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Reduced Exercise Endurance in Interleukin-6-Deficient Mice

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IL-6 is produced and released in large amounts from skeletal muscle during prolonged exercise in both mice and humans, but there are few data indicating the biological significance of this. IL-6 exerts metabolic effects such as stimulating energy expenditure and reducing body fat mass. We have now investigated the effects of IL-6 deficiency on exercise endurance and energy expenditure in preobese and obese IL-6-deficient (IL-6$^{-/-}$) mice. Four-month-old preobese and 7-month-old obese IL-6$^{-/-}$ male mice backcrossed to C57BL/6 and their littermate controls were exercised on a treadmill, and energy expenditure was measured as oxygen consumption with the use of indirect calorimetry. The preobese IL-6$^{-/-}$ mice were significantly leaner than the control mice, whereas the older IL-6$^{-/-}$ mice, as expected, had developed obesity. Resting young, but not older, IL-6$^{-/-}$ mice had an elevated respiratory exchange ratio (RER), indicating that they oxidize carbohydrates rather than fat for energy utilization. During exercise, the young and older IL-6$^{-/-}$ mice had a reduced endurance and a progressive decrease in oxygen consumption compared with control mice. There was no difference in RER in young IL-6$^{-/-}$ mice, whereas RER was enhanced in older IL-6$^{-/-}$, mice during exercise. In summary, IL-6$^{-/-}$ mice have reduced endurance and energy expenditure during exercise, suggesting that IL-6 is necessary for normal exercise capacity. (*Endocrinology* 145: 2680–2686, 2004)

THE CYTOKINE IL-6 is well known for its effects on the immune system and is released from immune cells during inflammation (1). However, IL-6 is also released from nonimmune tissues. In the absence of inflammation, about 10–35% of the circulating IL-6 may be derived from adipose tissue, and body mass index correlates with serum IL-6 levels (2–4). Both short- and long-term decreases in food intake result in decreased serum levels of IL-6 (5, 6). Moreover, Pedersen and co-workers as well as other authors (7–9) have shown that circulating levels of IL-6 in humans increase by up to 100-fold during prolonged muscular exercise to levels even considerably higher than those in severely obese, sedentary individuals (3, 4). In contrast, the release of proinflammatory cytokines, such as TNFα and IL-1β, are much lower or absent during exercise (8, 10, 11). On the other hand, other factors such as norepinephrine and glucocorticoids are released during exercise. Therefore, IL-6 is acting in a completely different context during exercise compared with both during inflammation and after injection of exogenous IL-6 to sedentary individuals. The high plasma levels of IL-6 observed during exercise are mainly due to an increased production of IL-6 in the working skeletal muscle, as shown by measurements of arterial-femoral venous differences in the exercising leg (11). This increased production is not a result of muscle cell damage or infiltration of immune cells, but instead seems to be a physiological response to exercise (11). Increased IL-6 concentrations in plasma and increased IL-6 expression in working muscle have also been shown in exercising mice and rats (12, 13). However, the physiological significance of exercise-induced increases in IL-6 remains unclear.

The above-described localization and regulation of IL-6 production are in line with a metabolic role for this cytokine. Indeed, short-term IL-6 treatment has been reported to increase lipolysis and fat oxidation in humans (14–16). Moreover, we have recently shown that IL-6-deficient (IL-6$^{-/-}$) mice develop obesity, which could partly be reversed by IL-6 replacement, suggesting a role for IL-6 in long-term regulation of adipose tissue mass (17). In addition, intracerebroventricular injections of a low dose of IL-6 decreased body fat mass and increased energy expenditure in rats, suggesting a central site of action of IL-6 (17, 18). The IL-6$^{-/-}$ mice had decreased glucose tolerance, probably secondary to the obesity (17). In humans, there is also an association between IL-6 in the cerebrospinal fluid and body fat mass (19), and IL-6 seems to affect glucose metabolism, although it is currently unclear how peripheral treatment with IL-6 affects blood glucose levels (14, 15, 20, 21).

As IL-6 is released during exercise, a state clearly associated with pronounced metabolic alteration (22, 23), it is possible that IL-6 is a novel player. Although we and others have hypothesized that IL-6 might exert metabolic effects (11, 16, 24), the physiological function of its production and release from working muscle is at present unknown. Thus, the aim of this study was to investigate the effects of endogenous IL-6 on endurance and energy metabolism during exercise by exploiting an IL-6$^{-/-}$ mouse strain that has been found not to release measurable levels of immunoreactive and bioac-

Abbreviations: DXA, Dual energy x-ray absorptiometry; RER, respiratory exchange ratio; RMR, resting metabolic rate; wt, wild-type.

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tive IL-6 into the blood circulation (17, 25, 26). As these animals develop mature-onset obesity, the exercise studies were mainly performed in young preobese IL-$6^{-/-}$ animals to avoid influence by the fat tissue load. We also investigated body composition and resting metabolism in the preobese animals to further evaluate factors that might predispose for the later development of obesity.

Materials and Methods

Animals

The IL-$6^{-/-}$ mice were originally generated by Kopf *et al.* (25) and have been bred onto a C57BL/6 background, as previously described (17), to reduce genetic heterogeneity. Backcrossed littermate wild-type (wt) mice were used as controls in all experiments. The young mice were 3–4 months old, and the older mice were 7–8 months old. Animals were maintained under standardized nonbarrier conditions and had free access to fresh water and food pellets (B&K Universal AB, Sollentuna, Sweden). All animal procedures were approved by the local ethics committee on animal care at Gothenburg University and were conducted in accordance with guidelines.

Dual energy x-ray absorptiometry (DXA)

DXA measurements in mice were performed with pDEXA Sabre (Norland, Fort Atkinson, WI) and Sabre Research software (version 3.9.2) as previously described (27). The total amount of body fat in the mice was calculated from the percent fat measured with DXA and body weight. This was performed by using the relation between percent extracted body fat and percent fat on DXA that previously has been determined in our laboratory (27). Fat-free mass was calculated in a similar way using the relation between percent fat-free mass (calculated as the difference between total body weight and extracted body fat in relation to total body weight) and percent fat-free mass measured with DXA. This calculation was performed using data from the previously described mice (27).

Exercise protocol

Mice were exercised on a motorized treadmill (Columbus Instruments, Columbus, OH) that had an adjustable belt speed (0–100 m/min) and adjustable inclination (−10 to 25°). The treadmill was connected to an Oxymax system (Columbus Instruments, Columbus, OH) for measurement of energy expenditure by indirect calorimetry. The mice were encouraged to run by gentle tapping on their back. Before the experiments, all mice were acclimatized to the treadmill during a 3-d period with 5 min of rest and 5 min of running at 10 m/min and 0° inclination each day. Two different exercise protocols were used as described below.

Endurance capacity. This test involved an incremental protocol with increasing workloads and is often used to test for cardiovascular disease. The mice were placed in individual treadmill lanes at room temperature (23 C). The test started with a 20° incline (4-month-old mice) or a 10° incline (8-month-old mice) and 10 m/min belt speed. The speed was increased to 14 m/min after 10 min and to 18 m/min after another 5 min. The mice continued to run at 18 m/min until exhaustion. We defined exhaustion as the inability to continue regular treadmill running despite the stimulus of repeated tapping on the back of the mouse. The range of total duration of exercise was 22–35 min in young animals and 17–30 min in older animals.

Energy expenditure during exercise. The mice ran at a fixed speed of 10 m/min with an inclination of 20°. Energy expenditure was defined as oxygen consumption measured by indirect calorimetry, using the Oxymax system. Before commencing to run, oxygen consumption was measured while the mice rested quietly for 1–2 h in the treadmill to reach stable oxygen consumption levels. There are a number of different ways of expressing oxygen consumption, which includes milliliters per minute per mouse, milliliters per minute per body weight$^{0.75}$, or milliliters per minute per fat-free mass$^{0.75}$. When oxygen consumption is expressed as milliliters per minute per body weight$^{0.75}$, the results may differ between animals because the values are influenced by fat mass, *i.e.* a metabolically rather inactive tissue (28). To express oxygen consumption as milliliters per minute per fat-free mass$^{0.75}$, fat-free mass must first be determined. However, the data are similar when compared with values obtained using milliliters per minute per mouse, and the determination of fat-free mass using DXA must be performed while the mouse is anesthetized and not exercising. Hence, in the current study we chose to express oxygen consumption as milliliters per minute per mouse.

Blood chemistry and glycogen measurements

Blood samples (35 μl) were collected from the tail vein of young mice after 60 min of running at a 20° inclination, for analysis of glucose and lactate (ABL 700 series, Radiometer Medical, Copenhagen, Denmark). The mice were then anesthetized by ip injections of 7.5 g/kg Ketalar (Pfizer AB, Täby, Sweden) and 0.1 g/kg Dormitor (Orion Pharma, Espoo, Finland) and blood, liver, and muscularis quadriceps were collected. The tissues were frozen in liquid nitrogen and then transferred to −80 C until use, while the blood were allowed to clot for two hours at room temperature before centrifugation. Sera were aliquoted and stored at −80 C until analysis.

Glycogen content in muscle and liver were measured by acidic hydrolysis of approximately 35 μg white muscularis quadriceps and approximately 5 μg liver tissue in 1 ml 1 M HCl at 100 C for 2 h. After neutralization with 1 M NaOH, samples were analyzed for glycogen-derived glucose by an enzymatic-colorimetric glucose assay kit (Glucose Hexokinase Liquid Stable Reagent, Thermo, Nobel Park, Victoria, Australia) and glucose standard solution (Sigma-Aldrich Corp., St. Louis, MO). The glycogen concentration is expressed as micromoles of glucose per gram of wet tissue.

Sera were analyzed for insulin (Crystal Chemical, Inc., Harris County, TX) and β-hydroxybuturate. Insulin analysis was performed according to the protocol provided by the manufacturer, whereas β-hydroxybuturate was analyzed enzymatically at Sahlgrenska University Hospital.

Resting metabolic rate (RMR)

The RMR, *i.e.* energy expenditure during rest, thermoneutrality, and in the absence of feeding, was measured as oxygen consumption at 30 C using the Oxymax system. The mice were placed in individual metabolic chambers, and oxygen consumption was measured every third minute over a 2-h period.

Respiratory exchange ratio (RER)

The RER is the ratio of carbon dioxide output (VCO_2) to oxygen uptake (VO_2), that is: RER = VCO_2/VO_2. The RER was calculated both during rest at 30 C and during exercise at room temperature. The value for RER will differ depending on the metabolic state; that is, when carbohydrates are being used exclusively for metabolism, RER rises to 1.00, whereas RER will fall to around 0.7 when fats are being used exclusively for metabolism. This holds true if the values are taken during steady state conditions with animals fed diets similar to those used in this well controlled experiment.

Statistical methods

All analyses were performed using an SPSS program (version 11.5.1, SPSS, Inc., Chicago, IL). Data were, when necessary, transformed by Blom's method to obtain both normally distributed data and normally distributed residuals. A *t* test was used to investigate the differences of single measured variables between the two groups (wt and IL-$6^{-/-}$), whereas two-way ANOVA (repeated measurement) was used to compare variables that were measured repeatedly in the same animal. Statistical calculations were also performed with nonparametric methods (*e.g.* Mann-Whitney), as the sample size was rather small. However, the use of nonparametric methods did not have any major effect on the outcome. Therefore, only the *P* values obtained with parametric results will be presented.

In the endurance protocol, a Kaplan-Meier survival curve was obtained, and the comparison of groups was performed using the log-rank test. Data in text and figures are given as the mean ± SEM. $P \leq 0.05$ was considered statistically significant.

Results

Body composition

At 4 months of age, the IL-6$^{-/-}$ mice had lower body weight compared with wt mice (31.3 ± 1.0 *vs.* 36.6 ± 1.3 g; $P < 0.001$). This weight difference was caused by a smaller fat mass in the IL-6$^{-/-}$ mice shown by *in vivo* measurements using DXA (Fig. 1, A and B). The relative fat mass was also reduced in IL-6$^{-/-}$ mice compared with wt (12.6 ± 1.2% *vs.* 19.9 ± 1.6%; $P < 0.01$). As in our previous study (17), the 8-month-old IL-6$^{-/-}$ mice had developed mature-onset obesity, as shown as a substantially increased relative (35.5 ± 2.6% *vs.* 25.6 ± 1.5%) and total (Fig. 1, C and D) fat mass. There was no effect of IL-6 deficiency on fat-free weight in either young or older IL-6$^{-/-}$ mice (Fig. 1, B and D).

Energy expenditure and RER during rest and thermoneutrality

There was no difference in the RMR between young IL-6$^{-/-}$ mice and wt mice (Fig. 2A). The mean RER during a period of 120 min at 30 C was higher in young IL-6$^{-/-}$ mice than in wt mice (0.83 ± 0.03 *vs.* 0.77 ± 0.02; $P < 0.05$), and the RER was also higher in IL-6$^{-/-}$ mice at several time points (Fig. 2B). In older obese animals, there were no differences in RMR or RER between the IL-6$^{-/-}$ and wt mice (data not shown).

Endurance during treadmill running

The young preobese IL-6$^{-/-}$ mice had significantly reduced endurance compared with wt mice ($P < 0.01$, by log-rank test; Fig. 3). All of the IL-6$^{-/-}$ mice stopped running after 35 min, whereas 60% of the wt mice were still running. A similar pattern with decreased endurance was seen in the 8-month-old obese IL-6$^{-/-}$ mice (mean running time: 23.7 ± 2.2 *vs.* 28.0 ± 1.4 min; $P = 0.08$).

Energy expenditure and RER during treadmill running

With the mice resting quietly on the treadmill at room temperature, there was no difference in energy expenditure (shown as oxygen consumption) between young preobese IL-6$^{-/-}$ and wt mice during the last hour of a 120-min rest period before running (Fig. 4). Moreover, there was no significant difference during the first hour of

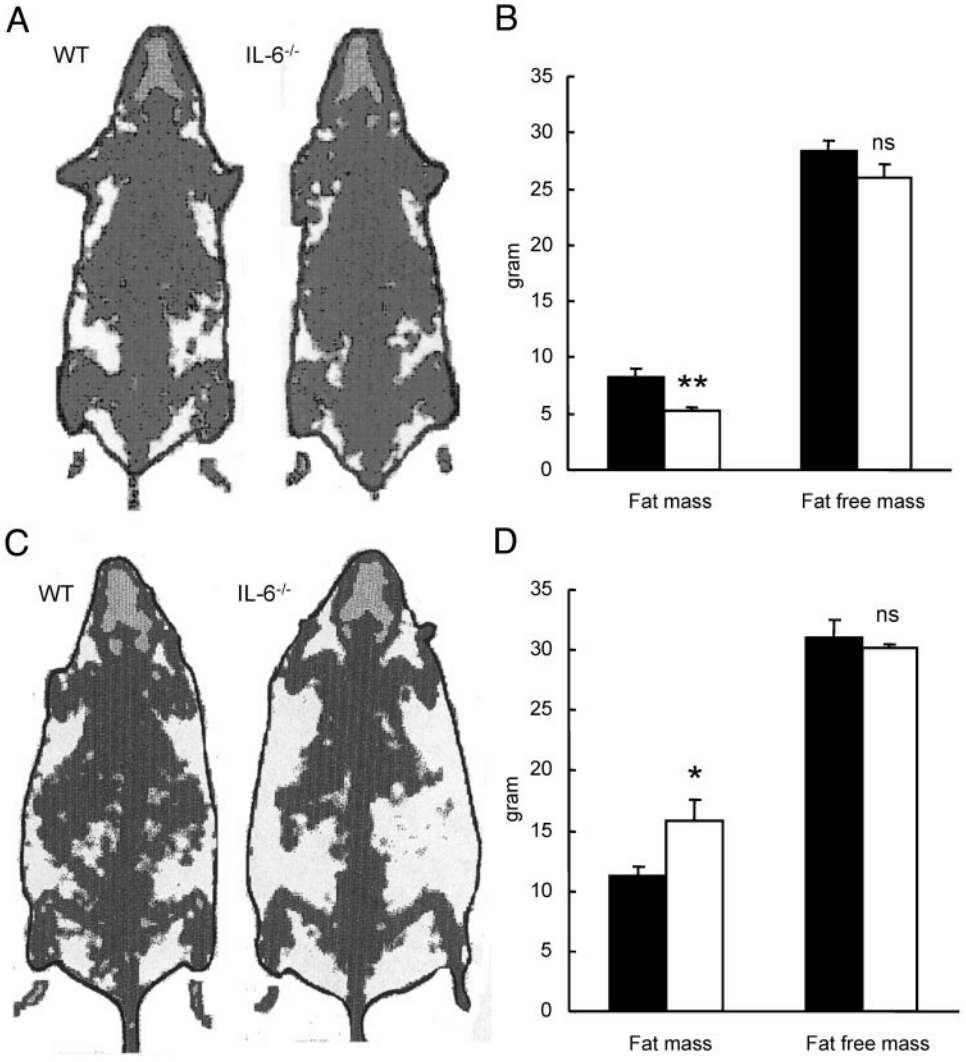

FIG. 1. Total body fat and fat-free mass, as measured using DXA, in 4-month-old, preobese (A and B) and 8-month-old obese (C and D) IL-6$^{-/-}$ and wt mice. A and C, DXA/Image analysis of fat content in representative IL-6$^{-/-}$ and wt mice. □, Areas with greater than 50% fat; ■, areas with less than 50% fat. B and D, Quantification of several DXA measurements for 4- and 8-month-old IL-6$^{-/-}$ (□) and wt mice (■). n = 6. ns, Not significant; *, $P < 0.05$; **, $P < 0.01$ (*vs.* corresponding wt mice, using *t* test).

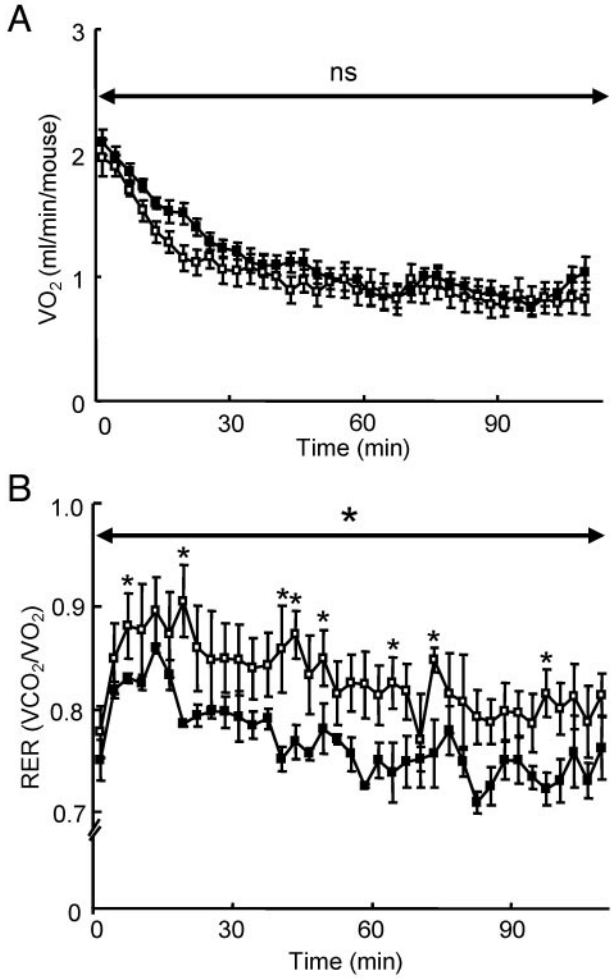

FIG. 2. Energy expenditure and RER during rest and thermoneutrality in IL-$6^{-/-}$ (□) and wt (■) mice. A, Basal metabolic rate (BMR), shown as oxygen consumption at 30 C. B, RER during rest at 30 C. Data were transformed by Blom's method to obtain both normally distributed data and normally distributed residual. Two-way ANOVA was used for comparison of RERs in the two groups during the entire resting period, whereas *t* test was used for comparison of individual time points. n = 6–8. *, $P < 0.05$.

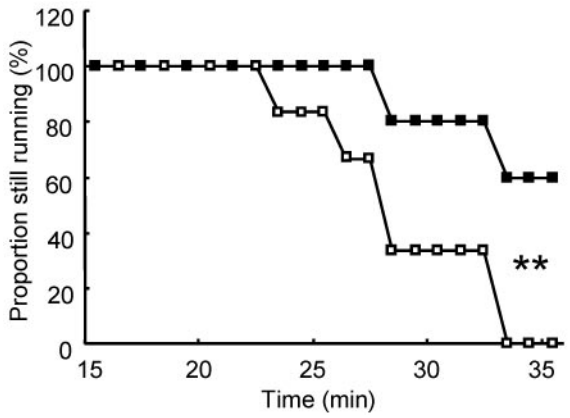

FIG. 3. Endurance capacity in 4-month-old preobese IL-$6^{-/-}$ (□) and wt (■) mice. The mice started on the treadmill at an inclination of 20° and a speed of 10 m/min, with incremental increases in speed until exhaustion of the mice. The results are shown as a Kaplan-Meier survival curve, and the comparison of the groups was performed using the log-rank test. n = 5–6. **, $P < 0.01$.

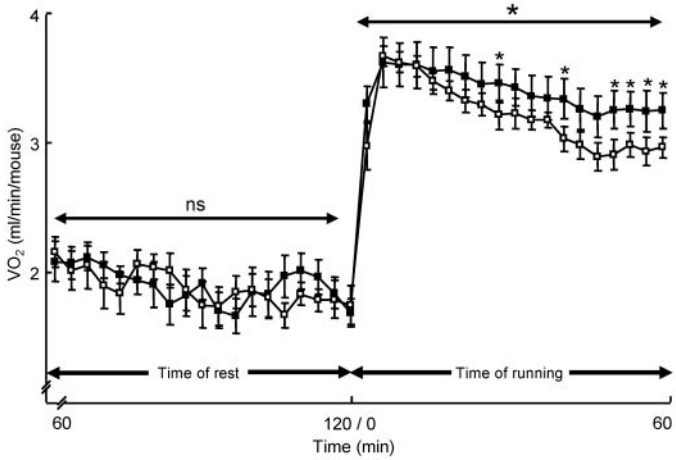

FIG. 4. Energy expenditure (shown as oxygen consumption) in 4-month-old preobese IL-$6^{-/-}$ (□) and wt (■) mice. The mice ran at a fixed speed of 10 m/min and an inclination of 20°. Before commencing running, the mice rested for 2 h in the treadmill, and oxygen consumption was measured. Data were transformed by Blom's method to obtain both normally distributed data and normally distributed residual. Two-way ANOVA was used for comparison of oxygen consumption in the two groups during the entire exercise period, and *t* test was used for comparison of individual time points. n = 9–12. ns, Not significant; *, $P < 0.05$.

rest, although the variability was greater (not shown). During the 60-min period of exercise, oxygen consumption was significantly reduced in the young preobese IL-$6^{-/-}$ mice compared with wt mice ($P < 0.05$). There was no difference in oxygen consumption during the first 12 min of exercise (3.33 ± 0.08 ml/min in IL-$6^{-/-}$ *vs.* 3.54 ± 0.16 ml/min in controls; $P > 0.05$), but the curves started to deviate after 12 min of running. Comparison of the time points after 15–60 min of running showed that the oxygen consumption was reduced in IL-$6^{-/-}$ mice. Moreover, the oxygen consumption of IL-$6^{-/-}$ mice was significantly reduced at many individual time points between 25 and 60 min of running (Fig. 4). A similar pattern with gradually reduced oxygen consumption in the IL-$6^{-/-}$ mice compared with the control mice was seen in the 8-month-old obese mice (data not shown).

During exercise, there was no difference in RER between preobese IL-$6^{-/-}$ mice and wt mice (Fig. 5A), whereas older obese IL-$6^{-/-}$ mice had a higher RER value than wt mice (Fig. 5B).

Blood chemistry and glycogen content

At rest, the 8-month-old obese IL-$6^{-/-}$ mice had, as we have seen previously (17), increased basal glucose levels, whereas there was no difference between the young animals (data not shown). Moreover, after 1 h of running at a fixed speed of 10 m/min and an inclination of 20°, there were no differences in the concentration of blood-glucose, blood-lactate, serum insulin, serum β-hydroxybuturate, muscle glycogen, or liver glycogen between young wt and IL-$6^{-/-}$ mice (Table 1).

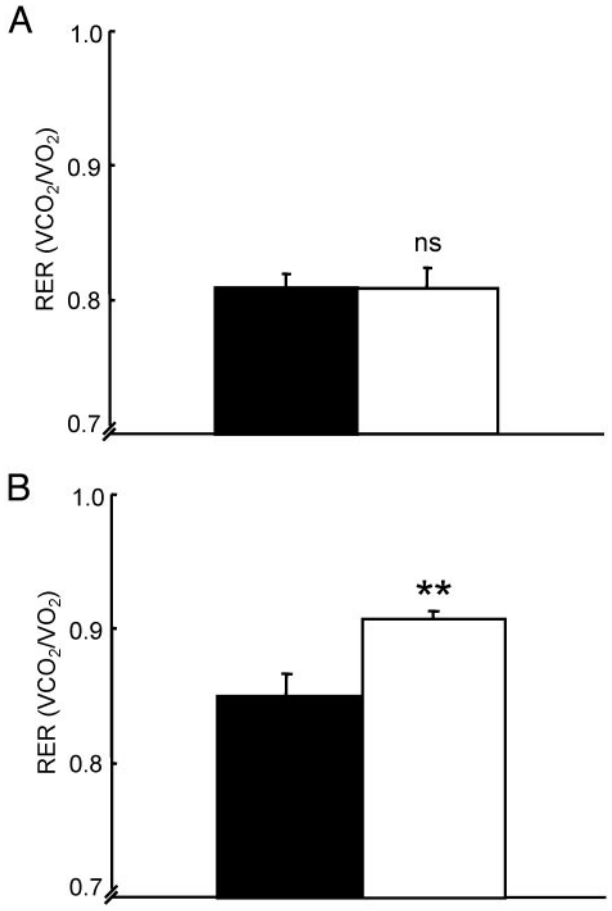

FIG. 5. Effects of exercise on RER in 4-month-old preobese (A) and 8-month-old obese (B) IL-$6^{-/-}$ (□) and wt (■) mice. The mice ran at a fixed speed of 10 m/min and an inclination of 20°. The figure shows the mean RER measured at peak oxygen consumption. n = 9–12. ns, Not significant; **, $P < 0.01$ (*vs.* corresponding control, by *t* test).

Discussion

We have shown for the first time that IL-$6^{-/-}$ mice have decreased endurance and energy expenditure during exercise. These findings suggest that endogenous IL-6 exerts a profound stimulatory effect on exercise capacity in mice. Several human studies have shown a marked increase in circulating IL-6 during prolonged exercise, mainly due to increased local production in the working skeletal muscle (7, 8, 11). In rodents there is also an increase in IL-6 expression in working muscle and plasma IL-6 concentration during exercise (12, 13). Although it has been proposed that increased IL-6 production during exercise exerts metabolic functions (7, 8, 11, 24), until now it has not been proven that IL-6 is indeed necessary for normal exercise capacity.

The mechanisms behind the reduced exercise endurance in IL-$6^{-/-}$ mice are not known, but one possibility is that the reduced oxygen consumption during exercise causes a progressive oxygen depletion in these animals, which impairs their ability to continue running (29–31). Another possible mechanism producing the reduced endurance capacity could be impaired heart function, as reduced heart muscle force may lead to an insufficient cardiac output for the increased requirement necessary during exercise. This is suggested by the findings that IL-6 stimulates the sympathetic nervous system (32), and this may exert an inotropic effect on heart function. Another possibility is reduced capillarization in the skeletal muscle, which would limit the transport of oxygen within the muscle and thereby impair the capacity to oxidize the fuel (33, 34). However, further studies are needed to elucidate the exact mechanism behind the decreased endurance in IL-$6^{-/-}$ mice.

TABLE 1. Blood chemistry and glycogen concentration in liver and muscle

Variable	Wt	IL-$6^{-/-}$
Glucose (mmol/liter)	11.6 ± 0.46	12.6 ± 2.60
Lactate (mmol/liter)	7.32 ± 0.76	6.37 ± 0.87
Insulin (pg/ml)	511 ± 56.5	584 ± 85.0
β-Hydroxy-buturate (mmol/liter)	0.20 ± 0.03	0.20 ± 0.03
Glycogen, muscle (μmol/g)	5.02 ± 0.56	5.50 ± 0.59
Glycogen, liver (μmol/g)	332 ± 47.6	342 ± 74.2

Data are the means ± SEM. Samples of blood, liver, and muscle (muscularis quadriceps) were obtained from young wt and IL-$6^{-/-}$ mice after 1 h of running at a fixed speed of 10 m/min and an inclination of 20°.

Our results in IL-$6^{-/-}$ mice suggest that endogenous IL-6 is of importance to keep persistently high oxygen consumption and thereby the ability to maintain skeletal muscle work during prolonged exercise. This finding is in accordance with earlier studies demonstrating that IL-6 treatment enhances energy expenditure in both rodents and humans (15, 17, 35, 36). At present it is not known in which organ endogenous IL-6, which promotes oxygen consumption, is produced and exerts its effects. Previously it has been shown that IL-6 treatment stimulates energy expenditure at the level of the brain in rodents (17, 36, 37), and it might be assumed that endogenous IL-6 also acts on the brain during exercise. The IL-6 exerting this effect during exercise could be produced by the brain itself, which has been shown to have increased IL-6 production during exercise (38). Alternatively, the large quantities of endocrine IL-6 produced from working skeletal muscle (10) might reach appropriate sites in the brain. It has also been suggested that the increased IL-6 level is a signal from working muscle to directly or indirectly release nutrients from storage organs, for instance, adipose tissue and liver (7, 8, 11).

In the present study we observed a higher RER in young preobese IL-$6^{-/-}$ mice at rest. In relation to the amounts of carbon dioxide produced, oxidation of fat requires more oxygen than oxidation of carbohydrates (RER ~0.7 for fat compared with ~1.00 for carbohydrates). Therefore, the elevated RER in the IL-$6^{-/-}$ mice indicates that they oxidize carbohydrate rather than fat. Interestingly, increased RER has been found to be a predictor for obesity in human populations (39, 40). It has been suggested that high RER might be a contributor to the development of obesity, as individuals with high RER oxidize carbohydrates and "save" the fat (39). In addition, high RER has been associated with both low sympathetic nerve activity (41) and the obesity-inducing effect of ghrelin treatment (42). However, the exact role of the presently observed increase in RER in preobese IL-$6^{-/-}$ mice needs to be elucidated further.

The older obese, but not the young, IL-$6^{-/-}$ mice exhibited higher RER compared with wt mice during exercise. High

RER during exercise has been shown to be a marker of reduced exercise capacity (29, 43), possibly because a limited amount of available oxygen results in reduced fat oxidation capacity. Therefore, the decrease in exercise endurance in older IL-$6^{-/-}$ mice is probably not only secondary to their mature-onset obesity and fat load, as also indicated by the fact that endurance was also reduced in young preobese IL-$6^{-/-}$ mice.

Careful analysis of young IL-$6^{-/-}$ mice showed, surprisingly, that they were actually leaner than wt controls. These results are in line with earlier findings that serum leptin levels are lower in young IL-$6^{-/-}$ mice (17). The reason for this remains unknown, although, the low body weight of young individuals during some circumstances has been associated with obesity later in life (44). The fat mass was very low in both young IL-$6^{-/-}$ mice and young control mice and probably had no major impact on exercise performance in those animals. The lean body mass was not affected by IL-6 deficiency in young or older animals, providing no support for the idea that muscle mass was reduced in IL-$6^{-/-}$ mice. Moreover, in line with results published by others (45), we have not observed any effect of IL-6 deficiency or IL-6 treatment on muscle weight (unpublished results).

In summary, we have found that the lack of endogenous IL-6 results in decreased exercise endurance and a less sustained increase in energy expenditure during exercise. These results clearly indicate that the marked increase in IL-6 production seen in animals and humans during exercise, *e.g.* by skeletal muscle, is crucial for exercise capacity. The mechanisms remain unclear, but are the subject of future studies.

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J.F. and I.W. contributed equally to this study.

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