

Enhanced exercise-induced muscle damage and muscle protein degradation in streptozotocin-induced type 2 diabetic rats

Hirohide Yokokawa¹, Ikiko Kinoshita², Takeo Hashiguchi², Masako Kako³, Kahoru Sasaki³, Akira Tamura⁴, Yuri Kintaka⁵, Yoko Suzuki³, Noriko Ishizuka³, Katsumi Arai³, Yoshiko Kasahara³, Mikiko Kishi³, Yoko Kobayashi³, Tosei Takahashi³, Hiroyuki Shimizu³, Shuji Inoue^{3*}

ABSTRACT

Aims/Introduction: The effects of 5-day voluntary exercise on muscle damage and muscle protein degradation were investigated in a streptozotocin-induced rat model of moderately glycemic, uncontrolled, type 2 diabetes.

Materials and Methods: In the preliminary experiment, an oral glucose tolerance (1.0 g/kg) test was carried out to confirm the development of diabetes 3 days after streptozotocin treatment (30 mg/kg). In the genuine experiment, rats were divided into four groups: (i) non-diabetic rats without exercise (controls); (ii) non-diabetic rats with exercise; (iii) diabetic rats without exercise; and (iv) diabetic rats with exercise. After 5 days of voluntary wheel running exercise, blood and 24-h urine were collected, and levels of serum creatine kinase, a marker of muscle damage, and 24-h urinary excretion of muscle degradation products were determined.

Results: Type 2 diabetic rats with insulin deficiency that exercised had higher serum creatine kinase and greater urinary excretions of creatinine, urea nitrogen and 3-methylhistidine compared with both type 2 diabetic rats with insulin deficiency and non-diabetic rats that did not exercise. However, there were no differences in serum creatine kinase and urinary excretions of creatinine, urea nitrogen and 3-methylhistidine between non-diabetic rats that did and did not exercise.

Conclusions: These findings suggest that muscle damage and muscle protein degradation are enhanced by chronic moderate exercise in moderately glycemic uncontrolled type 2 diabetic rats with insulin deficiency at an intensity level of exercise that does not affect muscle damage and muscle protein degradation in non-diabetic rats. (*J Diabetes Invest*, doi: 10.1111/j.2040-1124.2011.00130.x, 2011)

KEY WORDS: Type 2 diabetes mellitus, Muscle damage, Exercise

INTRODUCTION

The International Diabetes Federation (IDF)¹ predicts that the prevalence of people with diabetes mellitus will increase from 5.1% (194 million people worldwide) in 2003 to 6.3% (333 million people worldwide) by 2025. In this context, the United Nations passed a landmark resolution in December 2006 on diabetes mellitus, recognizing the disease as a chronic, debilitating and costly social burden². Multidimensional efforts to fight diabetes mellitus are taking place worldwide. In clinical practice for type 2 diabetes, exercise is one of three major treatments, along with diet and pharmacological treatment^{3–6}. Skeletal muscle mass plays an important role in glycemic control in type 2 diabetes,

especially through exercise. Acute (non-aerobic) exercise improves the glycemic level by increasing skeletal muscle glucose uptake through muscle contraction, whereas chronic resistance and/or aerobic exercise improves glycemic control by enhancing insulin sensitivity^{7,8}. Regular physical activity also increases cardiorespiratory fitness, maintains muscle mass, reduces the risk of cardiovascular mortality and enhances psychosocial well-being⁹.

Skeletal muscle is also important for maintaining normal body composition, because it comprises nearly 40% of body-weight, constitutes between 50 and 75% of all body proteins¹⁰ and is required for movement¹¹. Protein turnover in skeletal muscle normally occurs at a rate of 1–2% of protein synthesis and protein degradation daily¹², and skeletal muscle mass is maintained through a regulated balance between muscle protein synthesis (MPS) and muscle protein degradation (MPD)^{11,12}. Regular resistance exercise results in increased MPS in the post-exercise recovery period¹³. However, chronic intense unaccustomed exercise or endurance exercise might induce muscle damage by enhancing MPD through excessive muscle contraction, absolute or relative insulin deficiency, and inflammation^{14–16}.

¹Department of General Medicine, Juntendo University School of Medicine,

²Department of Nutrition, Faculty of Home Economics, Kyoritsu Women's University, Tokyo, ³Department of Nursing and Department of Nutrition, Faculty of Health Care, Kiryu University, Midori, ⁴Department of Nutrition, Faculty of Health Science, Chukyo Women's University, Nagoya, and ⁵Department of Food Science, Faculty of Dairy Science, Rakuno Gakuen University, Ebetsu, Japan

*Corresponding author. Shuji Inoue Tel: +81-277-48-9121 Fax: +81-277-48-9122 E-mail address: ishuj@kiryu-u.ac.jp

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Type 2 diabetes is characterized by a deficiency in insulin level or action and by inflammation^{16–18}. Therefore, patients with type 2 diabetes might be susceptible to exercise-induced muscle damage by enhanced MPD.

The treadmill or wheel exercise is commonly applied to test exercise tolerance in small animals^{19–22}. Wheel exercise involves voluntary running, but there is some difficulty in recording the details of the running behavior (intensity, duration or distance); however, several studies have used voluntary wheel running in rats^{20,21}. Rodnick *et al.*²⁰ characterized intermittent voluntary wheel running run by rats at fairly high speeds (40–50 m/min) as “sprint training”. Seburn and Gardiner²¹ estimated that the distance covered in wheel exercise was two to three times longer than that in treadmill exercise. Therefore, we applied voluntary wheel running in the current study as a moderate intensity exercise that might be sufficient enough to discriminate the effects of exercise in diabetic rats from that in non-diabetic rats.

The importance of muscle mass in exercise for glycemic control in type 2 diabetes has been widely reported^{3–6}. However, to the best of our knowledge, few studies have examined muscle protein damage and MPD by exercise in animals and humans with type 2 diabetes. In the current study, we investigated exercise-induced muscle damage and MPD in streptozotocin (STZ)-induced moderately glycemic uncontrolled type 2 diabetic rats placed in wheel-equipped cages by measuring serum creatine kinase (CK), a marker of muscle damage²³, and urinary excretions of creatinine (Cr), urea nitrogen (UN) and 3-methylhistidine (3-MH), which are muscle protein degradation products^{24–26}.

MATERIALS AND METHODS

Animals

Ten-week-old male Wistar rats were housed at 25°C and 50% humidity with a 12-h light/dark cycle (06.00 hours light on, 18.00 hours light off). They were allowed free access to food (carbohydrate 81.3%, protein 12.7%, fat 6.0%; Oriental East, Tokyo, Japan) and water. In the preliminary experiment, rats were given STZ (30 mg/kg; Sigma, St. Louis, MO, USA) or saline through the tail vein under ethanol anesthesia. On the third day after STZ injection, saline-treated normal (non-diabetic) and STZ-treated rats received an oral glucose tolerance test (OGTT; 1.0 g/kg) without anesthesia after an overnight fast. Diabetes was judged to be present when the blood glucose concentration at 120 min was >200 mg/dL²⁷. Rats found to be diabetic were then used as diabetic rats in the study. All animals were treated in accordance with the Kiryu University Guidelines for the Care and Use of Laboratory Animals, which are based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental Procedures

Normal (non-diabetic) and diabetic rats were divided into four groups: (i) non-diabetic rats without exercise (designated as controls); (ii) non-diabetic rats with exercise; (iii) diabetic rats

without exercise; and (iv) diabetic rats with exercise ($n = 5$ in each group). Exercise was carried out throughout for five consecutive days by placing rats in wheel-equipped cages. On the sixth day, rats were transferred into individual metabolic cages and 24-h urine samples were collected. Blood samples were taken in fed conditions from the abdominal aorta under pentobarbital anesthesia at the end of the experiment.

Wheel Running Exercise

The rats in the exercise groups were placed in wheel-equipped cages for five consecutive days. Each cage had an in-built stainless steel wheel (21 × 46 cm, circumference 1.445 m) mounted on a shaft containing a ball-bearing collar with a 4-mm wide running surface of stainless steel mesh²⁰. Rats intermittently played with the wheel voluntarily, arbitrarily and willingly during placement in wheel-equipped cages.

Measurements

Blood glucose was measured using the oxidase method and serum insulin was measured by ELISA (Rat Insulin ELISA kit; Cosmic Corporation, Tokyo, Japan). Serum CK was measured using a Hitachi 7450 automatic analyzer (Hitachi Medical System, Tokyo, Japan). Urine Cr and UN were measured using commercial kits (Eiken Chemical Co., Tokyo, Japan). 3-MH was separated from urine by high performance liquid chromatography using the method of Nagasawa *et al.*²⁸ and measured using a RF-535 spectrophotometer (Shimadzu, Kyoto, Japan) at 475 nm. Urinary Cr, UN and 3-MH excretion over 24 h was calculated from the urine concentration × 24-h urine volume.

Statistical Analysis

All data are presented as the mean ± SE. Significance was determined by one-way ANOVA with a Tukey's test or Student's *t*-test, as appropriate. A level of $P < 0.05$ was considered statistically significant.

RESULTS

In the preliminary OGTT, diabetic rats showed significantly higher blood glucose levels at 30, 60 and 120 min, and significantly lower serum insulin levels at 30 and 60 min compared with non-diabetic rats (Figure 1). This profile is similar to that of moderately glycemic uncontrolled type 2 diabetes.

Comparisons of parameters among the four groups in the genuine experiment at the end of the study are shown in Table 1. Bodyweight did not differ significantly among the four groups. Blood glucose levels in diabetic rats with and without exercise were significantly higher than those in both groups of non-diabetic rats. Blood glucose levels in diabetic rats with exercise tended to be higher than those in diabetic rats without exercise, but this difference did not reach statistical significance. Serum insulin levels in both groups of diabetic rats were significantly lower than those in both groups of non-diabetic rats, and serum insulin levels in diabetic rats with exercise were significantly lower than those in diabetic rats without exercise.

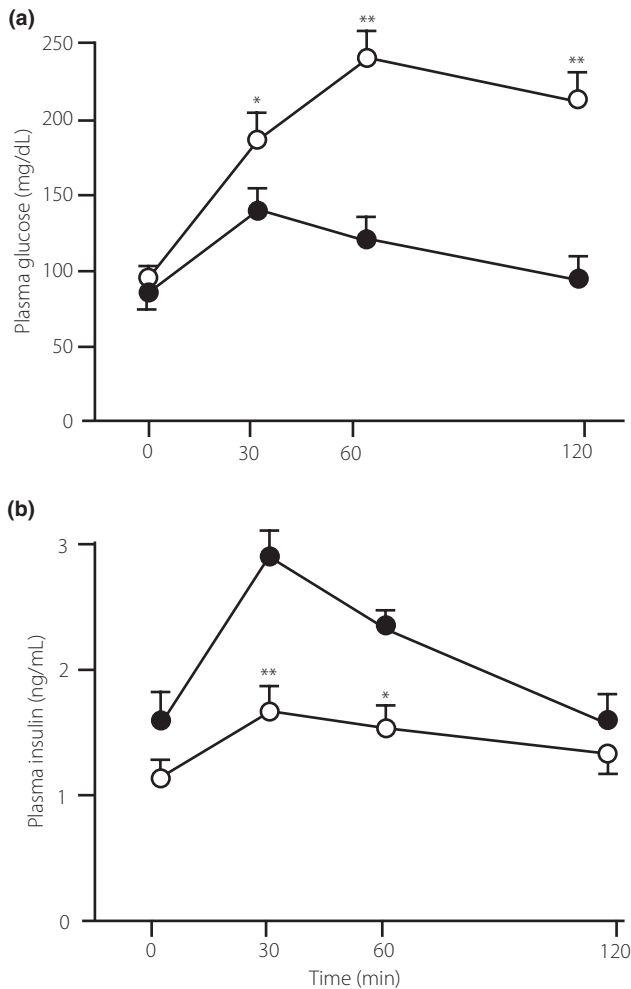


Figure 1 | (a) Blood glucose and (b) plasma insulin during an oral glucose tolerance test (1.0 g/kg) in control (non-diabetic) and streptozotocin-induced type 2 diabetic rats in the preliminary experiment. * $P < 0.05$, ** $P < 0.01$ vs non-diabetic rats (controls). (●) non-diabetic (control) group; (○) diabetic group.

Diabetic rats with exercise showed significantly higher serum CK levels than diabetic rats without exercise and both groups of non-diabetic rats. In contrast, serum CK did not differ significantly between non-diabetic rats with and without exercise.

The 24-h urinary excretions of Cr (Figure 2) in both groups of diabetic rats were significantly greater than those in both groups of non-diabetic rats (Figure 2). Cr excretion in diabetic rats with exercise was significantly greater than that in diabetic rats without exercise, but it did not differ significantly between non-diabetic rats with and without exercise. The 24-h urinary excretion of UN (Figure 3) in diabetic rats with exercise was significantly greater than that in diabetic rats without exercise and that in non-diabetic rats without exercise, but it did not differ significantly between non-diabetic rats with and without exercise. The 24-h urinary excretions of 3-MH (Figure 4) in both groups of diabetic rats were significantly greater than those in

Table 1 | Comparisons of bodyweight, serum glucose, serum insulin and serum creatine kinase

	Control (non-diabetic rats without exercise)	Non-diabetic rats with exercise	Diabetic rats without exercise	Diabetic rats with exercise
Bodyweight (g)	252 ± 3	253 ± 3	251 ± 4	250 ± 3
Serum glucose (mg/dL)	122 ± 17	144 ± 8	238 ± 41*†	268 ± 39*†
Serum insulin (ng/dL)	1.58 ± 0.7	1.08 ± 0.5	0.88 ± 0.3*†	0.33 ± 0.2**††§
Serum CK (IU/L)	533 ± 182	525 ± 315	595 ± 225	1213 ± 530*†§

CK, creatine kinase.

* $P < 0.05$, ** $P < 0.01$ vs controls, † $P < 0.05$, †† $P < 0.01$ vs non-diabetic rats with exercise, § $P < 0.05$ vs diabetic rats without exercise.

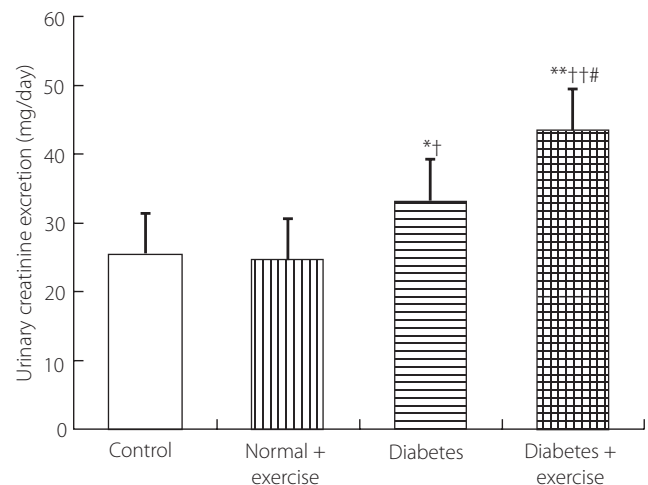


Figure 2 | The 24-h urinary excretion of creatinine in the genuine experiment. * $P < 0.05$, ** $P < 0.01$ vs non-diabetic rats without exercise (controls), † $P < 0.05$, †† $P < 0.01$ vs non-diabetic rats with exercise, # $P < 0.05$ vs diabetic rats without exercise.

both groups of non-diabetic rats. Diabetic rats with exercise had significantly greater 3-MH excretion than diabetic rats without exercise, but there was no significant difference between non-diabetic rats with and without exercise.

CONCLUSIONS

Streptozotocin selectively destroys pancreatic β -cells and is often used to produce models of type 1 diabetes. However, the extent of destruction of pancreatic β -cells by STZ treatment is dose-dependent^{29,30}. It is reported that high doses of STZ (40–60 mg/kg) produced type 1 diabetes, whereas low doses of STZ (35 mg/kg) produced type 2 diabetes^{31,32}. In the current study,

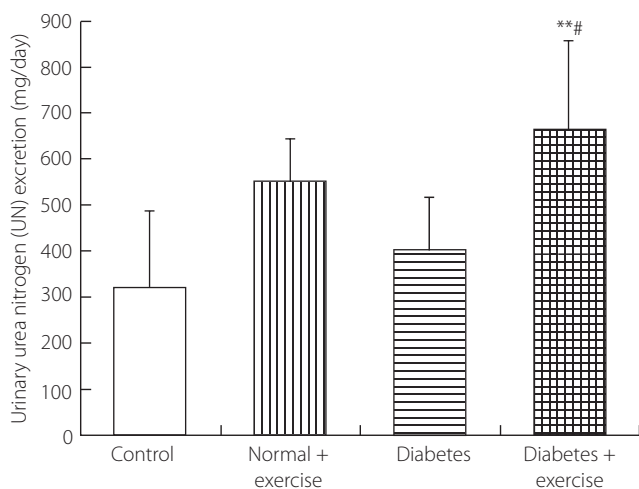


Figure 3 | The 24-h urinary excretion of urea nitrogen in the genuine experiment. $**P < 0.01$ vs non-diabetic rats without exercise (controls), $^{\#}P < 0.05$ vs diabetic rats without exercise.

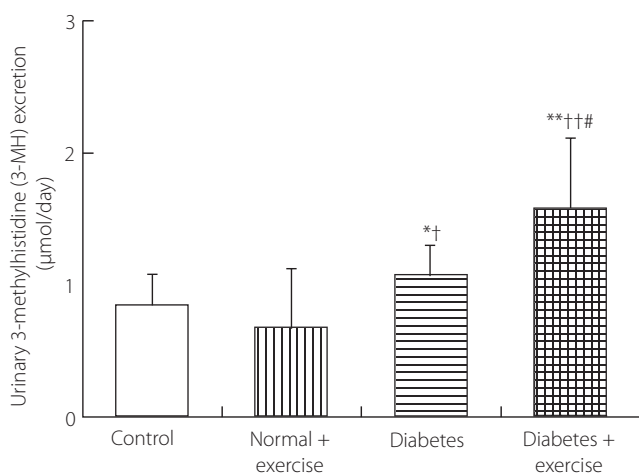


Figure 4 | The 24-h urinary excretion of 3-methylhistidine in the genuine experiment. $*P < 0.05$, $**P < 0.01$ vs non-diabetic rats without exercise (controls), $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs non-diabetic rats with exercise, $^{\#}P < 0.05$ vs diabetic rats without exercise.

we applied 30 mg/kg of STZ in male Wistar rats and produced moderately glycemic uncontrolled type 2 diabetes with insulin deficiency, which is a similar profile to that in Goto-Kakizaki (GK) diabetic rats³³ and that in spontaneously diabetic Torii (SDT) rats³⁴, which are animal models of non-obese type 2 diabetes with insulin deficiency. Using these rats, we found that serum CK, an enzyme marker of muscle damage, was elevated in diabetic rats after 5 days of voluntary exercise, compared with that in diabetic rats without exercise, whereas serum CK in non-diabetic rats with exercise was not elevated compared with that in non-diabetic rats without exercise, suggesting that this intensity level of exercise induced muscle damage in

diabetic rats, but did not induce muscle damage in non-diabetic rats.

Muscle tissues are damaged after intense prolonged exercise as a consequence of both mechanical (direct) and metabolic (indirect) factors²³. Exercise intensity might increase the membrane permeability of muscle cells, and CK and other enzymes in the muscle leak into the circulation, which then leads to leakage of intracellular muscle components by rhabdomyolysis^{23,35,36}. These components include muscle protein degradation (MPD) products, such as Cr, UN and 3-MH^{24–26}. It is likely that exercise-induced muscle damage in diabetic rats in the current study mainly occurs through MPD or muscle protein breakdown. Thus, we determined 24-h urinary excretions of Cr, UN and 3-MH, products of MPD, and found that these products were elevated in diabetic rats with exercise compared with those in diabetic rats without exercise. In contrast, changes in 24-h urinary Cr, UN and 3-MH excretion were not observed in non-diabetic rats with exercise compared with those in non-diabetic rats without exercise (controls). Serum Cr arises from damaged muscle²⁴, but is not always directly released into urine, as a creatine pool is present in the body³⁷. Serum UN formed from degradation of endogenous muscle proteins does not differ from serum UN derived from other organs and from ingested protein. Therefore, urinary excretion of UN transferred from serum only partly reflects MPD²⁵. Urinary 3-MH excretion is considered to be a reliable marker of muscle fiber protein degradation²⁶, but cannot be utilized as a marker of total MPD. Consequently, each marker has a limited capacity for identifying MPD; however, the consistent elevation of all three markers suggests that MPD was enhanced in moderately glycemic uncontrolled type 2 diabetic rats in the current study by 5-day voluntary moderate exercise at an intensity level that did not enhance MPD in non-diabetic rats.

Taken together, it might be reasonable to consider that muscle damage was induced mainly through enhanced MPD in moderately glycemic uncontrolled type 2 diabetic rats with insulin deficiency by moderate exercise at an intensity level that did not affect muscle integrity in non-diabetic rats.

The current study did not deal with the effect of exercise or muscle damage and MPD in uncontrolled type 2 diabetic rats with insulin resistance, and cannot explain the mechanisms of muscle damage and muscle protein degradation by exercise in uncontrolled type 2 diabetic rats with insulin resistance. In addition, we wonder whether a similar exercise effect on muscle damage and muscle protein degradation in diabetic rats in the current study might occur in type 2 diabetic patients with relative insulin secretory deficiency, whose profile consists of a considerably large number of Japanese, as well as Asian, type 2 diabetic patients³⁸. Further studies are needed to elucidate the effects on muscle damage and MPD in uncontrolled type 2 diabetic rats with insulin resistance or with relative insulin secretory deficiency.

In the current study, diabetic rats with exercise showed significant higher serum glucose and lower serum insulin than

diabetic rats without exercise. Because the diabetic rats in the current study were type 2 diabetic rats with insulin deficiency, we presume that these phenomena were caused by a decrease in serum insulin by exercise through increased sympathetic nerve activity³⁹.

Many studies have investigated the effects of exercise on type 2 diabetes, but the majority of reports have focused on the effects of exercise on blood glucose control^{3–6}. However, to date, there have been no reports that have addressed the effects of exercise on muscle damage and MPD in type 2 diabetic animals. In the current study, we unexpectedly found that uncontrolled type 2 diabetes was susceptible to exercise-induced muscle damage that probably occurred through enhanced MPD. The results suggest that moderate exercise might cause muscle damage and enhance MPD in moderately glycemic, uncontrolled type 2 diabetic patients. The results also suggest that the status of glycemic control should be considered carefully when implementing an exercise regimen in patients with type 2 diabetes in clinical practice.

This raises the question of the cause of induced muscle damage and enhanced MPD by exercise in uncontrolled type 2 diabetic rats with insulin deficiency. Insulin is an anabolic hormone^{40,41}. In an early study, Smith *et al.*⁴² reported that the total protein degradation rate was increased by 30% and myofibrillar catabolism was accelerated by 60% in STZ-induced diabetic rats, and that treatment with insulin within a physiological range completely reversed these changes. We suggest that reversal of induced muscle damage and enhanced MPD by insulin is mediated through the anabolic actions of insulin^{40,41}, directly, and through inhibition of increased hepatic gluconeogenesis, indirectly, because amino acids as substrates for gluconeogenesis are derived from whole body proteins, including muscle protein, in uncontrolled type 2 diabetes⁴³. This might make muscle structure and function susceptible to muscle damage and MPD enhancement by exercise. Subinflammation in type 2 diabetes might also contribute to these phenomena¹⁴. The results in the current study thus suggest that glycemic control is important to maintain muscle mass in diabetes. Further studies are also needed to investigate which molecular mechanisms underlie muscle damage and muscle protein degradation in type 2 diabetes.

In conclusion, exercise induced muscle damage and enhanced MPD in STZ-induced moderately glycemic, uncontrolled type 2 diabetic rats with insulin deficiency. These effects were caused by moderate exercise at an intensity level that did not induce muscle damage and did not enhance MPD in non-diabetic rats. These results suggest that in clinical practice an exercise regimen should be carefully prescribed in patients with uncontrolled type 2 diabetes, as well as in patients with type 1 diabetes, for maintaining muscle mass, as well as regulating glycemic control.

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