Hepatic FFAs has been shown to stimulate the liver to release cholesterol into the bloodstream (Brennan et al., 1996). It was suggested that acute lowering FFA is associated with decreased adiponectin concentrations (Bernstein et al., 2004). In light of the fact that catecholamines were reported to inhibit adiponectin mRNA expression in vitro (Fasshauer et al., 2001), it is tempting to speculate that they might affect adiponectin levels during exercise. In this study, we examined the effects of submaximal exercise on blood adiponectin, adiponectin receptor mRNA, urinary catecholamine, and blood lipids levels.

Materials and methods

Animals

All experimental procedures involving animals were approved by the University of Tsukuba Animal Care and Use Committee. Thirty, eight-week-old male Wistar rats were randomly assigned to either a sedentary control group ($n=10$, two groups of 5 animals) or exercise training groups ($n=5$ for each exercise regimen, 2 days/wk or 5 days/wk, at 30 m/min for 60 min or 25 m/min for 30 min; total number of rats=20). Rats were housed and maintained in a temperature-controlled room (22±2 °C) that was on a 12:12-h light–dark cycle (0600 to 1800), with food and water ad libitum. Exercise training sessions of 30 or 60 min were carried out just before the beginning of the dark cycle in the same room within which the animals were housed. Animals were weighed at the beginning and the end of the study, and sacrificed by blood harvesting after anesthetizing them.

Exercise training protocol

Animals were exercised for 12 weeks using the following regimens: 30 m/min for 60 min or 25 m/min for 30 min, 2 days/wk (E2) or 5 days/wk (E5) for 12 weeks on a motor-driven treadmill. Both the treadmill speed and grade were gradually increased over the course of the 12-week training period to provide training overload throughout the training regimen. This training intensity was designed to elicit 75% (30 m/min for 60 min) and 62% (25 m/min for 30 min) maximal oxygen uptake based on previous work in adult rats (Lawler et al., 1993), respectively.

Tissue samples

Animals were terminally anesthetized with diethyl ether and sodium pentobarbital (50 mg/kg ip), after which their blood was harvested from the abdominal aorta. Their adrenal glands, anterior tibial muscle, liver and adipose tissue from the epididymal fat pad were quickly removed, and the tissues were then quickly frozen by immersion in liquid nitrogen. Tissues were stored at −80 °C until use.

Determination of adiponectin and catecholamine levels

Plasma adiponectin concentration was determined by ELISA (Otsuka Pharmaceutical, Tokyo, Japan). Urine samples were collected for 24 h after the exercise (6:00 pm to 6:00 pm of the next day). Urine catecholamine (epinephrine, norepinephrine, dopamine) concentrations were determined using a catecholamine autoanalyzer (HLC-725CA, TOSOH, Tokyo).

Real-time PCR

Adiponectin mRNA expression in adipose tissue was assessed using Assay-On-Demand (Applied Biochemistry, Foster City, Calf) that was prepared using a Taqman PCR master reagent kit. TaqMan® Assay-On-Demand was also used to determine adiponectin receptors 1 & 2 mRNA expression in liver and muscle. The tyrosine hydroxylase (TH) mRNA expression was assessed as described previously (Kitaoka et al., 2003). The thermal cycling protocol was 2 min at 50 °C and 10 min at 95 °C, which was followed by 40 cycles of 95 °C for 15 s and 60 °C for

Table 1
Effects of 12 weeks of training on initial and final body weight and adrenal gland weight at sacrifice

	Body weight (g)		Adrenal gland weights (mg)
	Initial	Final	
30 m/min for 60 min			
Control	209±4.4	481±22.9	17.3±3.5
E2	203±6.7	432±15.8	17.5±1.6
E5	199±6.8	403*±16.7	23.1±5.6
F		3.915	2.986
df		2,13	2,10
25 m/min for 30 min			
Control	200±9.4	453±54.1	19.3±2.3
E2	191±13.2	404±42.2	22.14±3.69
E5	194±9.0	421±29	23.62±2.58
F		1.729	0.573
df		2,13	2,12

Data are means±SD. $*p<0.05$. Compared with control. E2, 2 days of exercise/wk; E5, 5 days of exercise/wk . *F*, *F* value; *df*, degrees of freedom.