Effect of Voluntary Wheel-Running on Insulin Sensitivity and Responsiveness in High-Fat-Fed Rats

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Abstract. The effect of voluntary wheel-running on insulin resistance was studied in high-fat-fed rats. A sequential hyperinsulinemic euglycemic clamp procedure was employed (insulin infusion rates: 3 and 30 mU/kg BW/min) in 14 high-fat-fed rats and 7 chow-fed rats under the awake condition. The high-fat-fed rats were further divided into a sedentary (n=7) and a voluntary wheel-running (n=7) groups. Blood glucose was clamped at the fasting level in each rat. Plasma insulin levels during the 3- and 30-mU/kg BW/min insulin infusions were 40-50 and 450-550 μU/ml, respectively. At both 3 and 30 mU/kg BW/min insulin infusions, high-fat-feeding showed a significant decrease in glucose infusion rate (GIR), compared with the chow-fed rats. However, decreased GIRs were restored by the 4-wk wheel-running and reached similar levels as the chow-fed rats. Therefore, it could be concluded that voluntary wheel-running prevents insulin resistance induced by high-fat feeding.

Key words: Euglycemic clamp technique, Insulin resistance, Voluntary running, High-fat diet

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INSULIN resistance is associated with various pathological states including type 2 diabetes mellitus, obesity, hypertension and atherosclerosis [1, 2]. Studies in rats have shown that high-fat feeding can cause profound and widespread tissue insulin resistance [3, 4]. On the other hand, physical exercise-training has been reported to improve insulin resistance [5, 6] and has been thought to be advantageous in syndromes of insulin resistance such as the above diseases [7]. With respect to the effect of exercise training on fat-induced insulin resistance, Kusunoki et al. [8] previously demonstrated that insulin resistance in high-fat-fed rats recovered after a single exercise bout.

Kraegen et al. [9] showed that wheel-running ameliorated both insulin sensitivity and responsiveness using the euglycemic clamp procedure in the awake and high-fat-fed rats. However the latter study was performed 72 hr after surgery for cannulation, in which condition the undesirable influence of surgical trauma on insulin action may have remained. As mentioned by Smith et al. [10], animals should be allowed at least five days to recover from the influence of surgery. Since it is unclear whether or not physical exercise-training can be effective for insulin resistant rats induced by high-fat feeding and if so, what kind of insulin action, insulin sensitivity and/or responsiveness, can be improved, the present study was conducted to clarify the above questions by use of the sequential euglycemic clamp procedure in the awake condition.
Materials and Methods

Animals

Female Wistar rats (n = 21) weighing between 120–130 g aged 6 wk were used for the study. Animals were housed in individual cages with or without a running wheel in a room maintained 23°C with 12 h light/dark cycle, where they had access to food and water ad libitum. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of Nagoya University.

After a 1-wk acclimation period, rats were randomly divided into two groups; a normal chow-diet (powdered rodent diet MF, Oriental Yeast Co., Chiba, Japan) and a high-fat diet. The caloric composition of the chow diet was 16% fat, 26% protein and 58% carbohydrate. The corresponding composition for the high-fat diet was 59% fat (mainly from lard), 21% protein and 20% carbohydrate. The high-fat diet was freshly made every 3 days and was stored at 4°C. The high-fat-fed rats were further assigned to either a sedentary (n = 7) or a wheel running (n = 7) group [11, 12]. The number of exercise wheel revolutions were recorded every day to determine running distance. Body weights of the animals were also measured daily.

After 3 wk on these conditions, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg BW). Thereafter, a middle ventral incision was made in the neck, and the right jugular vein and left carotid artery were cannulated with Silascon SH tubing (NO. 00, Kanaka Medix, Osaka, Japan). The catheters were tunneled subcutaneously to the back of the neck and flushed with 200 ml of saline containing heparin (100 U/ml) and sodium penicillin G (5,000 U/ml). They were then filled with a viscous solution of polyvinylpyrrolidone (PVP-30, Sigma, St. Louis, MO) and capped with a piece of polyethylene tubing melted and sealed at one end. After surgery, each rat was kept at the same conditions as the preoperative states. Food intake and wheel running recovered one day and 2-3 days after surgery, respectively.

Euglycemic clamp procedure

One week after surgery, a two-step hyperinsulinemic euglycemic clamp procedure [13, 14] was performed on each rat after an overnight fast and 24 h after cessation of wheel running to assess whole body insulin action. The rat was placed in a restraining cage to which it was accustomed, and extension tubing was attached to the jugular catheter by an adapter for continuous infusion of insulin (Actrapid MC, Novo Nordisk, Denmark) and glucose. The carotid catheter was used for blood sampling. A primed infusion was delivered at a rate of 3 mU/kg BW/min (low-dose) for 90 min and then at an increased rate of 30 mU/kg BW/min (maximal stimulation, high-dose) for an additional 90 min. Blood glucose concentration was kept constant at the basal level with a variable infusion of 20% (w/v) glucose solution, based on blood glucose concentration measured every 10 min. Additional blood samples were collected just before insulin infusion and at 90 min and 180 min after starting insulin infusion for the determination of plasma insulin concentrations. Glucose infusion rate (GIR) in mg/kg BW/min was calculated every 10 min during the clamp study. The means of GIR values from 60 to 90 min and 150 to 180 min for the two-step sequential euglycemic clamp procedure were regarded as an index of whole body insulin action since a plateau in GIR is achieved during these times [13].

Analytical methods

Blood glucose concentration was determined with a YSI 2300 STAT glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Samples were immediately centrifuged at 4°C and stored at −70°C until analyses. Cell fraction after plasma separation was suspended in saline and returned to the rat so as not to reduce the hematocrit level. Plasma insulin was assayed with a radioimmunological assay kit (Phadeseph Insulin RIA, Pharmacia AB, Stockholm, Sweden).

All values are presented as means ± SEM. Statistical analysis was performed using an analysis of variance followed by Fisher’s PLSD test. P values less than 0.05 were considered significant.
Results

Body weight and running distance

Final body weights in sedentary high-fat-fed rats were significantly greater than those in chow-fed rats, as shown in Table 1. Although body weights decreased in all rats after surgery for cannulation, they returned to preoperative levels after 3 days. With respect to the running activity pattern, the rats still reached approximately 3 km per day for the 1st wk and displayed a large increase in running activity during the 2nd wk, approaching 7 km per day. Thereafter, the rats maintained average running distances of 10 km per day through the end of the 3rd wk. Although the running activity decreased markedly for 2 days after surgery on the 4th wk, it was maintained at over 7 km per day for the last 4 days on the 4th week.

Whole body insulin action

Mean blood glucose and plasma insulin concentrations in chow-fed, high-fat-fed sedentary and wheel-running rats before and during the sequential euglycemic clamp studies are presented in Table 1. There was no significant difference in blood glucose concentration throughout the study. Plasma insulin concentrations during low-dose and high-dose insulin infusion rates were 40-50 and 450-550 μU/ml, respectively.

Average GIRs for the last 30 min during the low-dose and the high-dose insulin infusion rates are shown in Fig. 1 and 2. At the insulin infusion rate of 3 mU/kg BW/min, high-fat feeding resulted in a marked decrease in GIR (8.4 ±0.9 mg/kg BW/min), compared with chow-fed rats (18.4 ±1.3 mg/kg BW/min, p < 0.001). However, the wheel-running rats

Table 1. Body weight, blood glucose and plasma insulin levels before and during the sequential euglycemic clamp procedure at low-dose (3.0 mU/kg BW/min) and high-dose (30.0 mU/kg BW/min) insulin infusions.

<table>
<thead>
<tr>
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<th>Body wt (g)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (μU/ml)</th>
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<tr>
<td></td>
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<td>Basal clamp</td>
<td>Basal clamp</td>
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<td></td>
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<td>low-dose</td>
<td>high-dose</td>
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<tr>
<td>Chow-fed (7)</td>
<td>191 ± 4</td>
<td>75 ± 2</td>
<td>72 ± 2</td>
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<tr>
<td>High-fat-fed sedentary (7)</td>
<td>208 ± 4*</td>
<td>74 ± 1</td>
<td>73 ± 1</td>
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<tr>
<td>Wheel-running (7)</td>
<td>201 ± 5</td>
<td>71 ± 1</td>
<td>72 ± 1</td>
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Values are means ±SE. The number of rats is shown in parentheses. *p < 0.01 vs. chow-fed

Fig. 1. Glucose infusion rate (GIR) for euglycemic clamp procedure at low-dose (3.0 mU/kg BW/min) insulin infusion.

Fig. 2. Glucose infusion rate (GIR) for euglycemic clamp procedure at high-dose (30.0 mU/kg BW/min) insulin infusion.
treated with high-fat diet showed significantly higher GIR (18.6 ± 1.7 mg/kg BW/min, p < 0.001) than that of the sedentary rats given high-fat diet. There was no significant difference in GIR between chow-fed rats and wheel-running rats with high-fat diet.

Likewise, animals treated with high-fat diet demonstrated a significant decrease in GIR (39.2 ± 3.0 mg/kg BW/min) compared with chow-fed rats (49.2 ± 1.6 mg/kg BW/min) at insulin infusion rate of 30 mU/kg BW/min, whereas GIR was restored by wheel-running (48.1 ± 1.1 mg/kg BW/min) and reached similar levels as the chow-fed rats.

Discussion

The present study was undertaken to determine the effect of voluntary wheel-running on insulin resistance induced by high-fat feeding using a two-step hyperinsulinemic euglycemic clamp procedure. GIR at low-dose insulin infusion primarily reflects insulin sensitivity in peripheral tissues; changes in insulin sensitivity were thought to be caused by mainly changes in insulin receptor binding. High-dose insulin infusion leads to maximal insulin action, insulin responsiveness, predominantly indicating the capacity of post-receptor binding mechanisms [15, 16]. The present study demonstrated that 1) insulin resistance induced by high-fat feeding was due to impaired insulin sensitivity and responsiveness, and that 2) voluntary wheel-running increased insulin sensitivity and responsiveness, reaching the levels of the chow-fed rats.

Increased insulin sensitivity by physical training was reversed to the pre-training levels within a few days after cessation of training in humans and rats [11, 17]. It is possible that the improvement in insulin sensitivity is owing to the residual effect of the last exercise in the present study. Moreover, hyperinsulinemia during the low-dose insulin infusion (less than 100 μU/ml) could not sufficiently suppress hepatic glucose production [10]. Therefore, it is difficult to evaluate effects of wheel-running on peripheral insulin sensitivity in the current study.

However, Mikines et al. [19] previously estimated insulin sensitivity and responsiveness in well-trained men before and after detraining for 5 days, providing evidence that physical training induced an adaptation to insulin responsiveness. Nagasawa et al. [20], who used anesthetized rats, also showed that a rise in insulin responsiveness was maintained for 3 wk after cessation of training in wheel-running rats. From these reports, insulin responsiveness could not be influenced by surgery for cannulation or the last exercise bout. The high-dose insulin infusion resulted in the pharmacologic insulin range, which sufficiently suppressed hepatic glucose production [10]. Recently, impairments in insulin-mediated glucose transport by high-fat feeding is primarily due to changes in intracellular glucose metabolism [21] and GLUT-4 translocation [22] rather than changes in insulin receptor function. Moreover, insulin resistance induced by high-fat diet was caused by circulating lipids (such as FFA) [23] and TNF-α [24], which were generated or secreted by hypertrophic adipocytes. It seems likely that fat-induced insulin resistance is mainly decreased insulin responsiveness, and that improvement of insulin responsiveness is brought about by voluntary wheel-running.

The results of this investigation can be compared with two previous reports of the effect of training on insulin resistance induced by high-fat feeding. Kraegen et al. [9] determined insulin sensitivity and responsiveness using the euglycemic clamp procedure. Their results seem to be identical to ours. However, GIR determined during supraphysiologic insulin concentration was 40% reduced in their study compared with the present study. The difference may be explained by surgical stress since their study was performed 72 hr after surgery for cannulation. Smith et al. [10] affirmed that animals should be allowed at least five days to recover from the stress. Our study was conducted 1 wk after surgery. Finally, Kern et al. [25] evaluated maximal insulin action by use of hindquarter perfusion technique (in vitro), indicating that treadmill running can only partially ameliorate insulin resistance induced by high-fat feeding. In the present study (in vivo), decreased insulin responsiveness by high-fat feeding was completely restored, thus we came to somewhat different conclusions than them. The difference may be due to experimental design.

Based upon the results of the present study, it was concluded that voluntary wheel-running prevents insulin resistance induced by high-fat feeding.
Running Effect on Insulin resistance in rats

References