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Common single nucleotide polymorphisms in the *FNDC5* gene are associated with glucose metabolism but do not affect serum irisin levels in Japanese men with low fitness levels

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ABSTRACT

Objective. This cross-sectional study analyzed the association of serum irisin concentrations with cardiorespiratory fitness levels and common single nucleotide polymorphisms (SNPs) in the *FNDC5* gene and examined the relationships between cardiorespiratory fitness levels, common SNPs in *FNDC5*, and glucose metabolism.

Materials/Methods. Cardiorespiratory fitness was assessed by measuring peak oxygen uptake (VO_2peak) and serum irisin levels by ELISA in 163 Japanese men (age, 21–79 years). Subjects were divided into low- and high-fitness groups within each age group according to the median VO_2peak value. Common SNPs (rs3480 and rs16835198) of the *FNDC5* gene were genotyped with the TaqMan assay. Glucose metabolism was evaluated by measuring HbA1c, fasting plasma glucose (FPG), insulin levels, and HOMA-IR.

Results. Serum irisin levels were negatively correlated with age ($p < 0.001$) and not associated with the VO_2peak or HOMA-IR. In the low-fitness group, SNP analysis revealed that subjects with the rs3480 AG and GG genotypes had higher levels of insulin and HOMA-IR than those with the AA genotype ($p < 0.01$; no significant difference was observed in the high-fitness group). The GG genotypes of rs16835198 were associated with increased HbA1c and FPG in the low-fitness group only ($p < 0.05$). SNPs and both fitness groups were not associated with serum irisin levels.

Conclusions. In Japanese men, cardiorespiratory fitness levels and common SNPs in *FNDC5* are not associated with circulating irisin levels, whereas high cardiorespiratory fitness abolishes the association between the rs3480 and rs16835198 SNPs and glucose metabolism independent of serum irisin levels.

Abbreviations: BMI, body mass index; FFM, free fat mass; WC, waist circumference; VO_2peak, peak oxygen uptake; MVPA, moderate- and vigorous-intensity physical activity; FPG, fasting plasma glucose; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglyceride.

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1. Introduction

The benefits of exercise have been extensively documented. Regular physical exercise leads to functional adaptation of the cardiovascular system, while it has been suggested that cardiorespiratory fitness level is an independent predictor of type 2 diabetes incidence [1–3]. Although it is clear that high levels of cardiorespiratory fitness reduce the risk of type 2 diabetes, the mechanisms involved in glucose metabolism have not yet been completely elucidated.

Recently, Boström et al. [4] reported that PGC-1α expression in the skeletal muscle stimulates increased expression of fibronectin type III domain-containing protein 5 (FNDC5), a membrane protein that is cleaved and secreted as a novel myokine, irisin. The same study demonstrated that when an *FNDC5*-expressing adenovirus is administered to mice fed a high-fat diet, browning of subcutaneous fat is induced and total body energy expenditure increases, while obesity decreases [4]. Furthermore, ectopic overexpression of the *FNDC5* gene in mice fed a high-fat diet results in increased irisin plasma levels, which lead to an improvement in glucose tolerance [4]. Therefore, it is likely that the health benefits of exercise are, at least in part, due to exercise-induced *FNDC5* expression in the skeletal muscle and the consequent increase in the circulating irisin concentration. Since physical exercise leads to an improvement in cardiorespiratory fitness and high levels of cardiorespiratory fitness are associated with decreased risk of type 2 diabetes development, it is plausible that the extensive effects of cardiorespiratory fitness on glucose metabolism are mediated by an increase in the serum irisin level. Currently, the association between the cardiorespiratory fitness level and the circulating irisin concentration is not well understood.

A previous study suggested that common SNPs within the human *FNDC5* locus also contribute to glucose metabolism. Staiger et al. [5] revealed that the SNPs rs726344 and rs16835198 in *FNDC5* are associated with insulin sensitivity and resistance in white European individuals from Southern Germany. However, although irisin is proposed to be involved in glucose metabolism, they did not evaluate the relationship between the common *FNDC5* SNPs and the serum irisin level. Furthermore, even though previous studies suggest that the cardiorespiratory fitness level is associated with the risk of insulin resistance development [1,2], evaluation of whether a high level of cardiorespiratory fitness can offset the adverse effect of *FNDC5* SNPs on glucose metabolism has not yet been performed.

Therefore, the purpose of this study was to determine the association of the serum irisin concentration with the cardiorespiratory fitness level and common SNPs in the *FNDC5* gene and to examine the relationships between cardiorespiratory fitness levels, common SNPs in *FNDC5*, and glucose metabolism.

2. Methods

2.1. Subjects

One hundred and sixty-three Japanese men aged 21 to 79 years participated in this study. The subjects did not have a history of diabetes or cardiovascular diseases, and they were free of other chronic diseases such as cancer, chronic kidney failure, non-alcoholic steatohepatitis, and autoimmune disorders. Nine subjects (5.5%) were treated with lipid-lowering drug. Current or former smoking status was recorded with a questionnaire. Daily alcohol intake was assessed using a brief-type self-administered diet history questionnaire [6]. All subjects provided written informed consent before enrollment in the study. The research project was approved by the Ethical Committee of Waseda University.

2.2. Measurement of anthropometric characteristics

Body weight and body fat percentage were measured using an electronic scale (Inner Scan BC-600, Tanita, Tokyo, Japan), while height was measured with a stadiometer (YL-65, YAGAMI, Nagoya, Japan). BMI and FFM were calculated from measurements of body weight, percent body fat, and height. WC was measured at the umbilical region with an inelastic measuring tape.

2.3. Measurement of peak oxygen uptake

Cardiorespiratory fitness was assessed with a maximal graded exercise test using a cycle ergometer (Ergomedic 828E, Monark, Varberg, Sweden) and quantified as a VO_2peak. The graded cycle exercise began at a workload of 45–90 W, which was then increased by 15 W/min until the subjects could no longer maintain the required pedaling frequency of 60 rpm. Heart rate and ratings of perceived exertion were monitored minute by minute during exercise. During the incremental portion of the exercise test, expired gas was collected from the subjects: O_2 and CO_2 concentrations were measured and averaged over 30-s intervals using an automated gas analyzing system (Aeromonitor AE-300, Minato Medical Science, Tokyo, Japan). The highest value of VO_2 recorded during the exercise test was considered the VO_2peak ($mL \cdot kg^{-1} \cdot min^{-1}$). Subjects were subsequently divided into low cardiorespiratory fitness (low-fitness) and high cardiorespiratory fitness (high-fitness) groups according to the median VO_2peak value of each age group, which were as follows (in $mL \cdot kg^{-1} \cdot min^{-1}$): 45.4 for 21–29 years, 40.7 for 30–39 years, 36.4 for 40–49 years, 38.0 for 50–59 years, 32.6 for 60–64 years, 29.1 for 65–69 years, and 27.6 for 70–79 years.

2.4. Measurement of physical activity

Physical activity was measured using a uniaxial accelerometer (Kenz Lifecorder EX, SUZUKEN, Nagoya, Japan). Subjects were instructed on the use of the instrument, and then they continuously wore it on their belt or waistband at the right midline of the thigh for 10 days, except when sleeping or bathing. MVPA was used as the index of physical activity. On a scale with the points 0, 0.5, and 1–9, the Lifecorder system determined the level of physical activity intensity every 4 s. As described previously [7], the amount of time spent at intensity levels 4–9 was used as the amount of time spent in MVPA.

2.5. Collection and analysis of blood samples

Blood samples were collected between 0830 and 1100 after a 12-h overnight fast, and then centrifuged at 3000 × *g* for 15 min at