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Context: Adipose tissue insulin resistance is observed in obese subjects and is considered an early metabolic defect preceding insulin resistance in muscle and liver. While Asians readily develop metabolic disease without obesity, the clinical features of non-obese, apparently healthy Asians with reduced adipose tissue insulin sensitivity (ATIS) have not been elucidated.

Objective: To investigate the clinical parameters associated with reduced ATIS in non-obese, apparently healthy (body mass index <25 kg/m^2) Japanese men.

Methods: We studied 52 non-obese Japanese men with no cardiometabolic risk factors. Using two-step hyperinsulinemic euglycemic clamp with a glucose tracer, we evaluated insulin sensitivity in muscle, liver, and adipose tissue. ATIS was calculated as percent free fatty acid (FFA) suppression/insulin concentration during the first step of glucose clamp.

Results: Based on the median ATIS value, subjects were divided into low- and high-FFA suppression groups. The low-FFA suppression group had moderate fat accumulation in

abdominal subcutaneous adipose tissue and liver. Compared with the high-FFA group, they also had a lower fitness level, decreased insulin clearance, impaired insulin sensitivity in muscle, moderately elevated triglycerides, and lowered high-density lipoprotein cholesterol levels. All these factors were significantly correlated with ATIS. Hepatic insulin sensitivity was comparable between the two groups.

Conclusions: In non-obese, apparently healthy Japanese men, reduced ATIS was associated with moderate fat accumulation in subcutaneous fat and liver, lower insulin clearance, muscle insulin resistance, and moderate lipidemia. These data suggest that reduced ATIS may occur early in the development of metabolic syndrome, even in non-obese, apparently healthy men.

Even in non-obese, apparently healthy Japanese men, reduced adipose tissue insulin sensitivity is associated with clinical features of metabolic syndrome.

Introduction

Elevated levels of circulating free fatty acids (FFAs) are considered to be an important mechanism linking obesity and insulin resistance (1). For example, increased FFA concentrations are observed in insulin-resistant, obese subjects (2), and FFA elevation by lipid infusion acutely induces insulin resistance in muscle and liver in healthy subjects (3). Circulating FFA elevation in obesity is caused at least in part by increased FFA release from adipose tissue (4), which in turn results from an inadequate ability of insulin to suppress the release of FFAs from adipose tissue, a phenomenon known as adipose tissue insulin resistance (5,6). In obese subjects, adipose tissue insulin resistance is associated with fat accumulation in visceral adipose tissue and liver (1,7,8) as well as insulin resistance in muscle and liver (9). Thus, it has been hypothesized that adipose tissue insulin resistance is induced by weight gain and is the result of the reduced ability of subcutaneous adipose tissue to store lipids. Then, lipid spillover promote visceral and ectopic fat accumulation, and subsequently induce insulin resistance in muscle and liver (10,11); together these are considered to be the main pathogenic mechanisms underlying metabolic abnormalities such as hyperglycemia, dyslipidemia, and hypertension (12) (13).

It is known that Asians readily develop metabolic disease, even in the absence of obesity (body mass index (BMI) <25 kg/m^2) (14). Affected individuals exhibit increased adipose tissue, fat accumulation in the liver, and insulin resistance in muscle and liver (14-17). Given our previous finding that impaired insulin sensitivity occurred in muscle even in some non-obese, apparently healthy Japanese men (18), insulin sensitivity may be impaired even in the absence of overt symptoms in a considerable number of non-obese Asians. Indeed, several reports demonstrated that Asians, especially East Asians (16), have a lower fat storage capacity in their subcutaneous adipose tissue compared with other ethnicities (16,19). Thus, one may speculate that adipose tissue insulin sensitivity is easily impaired by only a modest increase of body fat content in non-obese, healthy East Asians, which may in turn impair

insulin sensitivity in muscle, at least in part by increasing the release of FFAs from adipose tissue and subsequently promoting moderate metabolic changes. However, there have been no reports of adipose tissue insulin sensitivity in non-obese, apparently healthy Asians.

Based on the above background, the present study was designed to identify the clinical features of reduced adipose tissue insulin sensitivity in non-obese, apparently healthy subjects. For this purpose, based on our previous data regarding tissue-specific insulin sensitivity derived using two-step euglycemic hyperinsulinemic clamp in non-obese (BMI <25 kg/m^2), apparently healthy Japanese men (15), we analyzed the association of various metabolic parameters with reduced insulin sensitivity in adipose tissue.

Research Design and Methods

Study subject

We studied participants of the Sportology Center Core Study, a prospective observational study involving hypothesis-driven, hypothesis-generating research on the underlying mechanisms of metabolic abnormalities in non-obese subjects (15). That study enrolled non-diabetic Japanese men with a BMI of 21 to 27.5 kg/m^2 (≥21.0 to <27.5 kg/m^2) who were between 30 and 50 years old. The current study analyzed subjects with a BMI of 21 to 25 kg/m^2 (≥21.0 to <25.0 kg/m^2) who were free of cardiometabolic risk factors based on the definition of metabolic syndrome in Japan (20) (Table 1). The cardiometabolic risk factors assessed in this study were hyperglycemia (fasting plasma glucose ≥110 mg/dl), dyslipidemia (triglycerides (TG) ≥150 mg/dl and/or high-density lipoprotein cholesterol (HDL-C) <40 mg/dl), and hypertension (systolic blood pressure ≥130 mmHg and/or diastolic BP ≥85 mmHg). All participants provided written informed consent to participate in the study, which was approved by the ethics committee of Juntendo University (No. 2011042). This study was carried out in accordance with the principles outlined in the Declaration of Helsinki.

Study design

The design of the Sportology Center Core Study was described previously in detail (15). Briefly, after the screening session, all participants visited our institute three times for baseline evaluation. At the first or second visit, each participant underwent an oral glucose tolerance test (OGTT) or peak oxygen uptake test (15). The participants were instructed to stop regular exercise for 10 days before the third visit, and the mean daily physical activity level was evaluated over 7 days with an accelerometer (Lifecorder; Suzuken, Nagoya, Japan). Then, each participant was asked to maintain their mean daily physical activity level (± 10%) as the past 3 days, which was monitored with an accelerometer. The participants were instructed to consume a weight-maintaining standard diet for the 3 days immediately preceding the clamp study. On the third visit, we measured intramyocellular lipid (IMCL) and intrahepatic lipid (IHL) by ^{1}H-magnetic resonance spectroscopy (MRS), and estimated the abdominal visceral fat area (VFA) and subcutaneous fat area (SFA) by magnetic

resonance imaging (MRI). The percent body fat and fat-free mass were measured by the bioimpedance method (InBody 720; Biospace, Tokyo). Then, euglycemic hyperinsulinemic clamp was performed to measure insulin sensitivity in muscle and liver. Surrogate markers of insulin resistance [i.e., the homeostasis model assessment of insulin resistance (HOMA-IR) and Matsuda index] were calculated as described previously (15).

Euglycemic hyperinsulinemic glucose clamp

A two-step euglycemic hyperinsulinemic glucose clamp study was performed with an artificial endocrine pancreas (STG-22; Nikkiso, Shizuoka, Japan) after overnight fasting (15). Briefly, after securing an intravenous cannula in the forearm, a bolus dose [200 mg/m^2 body surface area (BSA)] of [6,6-$^2H_2$]glucose (Cambridge Isotope Laboratories, Tewksbury, MA) was injected intravenously, followed by constant infusion of 2 mg/m^2 BSA per min for 3 h (−180 to 0 min) to measure fasting endogenous glucose production (EGP) (21). This was followed by primed insulin infusion (40 mU/m^2 per min followed by 20 mU/m^2 per min, each lasting 5 min) and continuous insulin infusion at 10 mU/m^2 per min for 3 h (first step) (0 to 180 min). In the second step of the clamp, after a priming insulin infusion (80 mU/m^2 per min followed by 40 mU/m^2 per min, each lasting 5 min), insulin was infused continuously at 20 mU/m^2 per min for 3 h (180 to 360 min). We used a warming blanket for arterialization of the hand vein, and plasma glucose level in arterialized blood was maintained at ~95 mg/dl by a variable 20% glucose infusion containing ~2.5% [6,6-$^2H_2$]glucose. Blood samples were obtained for biochemical analysis at 10-min intervals during the last 30 min of the steady-state period of the first and second steps of the clamp. We also performed blood sampling every 60 min. Enrichment of [6,6-$^2H_2$]glucose in plasma was measured by high-performance liquid chromatography (LTQ-XL-Orbitrap mass spectrometer, Thermo Scientific, CA) as described previously (15).

Calculations

A steady-state equation was used to calculate the rates of EGP and glucose disappearance (Rd) at each step (15). EGP and Rd were normalized by BSA and fat-free mass, respectively (15). Because EGP is known to be suppressed in accordance with insulin concentration at low insulin levels (~20 μU/ml) (22), we divided the percent reduction of EGP at the first step by the steady state serum insulin (SS_{SI}) level during glucose clamp, and used the result as an index of hepatic insulin sensitivity (7). Rd is also known to be enhanced in parallel with serum insulin concentration (22), and therefore Rd at the second step was divided by SS_{SI} and used as an index of muscle insulin sensitivity (23). Adipose tissue insulin sensitivity was calculated according to the degree of insulin-mediated suppression of circulating FFAs (7,24). Briefly, percent reduction of FFAs at the first step was calculated based on the basal and nadir FFA concentrations during the last 1 h of glucose clamp at the first step, then adjusted by the insulin concentration and used as an index of adipose tissue insulin sensitivity (7,24). The metabolic clearance rate for serum insulin (MCRI) during glucose clamp at the

second step was calculated by the following equation (18): MCRI = {IIR/[SS_{SI} − (B_{SI}×SS_{SC}/B_{SC})]}, where IIR = insulin infusion rate, SS_{SI} = steady-state serum insulin during glucose clamp, B_{SI} = basal serum insulin, SS_{SC} = steady-state serum C-peptide during glucose clamp, and B_{SC} = basal serum C-peptide.

^{1}H-MRS and MRI

The IMCL values of the right tibialis anterior and soleus muscles and the IHL of segment 6 in the liver were measured by ^{1}H-MRS (VISART EX V4.40, Toshiba, Tokyo) (25,26). After the measurements, IMCL was quantified by methylene signal intensity (S-fat) using the creatine signal (Cre) as the reference, and calculated as the ratio S-fat/Cre. IHL was quantified by S-fat with H_2O as the internal reference, and calculated as the percentage of H_2O + S-fat [S-fat × 100/(H_2O + S-fat)] (25,26). VFA and SFA were measured with MRI as described previously (26). Briefly, T1-weighted trans-axial scans were obtained, and VFA and SFA at the fourth and fifth lumbar interspaces were measured as described previously using specific software (AZE Virtual Place, Tokyo, Japan) (26).

Biochemical tests

Serum lipids such as total cholesterol, HDL-C, LDL-C, FFA, TG and liver function tests such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) were measured by enzymatic methods and UV methods, respectively (SRL Inc., Tokyo). Plasma insulin concentrations were evaluated by radioimmunoassay (LINCO Research, St Charles, MO). Serum adiponectin concentrations were measured by an 30 enzyme-linked immunosorbent assay (Daiichi Pure Chemicals, Tokyo).

Statistical analysis

Data are presented as mean ± SD or median (interquartile range: IQR). To approximate normal distribution, log-transformed values were used in the analysis, as appropriate. Data of two groups were compared by unpaired t-test or Mann-Whitney U test, as appropriate. Correlation analyses were performed using the Pearson or Spearman correlation coefficient, as appropriate. All statistical tests were two-sided with a 5% significance level. We used SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, NY, USA) for statistical analyses.

Result

Anthropometric characteristics of low- and high-FFA suppression groups

At first step during euglycemic hyperinsulinemic clamp study, SS_{SI} was moderately elevated (Table 2) and circulating FFA level was significantly suppressed (From 530.0 (423.5-637.0) to 67.0 (51.0-94.0) μEq/L, P<0.001) by 86.0 (80.0-90.0) % (Figure 1A, B, Table 2). However, we observed inter-individual differences of changes in circulating FFA levels (Figure 1B) and adipose tissue insulin sensitivity (Percent FFA suppression/insulin at first

step) (Table 2); therefore, to evaluate the clinical features of reduced adipose tissue insulin sensitivity in non-obese, apparently healthy subjects, we divided the subjects into a low-FFA suppression group (n = 26; red line in Figure 1) and high-FFA suppression group (n = 26; blue line in Figure 1) based on the median value of %FFA suppression/insulin at the first step [4.6 (3.3-5.9) (%・μU^{-1}・mL)]. As shown in Table 1, the following parameters were significantly higher in the low-FFA suppression group than the high-FFA suppression group: fasting serum insulin; area under the curve (AUC)-glucose and AUC-insulin during OGTT; HOMA-IR; TG; total body fat content; SFA; and IHL. In contrast, age, Matsuda index, HDL-C, VO_{2peak}, and daily physical activity were significantly lower in the low-FFA suppression group than the high-FFA suppression group. Other adipose tissue-associated factors such as FFA, VFA, IMCL, adiponectin, and C-reactive protein were comparable between the groups. These data suggest that subjects in the low-FFA suppression group were younger and had a lower fitness level, more fat accumulation in the subcutaneous adipose tissue and liver, and relatively reduced insulin sensitivity.

Insulin sensitivity in muscle and liver evaluated by glucose clamp

We evaluated insulin sensitivity in muscle and liver by the gold standard method: two-step hyperinsulinemic euglycemic clamp (Table 2). The SS_{SI} level during glucose clamp at the first and second steps was higher in the low-FFA suppression group than the high-FFA suppression group, due to impaired MCRI in the low-FFA suppression group. This difference is consistent with the finding of elevated fasting serum insulin, but not C-peptide, in the low-FFA suppression group compared with the high-FFA suppression group (Table 1).

As shown in Table 2, hepatic insulin sensitivity (percent reduction of EGP/SS_{SI} at the first step) (7) was comparable between the two groups. In addition, the basal EGP level was significantly lower in the low-FFA suppression group than in the high-FFA suppression group, probably due to higher insulin levels in the former group.

The Rd level at the second step, which indicates the amount of glucose uptake in the peripheral tissues (mainly muscle), was significantly lower in the low-FFA suppression group than in the high-FFA suppression group, although SS_{SI} was higher in the low-FFA suppression group. Thus, muscle insulin sensitivity (Rd/SS_{SI} at the second step) (23) was significantly lower in the low-FFA suppression group than in the high-FFA suppression group, and the difference of Rd/SS_{SI} between the groups was more prominent than the difference of Rd. These data suggest that the low-FFA suppression group was characterized by lower MCRI and impaired insulin sensitivity in muscle, but not in liver.

Correlations between %FFA suppression/insulin and other parameters

Correlation analysis was performed to further investigate the association between %FFA suppression/insulin, as a surrogate for adipose tissue insulin sensitivity, and various metabolic parameters (Table 3). In this analysis, parameters with p values less than 0.1 in Tables 1 and 2 and several parameters associated with adipose tissue (FFA, VFA, and IMCL)

were selected. %FFA suppression/insulin correlated significantly with percent body fat and SFA, but not with VFA. In terms of ectopic fat, %FFA suppression/insulin correlated significantly with IHL, but not with IMCL in the tibialis anterior and soleus muscles. In addition, insulin sensitivity in muscle, but not in liver, was significantly correlated with %FFA suppression/insulin. On the other hand, %FFA suppression/insulin was not significantly correlated with age, FFA, AUC-glucose during OGTT, daily physical activity, or basal EGP.

Discussion

Adipose tissue insulin resistance has been observed in obese subjects and is considered to be an early change in metabolic syndrome (6,13). Here, to investigate whether adipose tissue insulin resistance occurs before the onset of insulin resistance in other tissues, we investigated the characteristics of apparently healthy, non-obese Japanese subjects with reduced adipose tissue insulin sensitivity, because Asians are known to easily develop metabolic disease and insulin resistance without obesity. The results showed that compared with subjects in the high-FFA suppression group, those in the low-FFA suppression group had more fat accumulation in abdominal subcutaneous adipose tissue and liver and reduced insulin sensitivity in muscle. They also had a lower fitness level, decreased insulin clearance, moderately elevated TG, and lowered HDL-C. Correlation analysis demonstrated that all these factors were significantly correlated with adipose tissue insulin sensitivity.

In this study, we recruited only Japanese subjects. In Asians, unlike in Caucasians, the World Health Organization suggested defining overweight as BMI $\geq$23 kg/m^2 and obesity as BMI $\geq$25 kg/m^2 (27), because Asians easily develop metabolic disease at lower BMI (27). East Asians were found to have the lowest amount of subcutaneous fat and the highest amount of visceral fat with increasing adiposity compared with other ethnicities, including White, Black, Hispanic and Southeast Asian (16). In addition, Asians have relatively high body fat compared with Caucasians of similar BMI (16,17). These data imply that non-obese East Asians have a low subcutaneous adipose tissue capacity despite higher body fat content, and thus there are potentially many non-obese Asians with relatively high percent body fat who may be at high risk of lipid spillover and metabolic abnormalities. Indeed, our data showed that percent body fat was higher in the low-FFA suppression group (22.1%) than the high-FFA suppression group (18.6%), and this difference was associated with a greater amount of abdominal subcutaneous adipose tissue. It was shown that the percent body fat corresponding to BMI $\geq$25 kg/m^2 was 23% in Japanese men aged between 20 and 39 years, and 24% in those aged between 40 and 59 years (28). Thus, the percent body fat in the low-FFA suppression group was similar to that in obese Japanese men, while the high-FFA suppression group had normal body fat. Taken together, even non-obese, apparently healthy Japanese men with a relatively high percent body fat are already at high risk of exceeding the

capacity to store lipids in adipose tissue, and thus would be classified into the low-FFA suppression group.

According to the lipid spillover hypothesis, adipose tissue insulin resistance is induced by weight gain and leads to the reduced ability of subcutaneous adipose tissue to store lipids. Lipid spillover then occurs, promoting visceral and ectopic fat accumulation and subsequently inducing insulin resistance in muscle and liver (5,10-13). In fact, in this study, adipose tissue insulin sensitivity was negatively correlated with percent body fat and SFA, and positively correlated with muscle insulin sensitivity; however, our observations were not always consistent with the spillover hypothesis. For example, although the low-FFA suppression group had impaired insulin sensitivity in muscle, their IMCL level was similar to that of the high-FFA suppression group. On this point, the accumulation of IMCL also occurred in insulin-sensitive subjects with high maximum oxygen uptake (athlete's paradox) (29). Because the VO_{2peak} was significantly higher in the high-FFA suppression group than the low-FFA suppression group, the fact that subjects in the former group had high maximum oxygen uptake and elevated IMCL may have resulted in an overall reduction in IMCL difference between the two groups. In contrast, IHL was higher in the low-FFA suppression group than the high-FFA suppression group. Consistent with this data, previous studies demonstrated that FFAs released from adipose tissue were the main substrate for lipid accumulation in the liver in non-alcoholic fatty liver disease patients (30), and adipose tissue insulin resistance was associated with fat accumulation and insulin resistance in the liver (7,8). However, hepatic insulin sensitivity was comparable between the two groups in the present study. The level of IHL in the low-FFA suppression group was moderate and below the definition of fatty liver (IHL >5%), thus it might not have been sufficient to impair hepatic insulin sensitivity. In addition, it is also possible that IHL accumulation may not be a main determinant of hepatic insulin resistance. For example, hepatic insulin resistance was not evident in non-obese Japanese type 2 diabetes with fatty liver (31). Large cohort study (n=352) also demonstrated that IHL accumulation was not associated with hepatic insulin resistance (32).

It has been suggested that visceral adipose tissue is more resistant to insulin suppression of lipolysis than subcutaneous adipose tissue (33,34). Thus, compared with abdominal adipose tissue, abdominal visceral adipose tissue is likely to be more associated with low-FFA suppression; however, not visceral fat, but subcutaneous fat area was well associated with low-FFA suppression in the present study (Table 1, 3). Concerning, in non-obese adults, upper body subcutaneous fat is major source (>60%) of whole body FFA release at fasting and insulin-suppressed conditions (12,33,35), probably due to small amount of visceral fat volume in non-obese subjects. From these, we suppose that, in non-obese men without massive visceral fat accumulation, visceral fat does not much contribute to systemic FFA

concentration, thus visceral fat area was not associated with FFA suppression in the present study.

In this study, impaired adipose tissue insulin sensitivity was also closely associated with other metabolic changes, including hyperinsulinemia with decreased MCRI, moderately increased TG, and decreased HDL-C. Although our data could not identify causal relationships between the correlated parameters, previous reports suggested that circulating FFAs could induce all of these metabolic changes. For example, elevated FFA levels caused by lipid infusion decreased MCRI and elevated peripheral insulin concentrations (36,37). Also, FFA stimulated insulin secretion (38). Thus, theoretically, impaired adipose tissue insulin sensitivity enhances peripheral insulin levels and compensates for decreased muscle insulin sensitivity. It was also shown that adipose tissue insulin resistance was associated with hyper-triglyceridemia (39) and the overproduction of FFAs, and that very low density lipoprotein-TG reduced circulating HDL-C (40). Hence, although speculative, reduced adipose tissue insulin sensitivity could be upstream of associated clinical factors.

We found that the low-FFA suppression group was also characterized by younger age and lower fitness level than the high-FFA suppression group. Growth hormone (GH) and catecholamines were shown to increase lipolysis, and GH secretion and catecholamine responsiveness were decreased by aging (6,41) (42). Indeed, aging was associated with adipose tissue insulin sensitivity in the present study. In terms of fitness level, it was reported that high-intensity interval exercise improved adipose tissue insulin resistance in an animal model (43). However, aerobic exercise was reported not to change adipose tissue insulin resistance in human type 2 diabetes (44). Hence, the role of fitness level on adipose tissue insulin sensitivity is still unclear.

Our study has a few limitations. First, we did not directly measure FFA kinetics by tracer. Ra-FFA or Ra-glycerol suppression during glucose clamp has been recognized as the gold standard to evaluate adipose tissue insulin sensitivity, while %FFA suppression during glucose clamp and Adipo-IR (fasting FFA x fasting insulin) (24) were used as surrogate markers. Recent data suggested that %FFA suppression during glucose clamp was highly correlated ($r = 0.899$) and Adipo-IR was moderately correlated ($r = -0.526$) with tracer-determined suppression of Ra-glycerol by insulin (24). On the other hand, the insulin concentration required for 50% suppression of lipolysis (IC_{50}) has also been proposed as a gold standard for adipose tissue insulin sensitivity (6). We preliminarily calculated IC_{50} for 50% suppression of circulating FFAs by insulin and found that it was significantly correlated with adipose tissue insulin sensitivity as calculated in the present study ($r = -0.690$). This confirms the validity of the method used to determine adipose tissue insulin sensitivity in this study. Second, we included Japanese men only. Fat distribution and metabolism are different in women, and thus our data may not be generalizable to women. Similarly, our results may not be applicable to other ethnic groups in Asians. Indeed, fat distribution in East Asians was

different from that in South Asians (16). In addition, it is also still unclear whether our data is applicable to other East Asians. Finally, we included a relatively small number of subjects. However, to precisely evaluate tissue specific insulin sensitivity, we used the two-step clamp method to accurately measure insulin effects on muscle, liver, and adipose tissue. To the best of our knowledge, our cohort of over 50 non-obese, healthy subjects is the largest ever used with the 2-step glucose clamp technique.

In conclusion, in non-obese, apparently healthy Japanese men, reduced adipose tissue insulin sensitivity was associated with more fat accumulation in subcutaneous fat and liver, lower insulin clearance, muscle insulin resistance, and an increased risk of dyslipidemia. Thus, even in this population, adipose tissue insulin sensitivity may be impaired in correlation with body fat content and may cause moderate metabolic changes. However, the causal relationships involved have not been identified, and further longitudinal studies are required.

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Disclosure Summary:

All authors declare no conflict of interest.

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Figure 1. Individual free fatty acid (FFA) levels (A) and relative changes of FFA from baseline (B) during euglycemic hyperinsulinemic clamp study. Red line; subjects in low-FFA suppression group, Blue line; subjects in high-FFA suppression group.

Table 1. Clinical characteristics of the low- and high-FFA suppression groups.

	Total	Low-FFA suppression	High-FFA suppression	p value
n	52	26	26	
Age (years)	41.0 (36.0-45.8)	39.0 (33.8-41.3)	42.5 (38.8-46.3)	**0.003**
BMI (kg/m^2)	23.1±1.0	23.2±0.9	23.0±1.1	0.383
Systolic blood pressure (mmHg)	118.4±7.0	117.4±6.8	119.3±7.3	0.349
Diastolic blood pressure (mmHg)	75.3±5.6	74.3±5.6	76.2±5.6	0.239
Fasting plasma glucose (mg/dL)	93.4±6.8	93.2±6.6	93.6±7.1	0.810
Fasting serum insulin (μU/mL)	4.9±2.0	6.1±1.8	3.8±1.5	**<0.001**
Fasting serum C-peptide (ng/mL)	1.23±0.38	1.41±0.38	1.06±0.29	0.077
AUC-glucose during OGTT ($mg/dL \cdot min \cdot 10^3$)	21.3±27.8	22.4±25.8	20.2±25.7	**0.003**
AUC-insulin during OGTT ($IU/mL \cdot min \cdot 10^3$)	6.41±3.30	8.01±3.24	4.81±2.52	**<0.001**
HOMA-IR	1.14±0.49	1.41±0.46	0.87±0.35	**<0.001**

Matsuda index	6.9 (4.2-11.0)	4.7(3.6-6.7)	10.7(7.5-12.1)	**<0.001**
Free fatty acid (μEq/L)	345.4±112.0	370.4±102.1	320.5±117.6	0.109
Triglyceride (mg/dL)	105.5±46.5	121.7±51.7	89.4±34.5	**0.011**
High-density lipoprotein cholesterol (mg/dL)	58.6±13.5	54.2±12.0	63.1±13.6	**0.015**
HbA1c (%)	4.9±0.2	4.9±0.2	4.8±0.3	0.648
Aspartate aminotransferase (U/L)	19.0 (15.3-22.0)	19.0 (17.0-21.0)	20.0 (14.8-23.3)	0.308
Alanine aminotransferase (U/L)	19.0 (15.0-22.0)	19.5 (16.5-22.5)	19.0 (13.8-22.3)	0.893
Percent body fat (%)	20.4±4.9	22.1±4.7	18.6±4.6	**0.009**
Abdominal visceral fat area (cm^2)	75.2±27.5	80.8±22.6	69.3±31.2	0.139
Abdominal subcutaneous fat area (cm^2)	107.0±39.6	123.4±34.1	90.0±38.4	**0.002**
High molecular weight adiponectin (ng/mL)	1.78±1.18	1.74±0.88	1.83±1.44	0.786
C-reactive protein (ng/mL)	186.6 (114.7-496.4)	249.8 (138.2-527.5)	153.6 (84.0-427.2)	0.328
Intramyocellular lipid in TA (S-fat/Cre)	3.2±1.9	3.5±1.6	2.8±2.1	0.249
Intramyocellular lipid in SOL (S-fat/Cre)	12.6±6.7	12.6±7.2	12.7±6.3	0.959
Intrahepatic lipid (%)	0.99 (0.05-2.04)	1.61 (0.12-2.91)	0.33 (0.00-1.13)	**0.023**
VO_{2peak} (ml/kg per min)	35.7±7.1	33.7±6.5	37.7±7.1	**0.045**
Daily physical activity (METs･h)	4.95±2.19	4.27±1.39	5.65±2.61	**0.022**

Data are mean±SD or median (interquartile range).

AUC, area under the curve; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; TA, tibialis anterior muscle; SOL, soleus muscle; S-fat, methylene signal intensity; Cre, creatine signal; VO_{2peak}, peak oxygen consumption.

Table 2. Euglycemic hyperinsulinemic clamping data in the low- and high-FFA suppression groups.

	Total	Low-FFA suppression	High-FFA suppression	p value
n	52	26	26	
SS_{SI} at first step (μU/mL)	19.0±3.8	21.3±2.9	17.4±2.5	**<0.001**
SS_{SI} at second step (μU/mL)	36.2±5.1	38.6±4.0	33.6±4.9	**<0.001**
MCRI (ml/min per m^2)	594.1 (561-663)	569.7 (535-592)	621.3 (578-709)	**0.001**
Basal EGP ($mg/m^2 \cdot min^{-1}$)	80.3±6.4	78.2±5.2	82.4±6.9	**0.008**
Percent reduction of EGP at first step (%)	70.8±19.5	73.7±16.0	67.9±22.3	0.285
Percent reduction of EGP at second step (%)	89.5 (82.2-95.4)	89.6 (83.0-94.3)	86.2 (80.2-96.5)	0.159
Percent reduction of EGP/SS_{SI} at first step ($\%/\mu U \cdot ml^{-1}$)	3.7±1.0	3.5±0.7	4.0±1.3	0.105
Rd at first step (mg/kg FFM$\cdot min^{-1}$)	4.5±1.3	4.4±1.2	4.6±1.3	0.624
Rd at second step (mg/kg FFM$\cdot min^{-1}$)	8.6±2.0	7.9±2.0	9.4±1.8	**0.013**
Rd /SS_{SI} at second step (mg/kg FFM$\cdot min^{-1} \cdot \mu U^{-1} \cdot$mL)	0.23 (0.19-0.30)	0.20 (0.16-0.26)	0.28 (0.23-0.33)	**<0.001**
Percent FFA suppression at first step (%)	86.0 (80.0-90.0)	82.0 (73.3-87.0)	89.0 (85.3-91.0)	**0.006**
Percent FFA suppression/insulin at first step ($\%\cdot\mu U^{-1}\cdot$mL)	4.6±1.3	3.7±0.67	5.4±1.2	**<0.001**

Data are mean±SD or median (interquartile range).

SS_{SI}, steady state serum insulin; MCRI, metabolic clearance rate for serum insulin; EGP, endogenous glucose production; Rd, rate of disappearance; FFM, fat-free mass.

Table 3. Results of single correlation analysis between %FFA suppression/insulin and metabolic parameters.

	%FFA suppression/insulin	
	r	p
Age	0.25	0.078
Fasting serum insulin	-0.60	**< 0.001**
Fasting serum C-peptide	-0.41	**0.003**
HOMA-IR	-0.59	**<0.001**
OGTT-AUC Glucose	-0.18	0.19
OGTT-AUC insulin	-0.46	**0.001**
Free fatty acid	-0.20	0.16
Triglyceride	-0.31	**0.028**
Matsuda index	0.56	**<0.001**

High-density lipoprotein cholesterol	0.41	**0.002**
Percent body fat	-0.33	**0.016**
Abdominal visceral fat area	-0.10	0.48
Abdominal subcutaneous fat area	-0.29	**0.039**
Intramyocellular lipid in TA	-0.14	0.34
Intramyocellular lipid in SOL	0.036	0.81
Intrahepatic lipid	-0.34	**0.020**
VO_{2peak}	0.28	**0.045**
Daily physical activity	0.26	0.07
SS_{SI} at first step	-0.61	**<0.001**
SS_{SI} at second step	-0.47	**0.001**
MCRI	0.45	**0.001**
Basal EGP	0.23	0.100
Percent reduction of EGP/SS_{SI} at first step	0.11	0.440
Rd at second step	0.41	**0.003**
Rd/SS_{SI} at second step	0.58	**<0.001**
%FFA suppression at first step	0.50	**<0.001**

HOMA-IR, homeostasis model assessment of insulin resistance; AUC, area under the curve; OGTT, oral glucose tolerance test; TA, tibialis anterior muscle; SOL, soleus muscle; VO_{2peak}, peak oxygen consumption; SS_{SI}, steady state serum insulin; MCRI, metabolic clearance rate for serum insulin; EGP, endogenous glucose production; Rd, rate of disappearance.

Figure 1