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Physical exercise started before maturity prevents aging-related insulin resistance in rats

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To evaluate the effects of physical exercise on insulin resistance associated with aging, we investigated insulin-induced glucose disposal in mature trained rats (MT, 4 months of age), mature untrained ones (MU, 4 months of age), and young untrained ones (YU, 2 months of age), using the 2-step sequential euglycemic clamp technique (insulin infusion rates: 6 and 30 mU/kg/min). During 30 mU/kg/min clamp, metabolic clearance rates of glucose (MCR) in MU significantly declined compared with YU ($P<0.01$). MCR during 6 and 30 mU/kg/min clamp were, respectively, significantly higher in MT than in MU ($P<0.01$). There were no significant differences in MCR between MT and YU.

These results suggest that aging process of rats might decrease insulin action in their peripheral tissues predominantly because of post insulin receptor binding defect, and that physical exercise begun before maturity might prevent or delay insulin resistance associated with aging.

INTRODUCTION

Glucose intolerance which occurs with aging has been well documented[4,5]. The incidence of impaired glucose tolerance and non-insulin-dependent diabetes mellitus (NIDDM) increases with advancing age, especially after middle age. It has been reported that insulin action declines not only in humans but also in rats, as they grow older[6,14,15]. On the other hand, many investigators have demonstrated that exercise enhance insulin action in peripheral tissues[9,17]. As to older subjects, Kahn SE et al.[11] reported that exercise training was capable of improving their insulin action. Further, Yamanouchi et al.[22] presented that insulin resistance existed in aged subjects but that insulin responsiveness in aged athletic subjects reached to the same levels as those in young subjects. However, few studies have been made longitudinally. It has not been fully evaluated whether the initiation of physical exercise in early childhood could prevent or delay insulin resistance associated with aging. Using the insulin clamp technique in rats, therefore, we investigated in this study (1) whether aging process would induce glucose intolerance, and (2) whether the initiation of physical exercise before maturity would prevent aging-related insulin resistance.

METHODS

Animals

Eighteen male Wistar rats, 1 month of age were randomly assigned to three groups: mature trained rats (MT); mature untrained ones (MU); young untrained ones (YU). MT were housed individually for three months in wheelcages where voluntary access to running was available at all times. The untrained rats were kept in standardized individual cages, where they were not able to exercise: MU for 3 months, and YU for 1

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month. The euglycemic clamp studies were performed at 4 months of age for MT and MU, and at 2 months of age for YU. The distance of running in MT reached more than 4000 m/day respectively and their exercise was stopped at 48 hours before euglycemic clamp studies.

All rats were obtained from Chubu Kagaku Shizai (Nagoya, Japan). They were housed in an environmentally controlled room at 23°C with an alternating 12-hour light/dark cycle and received standard laboratory chow (Oriental East, Tokyo, Japan) and water ad libitum.

Euglycemic Clamp Procedures

The whole study was carried out in the postabsorptive state. The rats were anesthetized with pentobarbital sodium, 40 mg/kg intraperitoneally. Catheters (Silascon, Dow Corning; ID, 0.20 mm; OD 0.37 mm) were inserted in the left jugular vein and the right femoral vein. The tip of the catheter in the jugular vein was placed in the right atrium to sample whole-body venous return blood. The catheter in the femoral vein was attached to two infusion pumps (STC-521, Terumo, Japan), which were used for the infusion of insulin (Actrapid MC, Novo Nordisk, Denmark) and glucose solution. The insulin infusion rates were 6 mU/kg/min between 0 and 60 minutes, and 30 mU/kg/min between 60 and 120 minutes. During euglycemic clamps, blood glucose concentrations were determined by means of the samples drawn at 5-minute intervals and the infusion rates of glucose were adjusted to maintain the basal level of glucose. The glucose solution was 10%wt/vol during 6 mU/kg/min clamp and 20%wt/vol during 30 mU/kg/min clamp.

Analytical Procedures

Blood glucose concentration was measured by the glucose oxidase method (model 23A glucose analyzer, Yellow Springs Instrument, Ohio, USA). Samples for plasma insulin determination were obtained before insulin infusion, at 30, 60, 90, and 120 minutes after the start of insulin infusion. Plasma insulin concentration was determined by radioimmunoassay (Insulin Reabead, Dinabot, Japan).

Calculations

Glucose disposal rates (GDR) were calculated by multiplying the glucose infusion rates by the length of time the infusion rate was maintained. The products during 30 to 60 minutes and during 90 to 120 minutes of the clamp studies were averaged per minute respectively and adjusted by body weight. Metabolic clearance rates of glucose (MCR) were determined from GDR, which were divided by mean blood glucose levels during euglycemic clamp studies to reduce the influence of the different blood glucose concentrations. All values are presented as means ± SEM. Differences among the three groups were evaluated by using one way analysis of variance (ANOVA).

RESULTS

Body weight, blood glucose concentrations, and plasma insulin levels before and during euglycemic clamp studies

Mean body weight, blood glucose concentration, and plasma insulin level before and during euglycemic clamp studies are listed in Table 1. Mean body weights were significantly higher in MU and MT than in YU ($P<0.01$). There was also a significant difference in mean body weight between MU and MT ($P<0.05$). Before euglycemic clamp, blood glucose concentration was higher in MU than in MT and YU, but the differences were not statistically significant.

Table 1. Mean body weight, blood glucose concentrations, and plasma insulin levels before and during euglycemic clamp studies

	MT	MU	YU
n	6	6	6
Body weight (g)	$403 \pm 11^{a,b}$	$443 \pm 14^{a,c}$	$307 \pm 6^{b,c}$
Blood glucose (mg/dl)			
before euglycemic clamp	69 ± 6	82 ± 4	73 ± 3
6 mU/kg/min clamp	68 ± 4	82 ± 4	73 ± 6
30 mU/kg/min clamp	69 ± 6	82 ± 5	70 ± 3
Plasma insulin (μU/ml)			
before euglycemic clamp	14 ± 2^{b}	$21 \pm 2^{b,c}$	15 ± 1^{c}
6 mU/kg/min clamp	88 ± 6	114 ± 16	89 ± 14
30 mU/kg/min clamp	928 ± 70	1255 ± 134	923 ± 72

Values are means ± SEM. MT, mature trained rats; MU, mature untrained rats; YU, young untrained rats; [a] $P<0.05$; [b,c] $P<0.01$

Compared with the other two groups, plasma insulin level in MU increased significantly ($P<0.01$). During euglycemic clamp studies, blood glucose concentrations and plasma insulin levels were higher in MU than those in the other two groups, but there were no significant differences statistically.

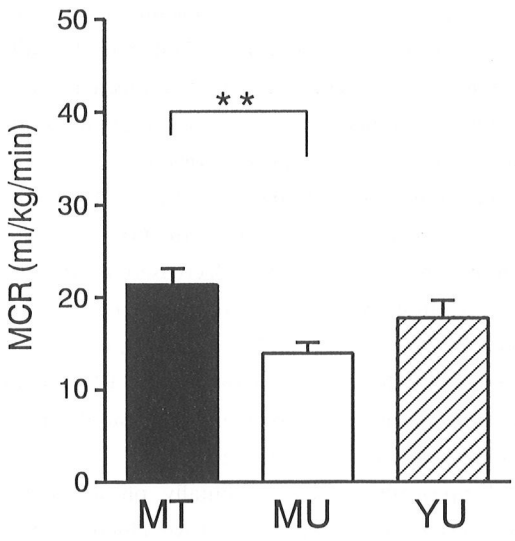

Figure 1. Metabolic clearance rates of glucose (MCR) during 6-mU/kg/min euglycemic clamp studies. Results are means ± SEM. MT, mature trained rats; MU, mature untrained rats; YU, young untrained rats; **$P<0.01$.

Metabolic clearance rates of glucose during euglycemic clamp studies

Figure 1 indicates metabolic clearance rates of glucose (MCR) during 6-mU/kg/min euglycemic clamp studies. MCR was significantly higher in MT than in MU (21.4 ± 1.8 vs. 13.9 ± 1.2 ml/kg/min; $P<0.01$). MCR was also higher in YU (17.4 ± 1.7 ml/kg/min) than that in MU, but there was no significant difference. The difference between MT and YU was also not statistically significant.

MCR during 30 mU/kg/min clamp study were drawn in Figure 2. Compared with MCR in MU, that in MT was significantly elevated at maximal hyperinsulinemia (40.5 ± 3.6 vs. 26.1 ± 1.1 ml/kg/min; $P<0.01$). MCR was also significantly higher in YU (39.9 ± 3.5 ml/kg/min) than those in MU ($P<0.01$). As during 6-mU/kg/min euglycemic clamp, no statistic difference was observed in MCR between MT and YU.

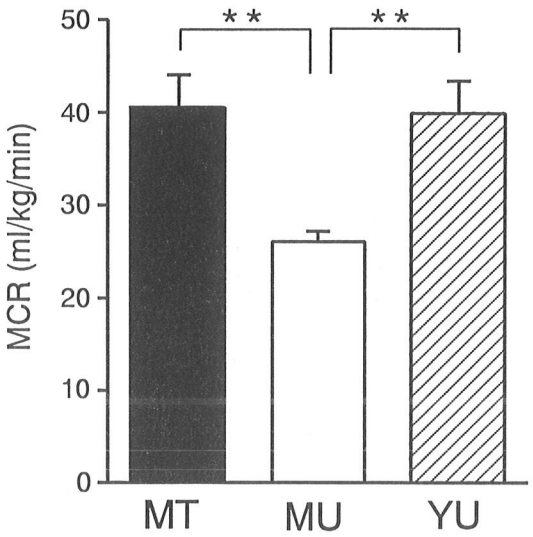

Figure 2. Metabolic clearance rates of glucose (MCR) during 30-mU/kg/min euglycemic clamp studies. Results are means ± SEM. MT, mature trained rats; MU, mature untrained rats; YU, young untrained rats; $^{**}P<0.01$.

DISCUSSION

In euglycemic clamp studies, MCR at submaximal insulin level is thought to reflect insulin sensitivity, which declines due to defects of insulin binding levels or certain kinds of post-binding levels involving coupling mechanisms in peripheral tissues[10,16]. At maximally effective insulin concentrations, MCR reflects insulin responsiveness, which indicates the capacity of post-receptor binding levels.

Compared with YU, MCR in MU at maximal insulin level declined significantly in this study. Nishimura et al.[15] reported that maximal induced glucose utilization decreased between 2 and 4 months of age using euglycemic clamp studies. Other studies in rats also presented that insulin resistance occurred markedly during their early growth[6,14]. Like these studies, our results indicated that the period of 4 months of age appeared to cause insulin resistance in rats, predominantly because of decreasing insulin responsiveness. On the other hand, MCR were significantly higher at any insulin infusion rate in MT than in MU. In addition, no significant difference was observed in MCR between MT and YU. These results suggested that physical exercise begun before maturity might prevent insulin resistance with aging and maintain the levels of insulin action similar to those in young rats.

Whether aging per se would reduce insulin action or not remains to be discussed. Other factors such as obesity and physical inactiveness may play a crucial role. In this study, we do not deny the influence of the different body weights. However, because there was no significant difference in MCR between MT and YU in spite of the difference of body weights, we suppose that other factors than body weight, probably aging process, must be one of the important factors which reduced insulin action in MU. Nishimura et al.[15] also reported that decreased insulin responsiveness in older rats was not explained by the difference of body composition. The mechanism of insulin resistance with aging also remains to be investigated. Many reports showed that post insulin binding defects mainly cause insulin resistance[2,5]. The reduced levels of GLUT4 with aging, which would cause decreased insulin responsiveness, were reported in adipocytes and skeletal muscles[12,13]. On the contrary, several investigators showed that muscle GLUT4 content in aged rats increased or maintained similar level compared with that in young rats[1,19].

Empirically and experimentally, physical exercise has been known to improve glucose tolerance. Many *in vivo* and *in vitro* studies have demonstrated that physical exercise enhance insulin action through various mechanisms in target tissues[8,9,20]. Recently, glucose transport system

has been well noticed. Several biochemical and morphological studies on the rat's skeletal muscle have shown that exercise increases its glucose uptake by the translocation of the GLUT4 transporter from an intracellular storage pool to the plasma membrane[7,20]. In addition, increased GLUT4 mRNA by exercise training was also shown in rat skeletal muscle homogenates[18,21]. In regard to the effects of exercise on aging-related insulin resistance, Cartee et al.[3] showed that substantial increase in the sensitivity of their muscle glucose transport system to insulin was observed after exercise regardless of age in rats . Although we did not make experiments on glucose transporter system and enzymatic activities in target tissues, our *in vivo* data indicated that the continuation of physical exercise before maturity might maintain the similar activity levels of glucose uptake in peripheral tissues to those in young rats.

In conclusion, our results suggested that (1) aging process would decrease insulin action in peripheral tissues predominantly because of post insulin receptor binding defect, and that (2) physical exercise started before maturity might prevent or delay aging-related insulin resistance. Further studies should be investigated, however, in order to reveal what kind of mechanism causes the delay of the occurrence of insulin resistance.

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