Original Article

Prevalence and features of impaired glucose tolerance in young underweight Japanese women

Motonori Sato[1], Yoshifumi Tamura[1,2], Takashi Nakagata[2], Yuki Someya[2], Hideyoshi Kaga[1], Nozomu Yamasaki[1], Mai Kiya[1], Satoshi Kadowaki[1], Daisuke Sugimoto[1], Hiroaki Satoh[1], Ryuzo Kawamori[2] and Hirotaka Watada[1, 2, 3, 4]

[1]Department of Metabolism & Endocrinology, [2]Sportology Center, [3]Center for Therapeutic Innovations in Diabetes, [4]Center for Identification of Diabetic Therapeutic Targets, Juntendo University Graduate School of Medicine, Tokyo, Japan

Short title: IGT in underweight Japanese women

Key words: underweight, impaired glucose tolerance, young women

Word count: 2,925 words

Number of figures and tables: 2 figures, 2 tables

Address correspondence and reprint requests to:

Yoshifumi Tamura, MD, PhD

Department of Metabolism & Endocrinology

Juntendo University Graduate School of Medicine

2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

Tel: +81-3-5802-1579

Fax: +81-3-3813-5996

E-mail: ys-tamur@juntendo.ac.jp

Funding: This work was supported by a Strategic Research Foundation at Private Universities; KAKENHI (Grant-in-Aid for Scientific Research (C)) (17K01868) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a grant from the Society for Women's Health Science Research.

Disclosure Summary: The authors have nothing to disclose.

Abstract

Objective: In Japan, while it is known that underweight women over the age 40 years have a high risk for type 2 diabetes, there is a lack of clarity on the association between glucose tolerance and underweight in younger women. Accordingly, we aimed to investigate the prevalence and features of impaired glucose tolerance (IGT) in young Japanese underweight women.

Designs and Methods: In this cross-sectional study, we recruited 56 normal weight and 98 underweight young Japanese women and evaluated their glucose tolerance levels using an oral glucose tolerance test. Then, we compared the clinical characteristics associated with normal glucose tolerance (NGT) and IGT in the underweight women. Insulin secretion, whole-body insulin sensitivity, and adipose tissue insulin resistance values were measured using the insulinogenic index, whole-body insulin sensitivity index (Matsuda index), and adipose insulin resistance index (Adipo-IR), respectively. Fitness level (peak VO_2) was measured using an ergometer.

Results: The prevalence of IGT was higher in the underweight women than the normal weight women (13.3% vs 1.8%). The underweight women with IGT showed a lower insulinogenic index, lower peak VO_2 and Matsuda index, and a higher fasting free fatty acid level and Adipo-IR than those with NGT. The whole-body composition was comparable between the NGT and IGT groups.

Conclusions: The prevalence of IGT was higher in young Japanese women with underweight than those with a normal weight. The underweight women with IGT showed impaired early-phase insulin secretion, low fitness levels, and reduced whole-body and adipose tissue insulin sensitivity levels.

Introduction

People with impaired glucose tolerance (IGT) are at a high risk for cardiovascular disease and type 2 diabetes mellitus (1). These individuals tend to exhibit impaired insulin secretion and resistance as a result of being obese or overweight (2-6). With the increase in the incidence of obesity, the prevalence of IGT in young people is now on the rise, worldwide (7-11). Accordingly, body weight reduction in obese youth is recommended for the prevention of cardiovascular disease and diabetes development later in life (12).

According to the NCD Risk Factor Collaboration database (13), in 2016, the prevalence of underweight in women was 9.3% in Japan, which was the highest value observed across developed countries. In particular, the prevalence of underweight among women in their 20s in Japan had increased from 12.7% in 1982 to 19.8% in 2018, based on a National Nutrition Survey report, potentially owing to a desire to be thin (14,15). Although this phenomenon seems to contribute to a lower incidence of abnormal glucose metabolism, a previous prospective study showed that underweight (body mass index [BMI] $\leq$18.5 kg/m^2) women aged 40-79 years have approximately double the risk for type 2 diabetes compared to their normal weight counterparts (16). Given the rapid increase in the prevalence of underweight in Japanese women, efforts are now being driven towards the identification of underweight women with abnormal glucose metabolism. However, the oral glucose tolerance test (OGTT) is rarely performed in underweight young women, and it remains unclear whether young underweight women in Japan are at a risk for abnormal glucose metabolism.

Accordingly, we aimed to investigate the prevalence and features of IGT in young Japanese underweight women.

Materials and methods

Study participants

We attempted to recruit ~100 young healthy underweight women aged 18-29 years with a BMI ranging from ≥16.0 to <18.5 kg/m^2 and ~50 normal weight women with a BMI ranging from ≥18.5 to <23.0 kg/m^2 as the control group through two outsourcing companies (Souken, Tokyo, Japan, and 3H medi solution, Tokyo, Japan). We excluded women with known diabetes, hypertension, dyslipidemia, hyperthyroidism, surgical menopause, multipara, and chronic disease, those who were taking medicines or supplements that may affect metabolism, and women with suspected anorexia nervosa based on the Eating Attitude Test (EAT-26, Japanese version) (17). During the recruiting period, we screened 160 candidates. Three participants with a BMI of 16 kg/m^2, two with a BMI of 23 kg/m^2, and one with suspicions of anorexia nervosa were excluded. We finally included 98 young and healthy underweight women and 56 normal weight women. This study was approved by the ethics committee of Juntendo University and performed in accordance with the principles outlined in the Declaration of Helsinki.

Study design

In this cross-sectional study, all measurements were performed at the Juntendo Sportology Center (Tokyo, Japan) from November 2018 to December 2019. The participants underwent examinations under overnight fasting conditions in the morning for 3-7 days during menstruation. Body composition was measured using the bioimpedance method (InBody; BIOSPACE) and dual-energy X-ray absorptiometry (DXA) (Hologic Discovery-A; Hologic, Inc., Bedford, MA). Blood samples were collected with patients in the supine position after at least a 15-minute rest, and then 75g OGTTs were performed. We also administered the

Brief-Type Self-Administered Diet History Questionnaire for energy intake (18) and International Physical Activity Questionnaire short form for physical activity (19). Hand grip strength was measured using a hand grip dynamometer (Takei Digital Grip Strength Dynamometer; Takei Scientific Instruments Co., Ltd, Tokyo, Japan) and peak oxygen uptake was estimated with incremental exercise testing using a cycle ergometer (AEROBIKE 75XL; COMBI, Tokyo, Japan).

OGTT

All participants underwent a standard 75g OGTT (20). Blood samples were obtained before and after the ingestion of 75g of glucose and, thereafter, every 30 min until 120 min. Fasting glucose tolerance and IGT were defined as a fasting glucose level ≥ 110 mg/dl and a 2-hour glucose level ≥ 140 mg/dl, according to World Health Organization criteria (21), respectively. Elevated 1-hour glucose level was defined as ≥ 155 mg/dL (22). The insulinogenic indices from insulin and C-peptide were calculated using the following equation: (insulin at 30 min - fasting insulin) or (C-peptide at 30 min - fasting C-peptide) / (plasma glucose at 30 min - fasting plasma glucose), respectively (23). We evaluated the adipose tissue insulin resistance degree using the adipose tissue insulin resistance index (Adipo-IR) (fasting insulin levels * fasting free fatty acid [FFA]) (24). The homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-β) (25) as well as the Matsuda index (26) were calculated as previously described.

Statistical analysis

We used IBM SPSS Statistics for Windows, version 25.0. (IBM Corp., Armonk, NY, USA) for all analyses. Data are presented as the mean ± standard deviation. Data were compared using Mann–Whitney U tests or x^2 tests. All statistical tests were two-sided, with a

significance level of 5%.

Results

Comparison between normal weight and underweight women

Table 1 summarizes the clinical characteristics of the underweight and normal weight women. The underweight women showed significantly lower body weight, BMI, body fat mass, and lean body mass values than the normal weight women, and weighed on average 7.2 kg less, predominantly owing to a lower lean body mass (4.6 kg). The waist, thigh, and lower leg circumferences were smaller in the underweight women than the normal weight women. The physical activity levels, as estimated using the corresponding questionnaire, were lower in the underweight women, as were the estimated total energy intake and levels of energy intake from protein, fat, and carbohydrate.

In terms of glucose metabolism, the glucose, insulin, and C-peptide concentrations at fasting state were lower and HOMA−IR was reduced in the underweight women compared to those in the normal weight women (Table 1). As shown in Figure 1, the levels of glucose (A), insulin (B), C-peptide (C), and FFA (D) during the OGTT were similar between the groups, except the aforementioned fasting values and glucose level at 60 min. In addition, the insulinogenic index, Matsuda index, a marker of whole-body insulin sensitivity, and HOMA-β value were comparable between the groups. However, the incidence of IGT was significantly higher in the underweight women than the normal weight women, and the proportion of those with a 1h glucose level >155 mg/dl mg/dl tended to be higher in the underweight women (Table 1).

Comparison between underweight women with NGT and IGT

To further elucidate the characteristics of IGT in underweight young women, we compared the clinical parameters between the underweight women with NGT and IGT (Table 2, Figure 2). The body composition was similar between the groups, while the peak VO_2 was lower in

158 those with IGT. The fasting glucose, FFA, and glycated hemoglobin levels were significantly
159 higher in the IGT participants, while the fasting insulin and C-peptide levels were comparable
160 between the groups. The carbohydrate intake level was lower and fat intake level was higher
161 in those with IGT.

162 Figure 2 presents the glucose (A), insulin (B), C-peptide (C), and FFA (D) levels
163 during the OGTT in the underweight women with NGT and those with IGT. The glucose
164 levels were higher from 0 to 120 min in the women with IGT than in those with NGT, while
165 the insulin and C-peptide levels were similar from 0 to 90 min and higher from 90 to 120 min
166 in the IGT population. Thus, the insulinogenic index and Matsuda index were significantly
167 lower in those with IGT (Table 2). In addition, the Adipo-IR was significantly higher in
168 association with IGT presence and the area under the FFA curve during the OGTT was
169 consistently higher in the women with IGT.

170

171

Discussion

Although the prevalence of underweight in young women has been increasing in Japan, the characteristics of glucose metabolism in this population have not been clarified yet. In the present study, which compared the degree of glucose tolerance between normal weight and underweight young women in Japan, we found that underweight was associated with a lower energy intake and physical activity level than normal weight and that the prevalence of IGT was significantly higher among underweight women. Intriguingly, the underweight women with IGT showed not only lower early-phase insulin secretion but also a lower peak VO_2, elevated fasting FFA levels, a lower Matsuda index, and elevated Adipo-IR values, suggesting the presence of decreased whole-body insulin sensitivity.

The OGTT is rarely performed in underweight young women; the present study is the first to demonstrate that the incidence of IGT was significantly higher in underweight young women (13.3%) than their counterparts with a normal weight (2%). In the United States, among people aged 19 to 34 years (mean BMI 27.7 kg/m^2), the prevalence of obesity was reported to be 29.8%; in these people, the prevalence of IGT was 5.8%, which increased in accordance with the BMI category (e.g., prevalence of IGT in normal or underweight people, 2.9%; overweight people, 4.9%; obese people; 10.6%) (7). Thus, the incidence of IGT in the underweight young women in the present study was higher than that observed in the same age group of obese people in the United States. Given that the proportion of underweight women in their 20s in Japan is ~20%, the number of underweight young women with IGT is considered large.

In the present study, the underweight young women with IGT had a lower early-phase insulin secretion and a reduced whole-body insulin sensitivity index (Matsuda index) compared to those without IGT. Previous studies have revealed that the rates of both insulin secretion and insulin sensitivity are reduced in people with IGT across ethnicities,

197 such as Caucasians (27), Japanese people (3,27), Mexican-Americans (3) and Arabs (3).
198 However, it is supposed that impaired insulin secretion rather than insulin resistance is
199 predominantly observed in underweight young women with IGT, since insulin sensitivity is
200 negatively associated with BMI and age; however, the Matsuda index and insulinogenic
201 index in these people was similar to those previously observed in middle-aged Japanese
202 people with IGT (age, 54 years; BMI, 26.3 kg/m^2; Matsuda index, ~6; insulinogenic index,
203 ~0.6) (27). Thus, our findings suggest that, even in underweight young women, both insulin
204 resistance and impaired insulin secretion are associated with IGT as strongly as in
205 middle-aged overweight people with IGT, and that the degree of insulin resistance is
206 relatively strong despite their low BMI. Enhanced endogenous glucose production due to
207 impaired early phase insulin secretion and insulin resistance causes postprandial
208 hyperglycemia in individuals with IGT (28,29).

209 We found that the FFA level, Adipo-IR, and Matsuda index were elevated in the
210 underweight young women with IGT, which is unexpected, as adipose tissue insulin
211 resistance and elevated FFA levels are generally observed in people with obesity (30,31). It
212 has been shown that the fasting FFA and Adipo-IR values are elevated in obese people
213 (30,31) and are considered upstream of insulin resistance in peripheral tissues (31). In fact,
214 artificial FFA elevations cause insulin resistance (32,33). On the other hand, we previously
215 showed, even in apparently healthy non-obese men, the presence of adipose tissue insulin
216 resistance, as evaluated using a two-step hyperinsulinemic euglycemic clamp, in correlation
217 with muscle insulin resistance (34). Thus, adipose tissue insulin resistance could be present in
218 people without obesity. The present study is the first to show that insulin resistance can
219 develop even in underweight young Japanese women and contribute to impaired glucose
220 metabolism.

221 Asians tend to develop type 2 diabetes even in the presence of a lower BMI

compared to Caucasians (35), partly due to a lower insulin secretory capacity (35,36) and lower adipose tissue capacity (37). Accordingly, underweight women with IGT may rarely be observed in non-Asians, however, the rate of glucose tolerance in underweight women in other ethnicities is worth testing. In addition, in the present study, the body fat mass was comparable between those with and without IGT; thus, adipose tissue insulin resistance mechanisms other than body fat accumulation may exist in people with IGT. The lack of difference in fat mass occurred in the face of higher fat intake and was accompanied by greater FFA, also suggesting a defect in adipose tissue metabolism. Thus, it is hypothesized that increased intramyocellular lipid accumulation is induced by FFA elevation due to impaired adipose tissue insulin resistance, resulting in muscle insulin resistance (32,33) (38). Further studies are required to clarify the role and mechanism of adipose tissue insulin resistance in underweight Japanese women.

We used same amount of glucose (75g) for OGTT in all subjects according to WHO definition, despite their different body weight. Thus, subjects with lower weight have a higher glucose load per body weight, which may partly cause hyperglycemia during OGTT. In fact, lean body mass reflects muscle volume and inversely predicts plasma glucose levels after oral glucose load (39). Thus, underweight women have a higher glucose load per body weight and lean body mass in OGTT compared with normal weight women, which may be partly the cause of higher prevalence of IGT in underweight women. However, body weight and lean body weight were comparable between NGT and IGT in underweight women, thus these were unlikely to be the cause of IGT in underweight women.

The present study demonstrated that underweight women with IGT had a low peak VO_2 and high fat/low carbohydrate intake level. Consistently, we previously showed that insulin sensitivity is positively correlated with peak VO_2 and negatively correlated with fat intake in non-obese men without diabetes (40). In addition, we also reported that the intake of

247 a three-day eucaloric low-carbohydrate/high-fat diet in non-obese healthy young men
248 increased the level of intramyocellular lipid and decreased the insulin sensitivity level, as
249 evaluated using a hyperinsulinemic euglycemic clamp (41,42). Thus, lower fitness levels and
250 dietary composition may also contribute to insulin resistance in underweight women with
251 IGT.

252 Although IGT prevalence was higher in underweight compared with normal weight,
253 fasting plasma glucose (FPG) level was lower in underweight compared with normal weight.
254 We do not the exact mechanism of low FPG in underweight. One possibility is that lower
255 energy intake in underweight compared with normal weight might contribute to the lower in
256 FPG level in underweight women compared with normal weight women. However, the FPG
257 level in underweight IGT was higher compared with that in underweight NGT. Thus, it is
258 unlikely that lower FPG contributes increased IGT prevalence in underweight young women.

259 Our data showed that underweight women had significantly lower total energy
260 intake and lower physical activity than those in normal weight (Table 1), thus, in terms of
261 energy balance, low energy intake could be main cause of thinness. The eating disorder was
262 ruled out based on the Eating Attitude Test in the present study, thus energy intake in
263 underweight women was declining for reasons other than eating disorder. Concerning,
264 previous report suggested that Japanese women strongly desire to be thin (14,15) and our
265 study subjects might also have such desire.

266 This study has several limitations. First, we used surrogate markers of insulin
267 secretion and insulin resistance; however, the Matsuda index (26) and Adipo-IR (30) have
268 been found to be sufficiently correlated with hyperinsulinemic euglycemic clamp findings in
269 the determination of peripheral and adipose tissue insulin sensitivity, respectively. Second, we
270 used the results of a single OGTT and did not confirm the reproducibility of the OGTT in
271 underweight women. Finally, this study had a cross-sectional design; further prospective and

interventional studies are required to confirm the direction of causality for the parameters identified.

In conclusion, the prevalence of IGT was higher in the underweight Japanese young women than their normal weight counterparts. Underweight young women with IGT tend to have low fitness levels, impaired insulin secretion, as well as reduced whole-body and adipose tissue insulin sensitivity. To improve insulin resistance may help prevent type 2 diabetes even in underweight women with IGT.

Acknowledgments

We thank Miyuki Iwagami and Naoko Daimaru for their excellent technical assistance.

Author Contributions: M.S., T.N., H.K., Y.S. and Y.T. researched the data and contributed to the study design, data collection, result interpretation, and manuscript preparation. N.Y., M.K., S.K., and D.S. participated in the data collection and analysis, and contributed to the discussion. H.S and R.K. contributed to the discussion. H.W. contributed to the study design and edited the manuscript.

References

1. Edelstein SL, Knowler WC, Bain RP, Andres R, Barrett-Connor EL, Dowse GK, Haffner SM, Pettitt DJ, Sorkin JD, Muller DC, Collins VR, Hamman RF. Predictors of progression from impaired glucose tolerance to NIDDM: an analysis of six prospective studies. *Diabetes*. 1997;46(4):701-710.
2. Fiorentino TV, Marini MA, Andreozzi F, Arturi F, Succurro E, Perticone M, Sciacqua A, Hribal ML, Perticone F, Sesti G. One-Hour Postload Hyperglycemia Is a Stronger Predictor of Type 2 Diabetes Than Impaired Fasting Glucose. *J Clin Endocrinol Metab*. 2015;100(10):3744-3751.
3. Abdul-Ghani MA, Matsuda M, Sabbah M, Jenkinson CP, Richardson DK, Kaku K, DeFronzo RA. The relative contributions of insulin resistance and beta cell failure to the transition from normal to impaired glucose tolerance varies in different ethnic groups. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2007;1(2):105-112.
4. Fretts AM, Howard BV, McKnight B, Duncan GE, Beresford SA, Calhoun D, Kriska AM, Storti KL, Siscovick DS. Modest levels of physical activity are associated with a lower incidence of diabetes in a population with a high rate of obesity: the strong heart family study. *Diabetes Care*. 2012;35(8):1743-1745.
5. Bell JA, Kivimaki M, Hamer M. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. *Obes Rev*. 2014;15(6):504-515.
6. Awede B, Lawani S, Adehan G, Akakpo J, Dossou E, Amoussou-Guenou M, Djrolo F. Glucose Tolerance in Non-Diabetic Adult Subjects of an Urban West-African Population. *Niger J Physiol Sci*. 2017;32(2):159-164.
7. Andes LJ, Cheng YJ, Rolka DB, Gregg EW, Imperatore G. Prevalence of Prediabetes Among Adolescents and Young Adults in the United States, 2005-2016. *JAMA Pediatr*. 2019;174(2):e194498.
8. Di Bonito P, Pacifico L, Chiesa C, Valerio G, Miraglia Del Giudice E, Maffeis C, Morandi A, Invitti C, Licenziati MR, Loche S, Tornese G, Franco F, Manco M, Baroni MG. Impaired fasting glucose and impaired glucose tolerance in children and adolescents with overweight/obesity. *J Endocrinol Invest*. 2017;40(4):409-416.
9. Soriguer F, Goday A, Bosch-Comas A, Bordiú E, Calle-Pascual A, Carmena R, Casamitjana R, Castaño L, Castell C, Catalá M, Delgado E, Franch J, Gaztambide S, Girbés J, Gomis R, Gutiérrez G, López-Alba A, Martínez-Larrad MT, Menéndez E, Mora-Peces I, Ortega E, Pascual-Manich G, Rojo-Martínez G, Serrano-Rios M, Valdés S, Vázquez JA, Vendrell J. Prevalence of diabetes mellitus and impaired glucose regulation in Spain: the Di@bet.es Study. *Diabetologia*. 2012;55(1):88-93.
10. Zorzi A, Wahi G, Macnab AJ, Panagiotopoulos C. Prevalence of impaired glucose

tolerance and the components of metabolic syndrome in Canadian Tsimshian Nation youth. *Can J Rural Med*. 2009;14(2):61-67.

11. Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, Savoye M, Rieger V, Taksali S, Barbetta G, Sherwin RS, Caprio S. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *N Engl J Med*. 2002;346(11):802-810.
12. Mizokami-Stout K, Cree-Green M, Nadeau KJ. Insulin resistance in type 2 diabetic youth. *Curr Opin Endocrinol Diabetes Obes*. 2012;19(4):255-262.
13. Collaboration NRF. BODY-MASS INDEX Evolution of BMI over time. Vol 2020.
14. Takimoto H, Yoshiike N, Kaneda F, Yoshita K. Thinness among young Japanese women. *Am J Public Health*. 2004;94(9):1592-1595.
15. Kaneko K, Kiriike N, Ikenaga K, Miyawaki D, Yamagami S. Weight and shape concerns and dieting behaviours among pre-adolescents and adolescents in Japan. *Psychiatry Clin Neurosci*. 1999;53(3):365-371.
16. Tatsumi Y, Ohno Y, Morimoto A, Nishigaki Y, Maejima F, Mizuno S, Watanabe S. U-shaped relationship between body mass index and incidence of diabetes. *Diabetology International*. 2012;3(2):92-98.
17. Garner DM, Olmsted MP, Bohr Y, Garfinkel PE. The eating attitudes test: psychometric features and clinical correlates. *Psychol Med*. 1982;12(4):871-878.
18. Kobayashi S, Honda S, Murakami K, Sasaki S, Okubo H, Hirota N, Notsu A, Fukui M, Date C. Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. *J Epidemiol*. 2012;22(2):151-159.
19. Lee PH, Macfarlane DJ, Lam TH, Stewart SM. Validity of the International Physical Activity Questionnaire Short Form (IPAQ-SF): a systematic review. *Int J Behav Nutr Phys Act*. 2011;8:115.
20. Bartoli E, Fra G, Schianca GC. The oral glucose tolerance test (OGTT) revisited. *European journal of internal medicine*. 2011;22(1):8-12.
21. Organization WH. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. 2006.
22. Abdul-Ghani MA, Abdul-Ghani T, Ali N, Defronzo RA. One-hour plasma glucose concentration and the metabolic syndrome identify subjects at high risk for future type 2 diabetes. *Diabetes Care*. 2008;31(8):1650-1655.
23. Tura A, Kautzky-Willer A, Pacini G. Insulinogenic indices from insulin and C-peptide: comparison of beta-cell function from OGTT and IVGTT. *Diabetes Res Clin Pract*. 2006;72(3):298-301.
24. Adams-Huet B, Devaraj S, Siegel D, Jialal I. Increased adipose tissue insulin resistance in metabolic syndrome: relationship to circulating adipokines. *Metab Syndr*

Relat Disord. 2014;12(10):503-507.

25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-419.

26. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;22(9):1462-1470.

27. Moller JB, Pedersen M, Tanaka H, Ohsugi M, Overgaard RV, Lynge J, Almind K, Vasconcelos NM, Poulsen P, Keller C, Ueki K, Ingwersen SH, Pedersen BK, Kadowaki T. Body composition is the main determinant for the difference in type 2 diabetes pathophysiology between Japanese and Caucasians. *Diabetes Care*. 2014;37(3):796-804.

28. Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, Gerich J. Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med*. 1992;326(1):22-29.

29. Göke B. Implications of blood glucose, insulin resistance and beta-cell function in impaired glucose tolerance. *Diabetes Res Clin Pract*. 1998;40 Suppl:S15-20.

30. Ter Horst KW, van Galen KA, Gilijamse PW, Hartstra AV, de Groot PF, van der Valk FM, Ackermans MT, Nieuwdorp M, Romijn JA, Serlie MJ. Methods for quantifying adipose tissue insulin resistance in overweight/obese humans. *Int J Obes (Lond)*. 2017;41(8):1288-1294.

31. Gastaldelli A, Gaggini M, DeFronzo RA. Role of Adipose Tissue Insulin Resistance in the Natural History of Type 2 Diabetes: Results From the San Antonio Metabolism Study. *Diabetes*. 2017;66(4):815-822.

32. Ferrannini E, Wahren J, Faber OK, Felig P, Binder C, DeFronzo RA. Splanchnic and renal metabolism of insulin in human subjects: a dose-response study. *Am J Physiol*. 1983;244(6):E517-527.

33. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, Loviscach M, Stumvoll M, Claussen CD, Schick F, Haring HU, Jacob S. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes*. 2001;50(11):2579-2584.

34. Sugimoto D, Tamura Y, Takeno K, Kaga H, Someya Y, Kakehi S, Funayama T, Furukawa Y, Suzuki R, Kadowaki S, Nishitani-Yokoyama M, Shimada K, Daida H, Aoki S, Kanazawa A, Kawamori R, Watada H. Clinical Features of Nonobese, Apparently Healthy, Japanese Men With Reduced Adipose Tissue Insulin Sensitivity. *J Clin Endocrinol Metab*. 2019;104(6):2325-2333.

35. Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, Hu FB. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. *JAMA*. 2009;301(20):2129-2140.

36. Shai I, Jiang R, Manson JE, Stampfer MJ, Willett WC, Colditz GA, Hu FB. Ethnicity, obesity, and risk of type 2 diabetes in women: a 20-year follow-up study. *Diabetes Care*. 2006;29(7):1585-1590.

37. Nazare JA, Smith JD, Borel AL, Haffner SM, Balkau B, Ross R, Massien C, Almeras N, Despres JP. Ethnic influences on the relations between abdominal subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: the International Study of Prediction of Intra-Abdominal Adiposity and Its Relationship With Cardiometabolic Risk/Intra-Abdominal Adiposity. *Am J Clin Nutr*. 2012;96(4):714-726.

38. Tamura Y. Ectopic fat, insulin resistance and metabolic disease in non-obese Asians: investigating metabolic gradation. *Endocr J*. 2019;66(1):1-9.

39. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes*. 1988;37(6):667-687.

40. Takeno K, Tamura Y, Kawaguchi M, Kakehi S, Watanabe T, Funayama T, Furukawa Y, Kaga H, Yamamoto R, Kim M, Nishitani-Yokoyama M, Shimada K, Daida H, Aoki S, Taka H, Fujimura T, Sawada SS, Giacca A, Kanazawa A, Fujitani Y, Kawamori R, Watada H. Relation Between Insulin Sensitivity and Metabolic Abnormalities in Japanese Men With BMI of 23-25 kg/m(2). *J Clin Endocrinol Metab*. 2016;101(10):3676-3684.

41. Kakehi S, Tamura Y, Takeno K, Sakurai Y, Kawaguchi M, Watanabe T, Funayama T, Sato F, Ikeda S, Kanazawa A, Fujitani Y, Kawamori R, Watada H. Increased intramyocellular lipid/impaired insulin sensitivity is associated with altered lipid metabolic genes in muscle of high responders to a high-fat diet. *Am J Phyiol Endocrinol Metab*. 2016;310(1):E32-40.

42. Sakurai Y, Tamura Y, Takeno K, Kumashiro N, Sato F, Kakehi S, Ikeda S, Ogura Y, Saga N, Naito H, Katamoto S, Fujitani Y, Hirose T, Kawamori R, Watada H. Determinants of intramyocellular lipid accumulation after dietary fat loading in non-obese men. *J Diabetes Investig*. 2011;2(4):310-317.

438 **Figure Legends**

439

440 **Figure 1.** Glucose (A), insulin (B), C-peptide (C) and free fatty acid (D) levels during an oral

441 glucose tolerance test in young normal weight and underweight women. *P <0.05 for

442 significant differences between young normal weight and underweight women.

443 FFA, free fatty acid

444 **Figure 2.** Glucose (A), insulin (B), C-peptide (C) and free fatty acid (D) levels during an oral

445 glucose tolerance test in young underweight women with NGT and IGT. *P <0.05 for

446 significant difference between underweight women with NGT and those with IGT.

447 IGT, impaired glucose tolerance; NGT, normal glucose tolerance; FFA, free fatty acid

Figure 1.

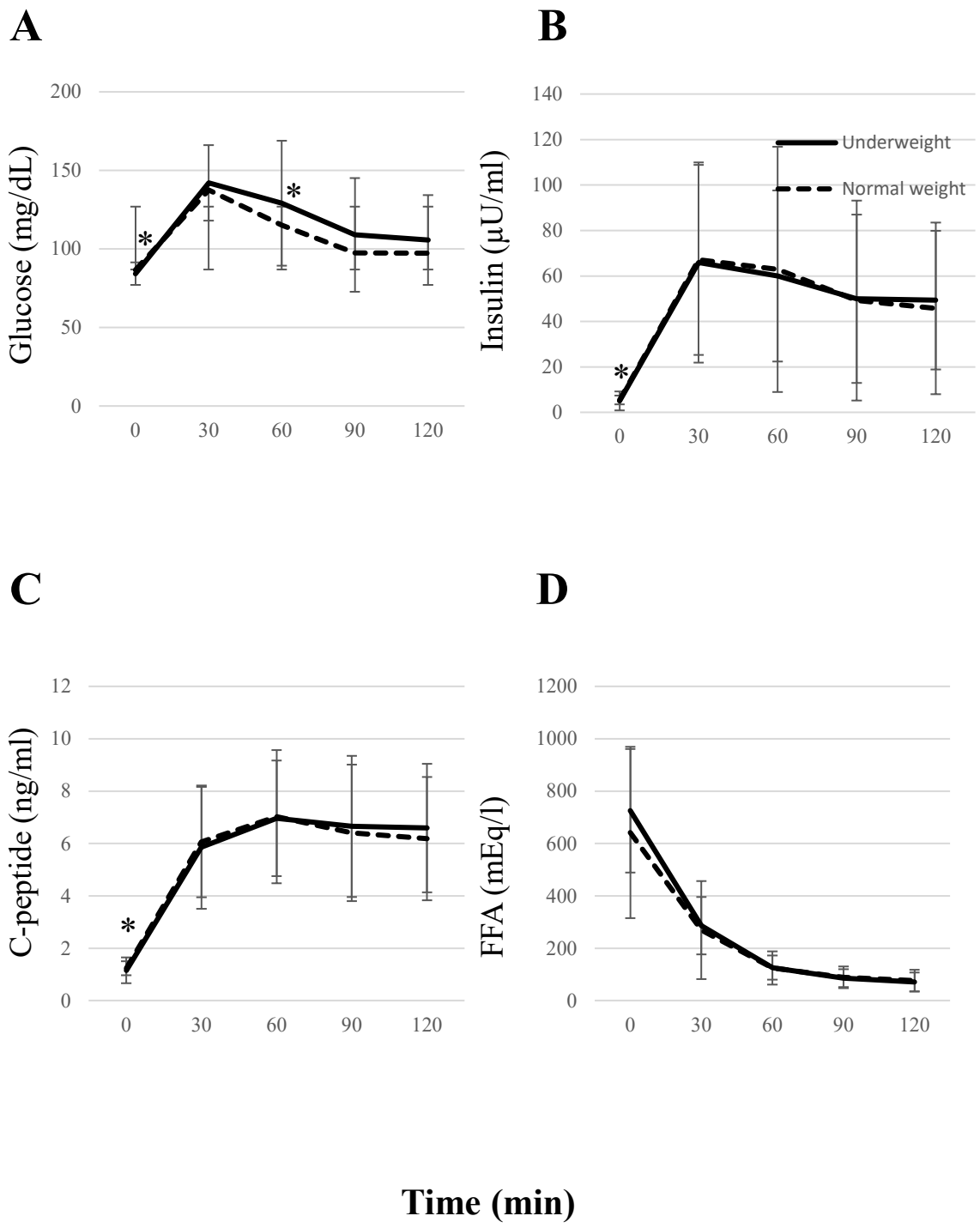
A
B
C
D
Glucose (mg/dL)
Insulin (μU/ml)
C-peptide (ng/ml)
FFA (mEq/l)
Underweight
Normal weight
Time (min)

Figure 2.

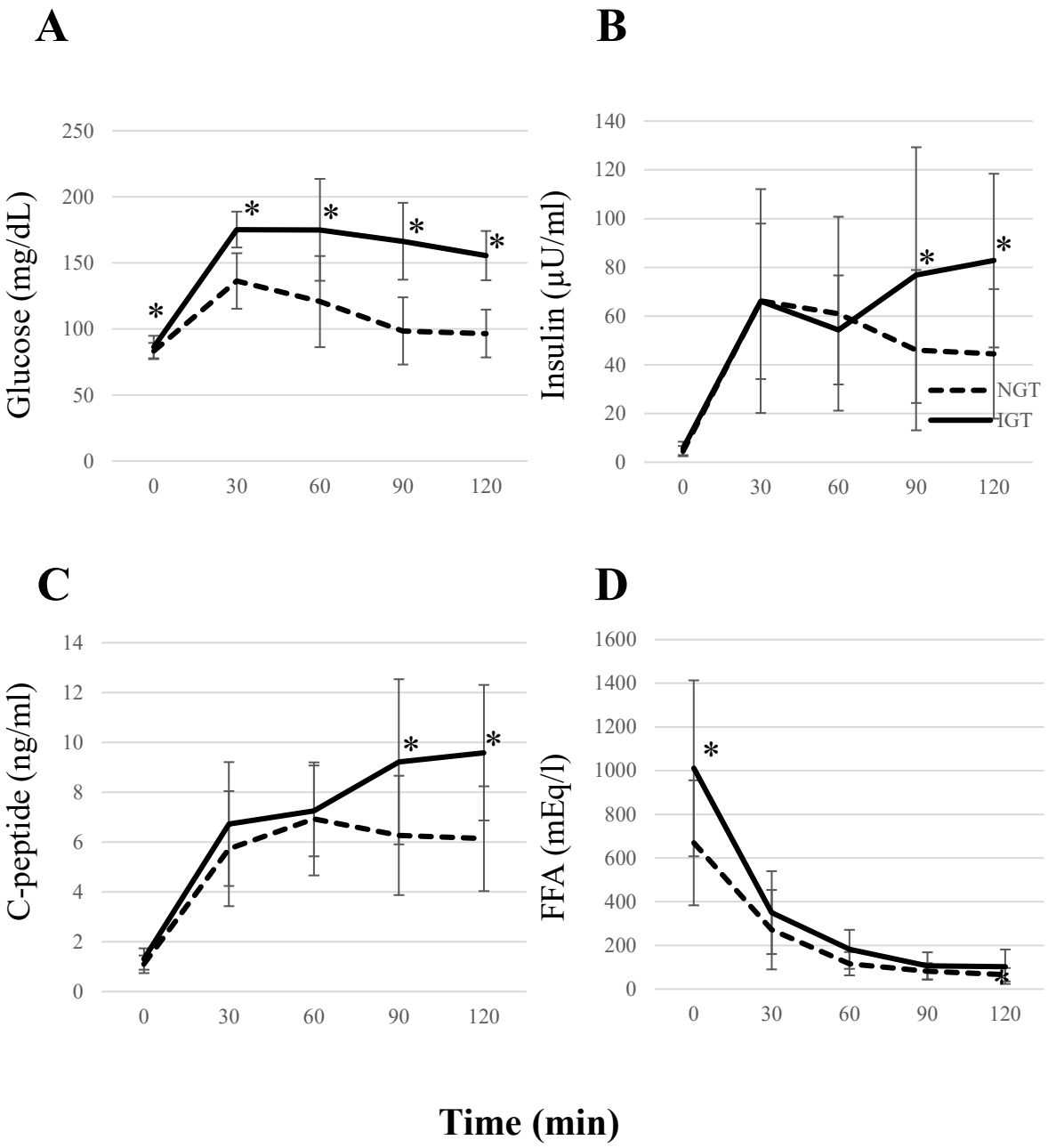
A
B
C
D
Glucose (mg/dL)
Insulin (μU/ml)
C-peptide (ng/ml)
FFA (mEq/l)
NGT
IGT
Time (min)

Table 1. Clinical characteristics of the normal weight and underweight young women

	Normal weight (n=56)	Underweight (n=98)	*P*
Age (years)	22.6 ± 3.2	23.6 ± 3.0	**0.046**
Height (cm)	159.2 ± 6.1	159.6 ± 5.2	0.754
Weight (kg)	51.5 ± 5.1	44.3 ± 3.2	**<0.001**
Systolic blood pressure (mmHg)	105.3 ± 8.8	105.3 ± 8.7	0.653
Diastolic blood pressure (mmHg)	65.4 ± 9.3	65.7 ± 8.2	0.492
Hypertension (≥ 130/80 mmHg) (n, %)	3 (5.4)	4 (4.1)	0.500
Smoking history: current smoker (n, %)	1 (1.8)	6 (6.1)	0.205
past smoker (n, %)	3 (5.4)	4 (4.1)	0.500
Body mass index (kg/m^2)	20.3 ± 1.4	17.4 ± 0.7	**<0.001**
Total body fat content (%)	22.6 ± 5.3	21.0 ± 3.7	0.061
Total body fat mass (kg)	11.9 ± 3.1	9.5 ± 1.8	**<0.001**
-Arm fat mass (kg)	1.4 ± 0.5	1.1 ± 0.3	**<0.001**
-Trunk fat mass (kg)	4.3 ± 1.5	3.2 ± 0.8	**<0.001**
-Leg fat mass (kg)	5.3 ± 1.4	4.4 ± 0.9	**<0.001**
Lean body mass (kg)	38.4 ± 4.5	33.8 ± 2.9	**<0.001**
Waist circumference (cm)	71.6 ± 5.6	65.8 ± 3.9	**<0.001**
Thigh circumference (cm)	49.3 ± 4.2	45.2 ± 3.3	**<0.001**
Lower leg circumference (cm)	34.2 ± 3.1	31.2 ± 2.0	**<0.001**
Fasting plasma glucose (mg/dl)	86.6 ± 5.4	84.2 ± 7.2	**0.014**
Fasting plasma insulin (μU/ml)	5.5 ± 2.0	5.1 ± 4.1	**0.021**
AUC-glucose during OGTT (mg·min/dl·10^3)	13.3 ± 2.1	14.3 ± 3.1	0.062
AUC-insulin during OGTT (μU·min/ml·10^3)	6.1 ± 4.4	6.1 ± 3.1	0.833
C-peptide index	1.4 ± 0.3	1.4 ± 0.5	0.086
Insulinogenic index	1.1 ± 2.2	1.4 ± 2.0	0.211

HOMA-β	86.2 ± 33.3	103.5 ± 169.2	0.651
Matsuda Index	7.2 ± 2.8	8.2 ± 4.1	0.357
HOMA-IR	1.18 ± 0.5	1.09 ± 1.1	**0.009**
Impaired fasting glucose (n, %)	0 (0)	1 (1.0)	0.448
Impaired glucose tolerance (n, %)	1 (1.8)	13 (13.3)	**0.016**
2-hour glucose level ≥ 200 mg/dl (n, %)	0 (0)	1 (1.0)	0.448
HbA1c (%)	5.3 ± 0.3	5.2 ± 0.3	**0.015**
Hb (g/dl)	13.1 ± 1.0	12.8 ± 0.9	0.101
Triglyceride (mg/dl)	53.6 ± 22.5	51.8 ± 22.8	0.578
Hypertriglyceridemia (n, %)	0	0	
HDL-cholesterol (mg/dl)	68.6 ± 13.4	69.0 ± 12.8	0.723
Low HDL-cholesterol level (n, %)	0	0	
LDL-cholesterol (mg/dl)	96.6 ± 20.8	100.8 ± 29.2	0.608
High LDL-cholesterol level (n, %)	2 (3.6)	9 (9.2)	0.165
Free fatty acid (mEq/l)	642.1 ± 236.1	725.3 ± 327.2	0.159
Adiponectin (μg/ml)	11.8 ± 4.6	12.3 ± 5.0	0.675
Total physical activity (MET hours/week)	42.7 ± 45.7	32.8 ± 44.7	**0.025**
VO_2 peak (ml/min·kg)	31.8 ± 4.8	33.4 ± 5.0	0.072
Hand grip strength (kg)	23.7 ± 4.5	21.3 ± 3.8	**0.002**
Total energy intake (kcal)	1589 ± 461	1333 ± 504	**0.046**
Protein intake per total energy intake (%)	14.7 ± 2.7	14.6 ± 3.1	**0.384**
Fat intake per total energy intake (%)	28.0 ± 5.6	29.0 ± 5.8	**0.249**
Carbohydrate intake per total energy intake (%)	52.6 ± 7.2	52.8 ± 8.2	**0.625**

Data are means ± SD and number (%). P-values are based on Mann–Whitney U tests or Chi-squared tests.

OGTT, oral glucose tolerance test; SD, standard deviation; AUC, area under the curve; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycated hemoglobin; Hb, hemoglobin; VO_{2peak},

peak oxygen consumption; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone

Table 2. Clinical characteristics of the NGT and IGT groups in underweight.

	NGT (n=83)	IGT (n=13)	*P*
Age (years)	23.7 ± 3.1	23.3 ± 2.9	0.755
Height (cm)	159.7 ± 5.4	159.3 ± 4.1	0.936
Weight (kg)	44.4 ± 3.2	44.3 ± 2.6	0.877
Systolic blood pressure (mmHg)	105.1 ± 8.9	104.5 ± 6.3	0.923
Diastolic blood pressure (mmHg)	65.6 ± 8.4	66.5 ± 7.3	0.566
Hypertension (≥ 130/80 mmHg) (n, %)	4 (4.8)	0	0.553
Smoking history: current smoker (n, %)	6 (7.2)	0	0.407
past smoker (n, %)	4 (4.8)	0	0.553
Body mass index (kg/m^2)	17.4 ± 0.6	17.5 ± 0.7	0.599
Total body fat content (%)	20.8 ± 3.5	21.7 ± 5.2	0.680
Total body fat mass (kg)	9.4 ± 1.7	9.8 ± 2.6	0.728
-Arm fat mass (kg)	1.0 ± 0.3	1.1 ±0.4	0.680
-Trunk fat mass (kg)	3.1 ± 0.7	3.4 ± 1.3	0.510
-Leg fat mass (kg)	4.4 ± 0.9	4.5 ± 1.0	0.843
Lean body mass (kg)	33.9 ± 3.0	33.3 ± 2.6	0.657
Waist circumference (cm)	66.0 ± 3.9	65.4 ± 4.4	0.756
Thigh circumference (cm)	45.1 ± 3.3	45.5 ± 3.5	0.876
Lower leg circumference (cm)	31.2 ± 2.1	31.0 ± 1.3	0.830
Fasting plasma glucose (mg/dl)	83.4 ± 6.1	86.4 ± 8.5	**0.040**
Fasting plasma insulin (μU/ml)	4.6 ± 2.1	5.8 ± 2.7	0.151
AUC-glucose during OGTT (mg·min/dl·10^3)	13.4 ± 2.3	19.1 ± 2.2	**<0.001**
AUC-insulin during OGTT (μU·min/ml·10^3)	6.0 ± 3.2	7.2 ± 2.9	0.073
C-peptide index	1.3 ± 0.4	1.5 ± 0.5	0.135
Insulinogenic index	1.6 ± 2.1	0.7 ± 0.3	**0.044**
HOMA-β	86.1 ± 43.0	204.5 ± 450.7	0.826
Matsuda Index	8.7 ± 4.2	5.5 ± 2.3	**0.004**

Adipo-IR	3116.5 ± 2147.6	6382.6 ± 5031.1	**0.008**
HOMA-IR	0.95 ± 0.47	1.23 ± 0.58	0.114
HbA1c (%)	5.1 ± 0.2	5.3 ± 0.2	**0.020**
Triglyceride (mg/dl)	51.9 ± 22.1	47.6 ± 22.6	0.394
Hypertriglyceridemia (n, %)	0	0	
HDL-cholesterol (mg/dl)	68.7 ± 12.7	73.1 ± 12.7	0.195
Low HDL-cholesterol level (n, %)	0	0	
LDL-cholesterol (mg/dl)	99.5 ± 30.0	106.6 ± 25.3	0.228
High LDL-cholesterol level (n, %)	6 (7.2)	2 (15.4)	0.295
Free fatty acid (mEq/l)	669.5 ± 285.8	1010.9 ± 403.1	**0.001**
Adiponectin (μg/ml)	12.3 ± 5.2	12.6 ± 3.5	0.444
Total physical activity (MET hours/week)	34.7 ± 47.8	23.2 ± 20.3	0.805
VO_2 peak (ml/min·kg)	33.7 ± 5.1	30.8 ± 3.4	**0.034**
Hand grip strength (kg)	21.3 ± 3.7	21.0 ± 4.4	0.797
Total energy intake (kcal)	1369 ± 518	1152 ± 376	0.197
Protein intake per total energy intake (%)	14.2 ± 2.5	16.9 ± 5.2	0.053
Fat intake per total energy intake (%)	28.5 ± 5.6	32.2 ± 6.6	**0.033**
Carbohydrate intake per total energy intake (%)	53.7 ± 7.4	46.7 ± 10.7	**0.015**

Data are means ± SD. P-values are based on the Mann–Whitney U test.

OGTT, oral glucose tolerance test; SD, standard deviation; HbA1c, glycated hemoglobin A1c; Adipo-IR, adipose insulin resistance index; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; AUC, area under the curve; HOMA-β, homeostasis model assessment of β cell function; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VO_{2peak}, peak oxygen consumption