Higher C-Peptide Level During Glucose Clamp Is Associated With Muscle Insulin Resistance in Nonobese Japanese Men

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Context: Circulating C-peptide is generally suppressed by exogenous insulin infusion. However, steady-state serum C-peptide (SS_{SC}) levels during hyperinsulinemic-euglycemic clamp in obese subjects are higher than in healthy subjects, which may contribute to hyperinsulinemia to compensate for insulin resistance. Even in healthy subjects, interindividual variations in SS_{SC} levels are present; however, the characteristics of subjects with high SS_{SC} levels in those populations have not been fully elucidated.

Objective: To investigate the clinical parameters associated with interindividual variations in SS_{SC} levels in apparently healthy, nonobese Japanese men.

Design and Participants: We studied 49 nonobese (BMI < 25 kg/m^2), healthy Japanese men. We evaluated SS_{SC} and insulin sensitivity using hyperinsulinemic-euglycemic clamp with tracer. Intrahepatic lipid (IHL) was measured using proton magnetic resonance spectroscopy.

Results: We divided subjects into high and low SS_{SC} groups based on the median SS_{SC} value and compared their clinical parameters. Compared with the low SS_{SC} group, the high SS_{SC} group had IHL accumulation, impaired muscle insulin sensitivity, reduced insulin clearance, and hyperinsulinemia during a 75-g oral glucose tolerance test (OGTT). All of these factors were significantly correlated with SS_{SC}.

Conclusions: In healthy, nonobese men, higher SS_{SC} was associated with impaired muscle insulin sensitivity, IHL accumulation, and hyperinsulinemia during OGTT. These findings suggest that higher endogenous insulin secretion during hyperinsulinemia, along with reduced insulin clearance, may be an

Abbreviations: AUC-insulin, area under the curve of insulin; BMI, body mass index; BSA, body surface area; Cre, creatine signal; DPG, diastolic blood pressure; EGP, endogenous glucose production; FFA, free fatty acid; FFM, fat-free mass; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; MCRI, metabolic clearance rate of insulin; MRS, magnetic resonance spectroscopy; OGTT, oral glucose tolerance test; Rd, rate of glucose disappearance; *S*-fat, methylene signal intensity; SBP, systolic blood pressure; SFA, subcutaneous fat area; SS_{SC}, steady-state serum C-peptide; SS_{SI}, steady-state serum insulin; TG, triglyceride; VFA, visceral fat area.

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early change to maintain metabolic status in the face of moderate muscle insulin resistance, even in healthy, nonobese men.

Freeform/Key Words: hyperinsulinemia, insulin resistance, nonobese, feedback inhibition of insulin secretion

Insulin resistance is important in the pathogenesis of type 2 diabetes and metabolic syndrome [1]. Hyperinsulinemia, observed in individuals with insulin resistance, is considered as a compensatory mechanism for insulin resistance, resulting from increased insulin secretion [2, 3] and decreased insulin clearance [4–6]. Insulin secretion is regulated by complex stimulatory and inhibitory mechanisms in β cells [7]. Whereas blood glucose levels are a most important stimulator of insulin secretion [8–10], previous studies have shown that endogenous insulin secretion is directly or indirectly suppressed by exogenous insulin infusion [11–18]. Whereas endogenous insulin secretion during exogenous insulin infusion was evaluated by steady-state serum C-peptide (SS_{SC}) levels during hyperinsulinemic-euglycemic clamp, obese subjects with insulin resistance had higher SS_{SC} levels than healthy subjects [12–15]. This phenomenon suggests that individuals with insulin resistance have the ability to secrete endogenous insulin, even in hyperinsulinemic-euglycemic conditions, which may contribute to hyperinsulinemia during fasting and postprandial states; this seems to be a compensatory mechanism for insulin resistance.

C-Peptide levels during hyperinsulinemic-euglycemic clamp are generally lower in healthy vs insulin-resistant obese individuals [12–15]; however, SS_{SC} levels during clamp are highly variable, even in healthy controls, suggesting the presence of interindividual variations in SS_{SC}. Indeed, at least previous three reports failed to detect substantial differences in SS_{SC} levels between healthy subjects and insulin-resistant obese subjects [18–20]. On the contrary, Mari *et al.* [21] has demonstrated that endogenous insulin secretion is enhanced during the hyperinsulinemic isoglycemic clamp in insulin-sensitive subjects compared with insulin-resistant subject, which is completely opposite to previous studies [12–15]. Thus, the association of SS_{SC} and insulin sensitivity is still unclear. Furthermore, insulin sensitivity could be impaired in muscle, liver, and adipose tissue in nonobese subjects, independently [22]; however, it is also unclear which site of insulin resistance is associated with SS_{SC} levels during glucose clamp. Finally, although Asians secrete much less insulin than other ethnicities [23], no studies evaluated the significance of SS_{SC} levels in Asians.

In this context, the current study was designed to examine SS_{SC} levels during hyperinsulinemic-euglycemic clamp in nonobese, healthy Japanese individuals and clarify the association between SS_{SC} levels and metabolic characteristics. We measured tissue-specific insulin resistance as well as SS_{SC} levels in apparently healthy, nonobese [body mass index (BMI) $<$ 25 kg/m^2] Japanese men using a two-step hyperinsulinemic-euglycemic clamp.

1. Research Design and Methods

A. Study Subjects

SS_{SC} was assessed in participants of the Sportology Center Core Study, a prospective observational study to support hypothesis-driven, hypothesis-generating research on the mechanisms underlying metabolic abnormalities in nonobese subjects [22]. The study participants were described in detail previously [22]. To assess the role of SS_{SC} in apparently healthy, nonobese men, we selected those with a BMI of 21.0 to $<$25.0 kg/m^2 who did not have cardiometabolic risk factors, such as elevated fasting plasma glucose (FPG) $\geq$ 110 mg/dL, dyslipidemia [triglycerides (TG) $\geq$ 150 mg/dL or high-density lipoprotein-cholesterol

(HDL-C) < 40 mg/dL], or hypertension [systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg] [24]. Among the 52 subjects meeting these criteria, C-peptide values were not available in three participants, leaving 49 apparently healthy, nonobese Japanese men in the study. All participants gave written, informed consent for study participation. The study was approved by the Ethics Committee of Juntendo University and carried out in accordance with the principles outlined in the Declaration of Helsinki.

B. Study Design

The design of the Sportology Center Core Study was described previously in detail [22]. In brief, after the screening session, all participants visited our institution three times for baseline evaluation. During the first two visits, each participant underwent a 75-g oral glucose tolerance test (OGTT) or peak oxygen uptake test, as described previously [22, 25]. Participants were instructed to discontinue regular exercise for 10 days before the third visit. The mean daily physical activity level was evaluated over 7 days with an accelerometer (Lifecorder; Suzuken, Nagoya, Japan). Next, each participant was asked to maintain his daily physical activity level at the mean daily physical activity level ± 10% during the last 3 days. Daily physical activity during these 3 days was monitored using an accelerometer. Participants were asked to fast overnight on the eve of the experiment. On the day of the experiment, we measured intramyocellular lipid (IMCL) and intrahepatic lipid (IHL) levels using ^{1}H-magnetic resonance spectroscopy (MRS). Total body fat content and fat-free mass (FFM) were measured using the bioimpedance method (InBody; Biospace, Tokyo, Japan) [26]. Furthermore, visceral fat area (VFA) and subcutaneous fat area (SFA) were estimated using MRI. Next, hyperinsulinemic-euglycemic clamp was performed to measure insulin sensitivity in muscle, liver, and adipose tissue, as described below. Homeostasis model assessment of insulin resistance (HOMA-IR) and Matsuda index were calculated as surrogate markers of insulin resistance, as described previously [22, 27].

C. Hyperinsulinemic-Euglycemic Clamp

Participants were instructed to consume a standard weight-maintaining diet on the 3 days immediately preceding the clamp study. In addition, they were asked to refrain from alcohol starting the day before the clamp study. After an overnight fast, a two-step hyperinsulinemic-euglycemic glucose clamp study was performed with an artificial endocrine pancreas (STG 22; Nikkiso, Shizuoka, Japan) [22]. In brief, after securing an intravenous cannula in the forearm, a bolus dose [200 mg/m^2 body surface area (BSA)] of [6,6-^{2}H$_2$]glucose (Cambridge Isotope Laboratories, Tewksbury, MA) was injected intravenously, followed by constant infusion of 2 mg/m^2 BSA per minute for 3 hours (−180 to 0 minutes) to measure fasting endogenous glucose production (EGP) [28]. This was followed by the first step of the clamp, which consisted of primed insulin infusion (40 mU/m^2 per minute for 5 minutes, followed by 20 mU/m^2 per minute for 5 minutes) and continuous insulin infusion at 10 mU/m^2 per minute for 3 hours (0 to 180 minutes). In the second step of the clamp, after a priming insulin infusion (80 mU/m^2 per minute for 5 minutes, followed by 40 mU/m^2 per minute for 5 minutes), insulin was infused continuously at 20 mU/m^2 per minute for 3 hours (180 to 360 minutes). The infusion of [6,6-^{2}H$_2$]glucose was decreased by 75% of the initial infusion rate during the first step and 85% of the basal rate during the second step to maintain constant plasma glucose enrichment [29]. We used a warming blanket for arterialization of the hand vein. Plasma glucose levels in arterialized blood were maintained at ~95 mg/dL by a variable 20% glucose infusion containing ~2.5% [6,6-^{2}H$_2$]glucose. Blood samples were drawn for biochemical analysis at 10-minute intervals at 30 minutes before the clamp and during the steady-state periods of the two clamp steps. Enrichment of [6,6-^{2}H$_2$]glucose in plasma was measured by HPLC (LTQ-XL-Orbitrap mass spectrometer; Thermo Fisher Scientific, MA), as described previously [22]. A steady-state equation was used to calculate the rate of EGP and rate of glucose disappearance (Rd) at each step [30]. EGP and Rd were normalized by BSA and FFM,

respectively [22]. We divided percent reduction of EGP at the first step by steady-state serum insulin (SS_{SI}) and used it as an index of hepatic insulin sensitivity [31]. Likewise, Rd at the second step was divided by SS_{SI} and used as an index of muscle insulin sensitivity [32]. Adipose tissue insulin sensitivity was reflected by the degree of insulin-mediated suppression of circulating free fatty acid (FFA) [31, 33]. In brief, percent reduction of FFA at the first step was calculated by basal and nadir FFA concentrations during the last hour of glucose clamp during the first step and adjusted by insulin concentration; this was used as an index of adipose tissue insulin sensitivity. The metabolic clearance rate of insulin (MCRI) during the second step of the glucose clamp was calculated using the following equation [12, 34]: MCRI = **(**$IIR/[SS_{SI} - (B_{SI} \times SS_{SC}/B_{SC})]$**)**, where IIR = insulin infusion rate, SS_{SI} = SS_{SI} during glucose clamp, B_{SI} = basal serum insulin, SS_{SC} = SS_{SC} during glucose clamp, and B_{SC} = basal serum C-peptide.

D. ^{1}H-MRS

IMCL values of the right tibialis anterior and soleus muscles and IHL of segment 6 of the liver were based on ^{1}H-MRS (VISART EX V4.40; Toshiba, Tokyo, Japan) [35, 36]. After making these measurements, IMCL was quantified by methylene signal intensity (*S*-fat) using a 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 a percentage of H_2O + *S*-fat [*S*-fat × 100/(H_2O + *S*-fat)] [35, 36].

E. Abdominal VFA and SFA

The area of abdominal visceral and subcutaneous fat was measured using MRI, as described previously [36]. In brief, T1-weighted transaxial scans were obtained, and the area of abdominal visceral and subcutaneous fat at the fourth and fifth lumbar interspaces was measured, as described previously, using specific software (AZE Virtual Place, Tokyo, Japan) [36].

F. Statistical Analysis

Data are presented as means ± SD or medians (range: 25% to 75%). To approximate the normal distribution, log-transformed values were used in the analysis as appropriate. Data were compared using the unpaired Student's *t*-test. The relationship between SS_{SC} levels during the second step and various metabolic parameters was assessed using Pearson or Spearman correlation coefficients as appropriate. All statistical tests were two sided with a significance level of 5%.

2. Results

A. Insulin and C-Peptide Levels During Hyperinsulinemic-Euglycemic Clamp

Table 1 summarizes the clinical characteristics of the study subjects. The entire group's mean values for cardiometabolic risk factors and renal function were within the normal range. SS_{SI} levels during the second step of the glucose clamp in all subjects reached 36.4 ± 5.2 μU/mL (Table 2). The mean SS_{SC} level during the second step was 0.87 ± 0.39 ng/mL (Table 2). We then divided the subjects into the high SS_{SC} group (n = 24) and low SS_{SC} group (n = 25), based on the median value of SS_{SC} (0.88 ng/mL) during the second step of the glucose clamp. There was no significant difference in basal C-peptide levels between the two groups (Table 1). On the other hand, during the second step of the glucose clamp, the high SS_{SC} group had a mean SS_{SC} level that was 2.1 times higher than the level in the low SS_{SC} group (Table 2).

Table 1 summarizes the clinical characteristics in each group. There were no significant differences in the prevalence of risk factors for metabolic syndrome, including SBP, FPG, TG, and HDL-C between the low SS_{SC} group and the high SS_{SC} group, except for DBP. Whereas fasting C-peptide levels were comparable between the groups, fasting serum insulin levels

Table 1. Clinical Characteristics of the Low SS_{SC} and High SS_{SC} Groups

	Overall	Low SS_{SC}	High SS_{SC}	*P* Value
n	49	25	24	
Age, y	40.0 (36.0–45.0)	41.0 (39.0–46.0)	38.5 (34.8–42.0)	0.078
BMI, kg/m^2	23.1 ± 1.0	23.1 ± 1.2	23.1 ± 0.9	0.917
SBP, mmHg	118.6 ± 7.0	118.9 ± 6.7	118.3 ± 7.5	0.774
DBP, mmHg	75.4 ± 5.7	77.1 ± 5.0	73.6 ± 5.9	**0.032**
FPG, mg/dL	93.2 ± 6.8	94.7 ± 6.7	91.7 ± 6.7	0.123
Fasting serum insulin, μU/mL	4.9 ± 2.1	4.15 ± 1.83	5.67 ± 2.02	**0.008**
Fasting serum C-peptide, ng/mL	1.23 ± 0.38	1.14 ± 0.39	1.34 ± 0.34	0.065
Blood urea nitrogen, mg/dL	12.8 ± 2.8	12.7 ± 3.0	13.0 ± 2.7	0.725
Creatinine, mg/dL	0.80 ± 0.09	0.80 ± 0.10	0.79 ± 0.09	0.537
AUC-glucose during OGTT, mg · min/dL · 10^3	21.3 ± 2.9	21.0 ± 2.2	21.6 ± 3.5	0.497
AUC-insulin during OGTT, μU · min/mL · 10^3	5.2 ± 2.8	3.9 ± 2.0	6.6 ± 3.0	**0.001**
HOMA-IR	1.13 ± 0.49	0.97 ± 0.43	1.29 ± 0.51	**0.021**
Insulinogenic index	0.95 ± 0.68	0.81 ± 0.50	1.09 ± 0.81	0.142
Matsuda index	6.7 (4.2–10.9)	8.9 (6.5–12.1)	4.7 (3.7–6.9)	**0.029**
FFAs, μEq/L	335 ± 105	317.4 ± 112.0	353.8 ± 96.8	0.231
TG, mg/dL	108 ± 46	98.4 ± 49.9	107 ± 57	0.134
HDL-C, mg/dL	59.0 ± 13.8	59.6 ± 15.0	58.4 ± 12.7	0.776
HbA1c (%)	4.9 ± 0.2	4.9 ± 0.2	4.9 ± 0.3	0.708
High molecular-weight adiponectin, ng/mL	1.82 ± 1.21	1.73 ± 1.15	1.92 ± 1.29	0.593
C-Reactive protein, ng/mL	177 (125–490)	141 (93–318)	314 (160–527)	0.485
IMCL in TA, *S*-fat/Cre	3.2 ± 1.9	2.8 ± 1.8	3.6 ± 1.9	0.133
IMCL in SOL, *S*-fat/Cre	12.8 ± 6.8	12.4 ± 8.0	13.3 ± 5.4	0.662
IHL, %	0.99 (0.05–2.04)	0.21 (0.01–1.02)	1.51 (0.23–2.77)	**0.025**
% Body fat	20.1 ± 5.0	19.5 ± 4.8	20.8 ± 5.3	0.396
Abdominal VFA, cm^2	75.3 ± 28.0	73.1 ± 30.6	77.6 ± 25.6	0.582
Abdominal SFA, cm^2	106 ± 40	96.4 ± 42.7	116.9 ± 34.8	0.072
VO_{2peak}, mL/kg/min	36.0 ± 7.0	37.3 ± 8.0	34.5 ± 5.6	0.171
Daily physical activity, METs· h	4.98 ± 2.24	5.38 ± 2.85	4.56 ± 1.28	0.201

Data are means ± SD or medians (interquartile range). Boldface represents statistical significance ($P < 0.05$).
Abbreviations: AUC-glucose, area under the curve of glucose; AUC-insulin, area under the curve of insulin; HbA1c, hemoglobin A1c; METs, metabolic equivalents; SOL, soleus; TA, tibialis anterior; VO_{2peak}, peak oxygen uptake.

were significantly higher in the high SS_{SC} group (Table 1), probably as a result of the difference in insulin clearance between the two groups. Whereas FPG levels were comparable between the two groups, HOMA-IR was higher in the high SS_{SC} group compared with the low SS_{SC} group. Whereas % body fat, abdominal subcutaneous, and visceral adipose tissue and IMCL were comparable between the two groups, IHL was significantly higher in the high SS_{SC} group compared with the low SS_{SC} group. In addition, as shown in Fig. 1, whereas glucose excursion during the 75-g OGTT was similar between the two groups, the high SS_{SC} group had a significantly higher area under the curve of insulin (AUC-insulin) than the low SS_{SC} group (Table 1 and Fig. 1). Thus, the Matsuda index, an index of insulin sensitivity, was lower in the high SS_{SC} group compared with the low SS_{SC} group (Table 1). These data suggest that the high SS_{SC} group was characterized by impaired insulin sensitivity, hyperinsulinemia, and moderate IHL accumulation.

B. Insulin Sensitivity in Adipose Tissue, Muscle, and Liver Evaluated by Glucose Clamp

We evaluated insulin sensitivity using the gold-standard method, the two-step hyperinsulinemic-euglycemic clamp (Table 2). The high SS_{SC} group had significantly higher SS_{SI} levels than the low SS_{SC} group during both steps of the glucose clamp. In theory, higher endogenous insulin secretion during a hyperinsulinemic-euglycemic state in the high SS_{SC} group contributed to this difference (Table 2). On the other hand, insulin clearance was decreased in the high SS_{SC} group, which also contributed to elevated insulin levels during the clamp study. Based on the calculated

Table 2. Hyperinsulinemic-Euglycemic Clamp Data in the Low SS_{SC} and High SS_{SC} groups

	Overall	Low SS_{SC}	High SS_{SC}	*P* Value
n	49	25	24	
SS_{SI} at the first step, μU/mL	19.2 ± 3.5	17.6 ± 3.1	20.8 ± 3.3	**0.001**
SS_{SI} at the second step, μU/mL	36.4 ± 5.2	33.5 ± 4.5	39.4 ± 4.0	**<0.001**
SS_{SC} at the second step, ng/mL	0.87 ± 0.39	0.57 ± 0.24	1.19 ± 0.24	**<0.001**
% Suppression of C-peptide at second step, %	28.1 ± 30.1	48. 4 ± 20.8	7.0 ± 24.9	**<0.001**
MCRI at the second step	594.1 (561–663)	621.3 (579–703)	574.6 (535–587)	**0.001**
Basal EGP, $mg/m^2 \cdot min^{-1}$	80.6 ± 6.4	81.4 ± 6.7	79.7 ± 6.2	0.341
% Reduction of EGP at the first step	71.7 ± 15.8	68.4 ± 15.1	75.1 ± 16.0	0.145
% Reduction of EGP at the second step	89.5 (82.2–93.9)	86.2 (77.0–93.7)	89.9 (83.8–94.3)	0.102
% Reduction of EGP/SS_{SI} at the first step, $\%/\mu U \cdot mL^{-1}$	3.7 ± 1.0	3.9 ± 0.9	3.7 ± 0.8	0.295
Rd at the first step, $mg/kg\ FFM \cdot min^{-1}$	4.4 ± 1.2	4.5 ± 1.4	4.3 ± 1.0	0.53
Rd at the second step, $mg/kg\ FFM \cdot min^{-1}$	8.6 ± 2.0	9.3 ± 2.1	7.7 ± 1.6	**0.005**
Rd /SS_{SI} at the second step, $mg/kg\ FFM \cdot min^{-1} \cdot \mu U^{-1} \cdot mL$	0.22 (0.19–0.29)	0.29 (0.23–0.33)	0.20 (0.17–0.21)	**<0.001**
% FFA suppression at the first step	86.2 (80.4–89.5)	86.4 (81.0–89.3)	85.2 (79.8–90.6)	0.638
% FFA suppression/insulin at the first step, $\%/\mu U \cdot mL^{-1}$	4.54 ± 1.35	4.88 ± 1.51	4.19 ± 1.08	0.073

Data are means ± SD or median (interquartile range). Boldface represents statistical significance ($P < 0.05$).

basal insulin/C-peptide ratio [12], ~40% of the difference in insulin concentration during the second step of the glucose clamp between the groups was explained by higher endogenous insulin secretion and the remaining ~60% was explained by lower insulin clearance. In terms of insulin resistance, muscle insulin sensitivity (Rd/SS_{SI} at the second step) was significantly lower in the

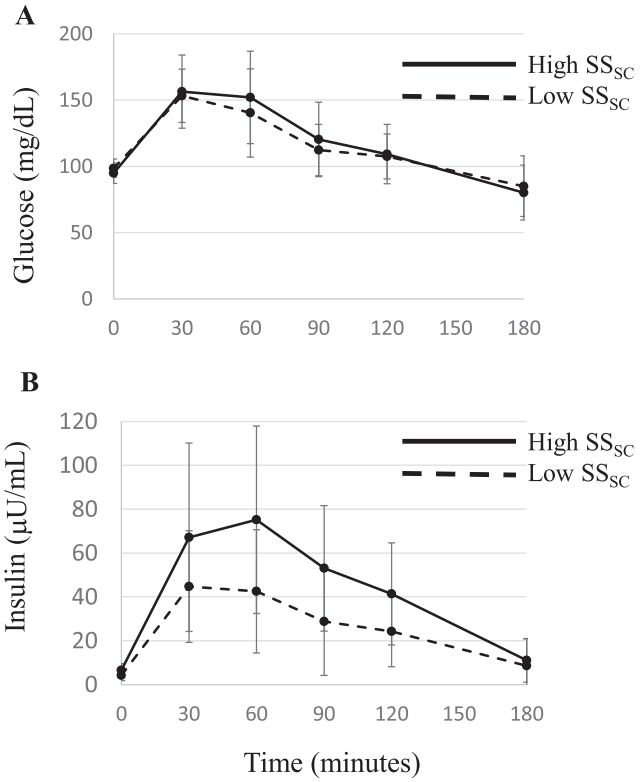

Figure 1. (A) Glucose and (B) insulin levels during OGTT in the high and low SS_{SC} groups.

high SS_{SC} group compared with the low SS_{SC} group. There were no significant differences in adipose tissue insulin sensitivity (% suppression of FFA/insulin at the first step) between the groups; however, the high SS_{SC} group tended to have lower adipose tissue insulin sensitivity. On the other hand, hepatic insulin sensitivity was similar between the two groups.

C. Correlations Between SS_{SC} at the Second Step and Other Parameters

To investigate further the association between SS_{SC} and various metabolic parameters, correlation analysis was performed (Table 3). In this analysis, parameters with $P < 0.1$, shown in Table 1, and glucose clamp data, shown in Table 2, were selected. SS_{SC} was negatively correlated with age and DBP. In addition, SS_{SC} was positively correlated with SFA and IHL. SS_{SC} was also positively correlated with both fasting serum insulin and AUC-insulin during OGTT and negatively correlated with MCRI, again suggesting higher endogenous insulin secretion contributed to hyperinsulinemia during a hyperinsulinemic-euglycemic state. In terms of insulin sensitivity, SS_{SC} was negatively correlated with muscle insulin sensitivity (Rd/SS_{SI} at the second step) and adipose tissue insulin sensitivity (% FFA suppression/insulin at the first step) but not with hepatic insulin sensitivity (% reduction of EGP/SS_{SI} at the first step).

3. Discussion

Interindividual variations in SS_{SC} exist even in healthy subjects; however, the characteristics of individuals with higher and lower SS_{SC} levels have not been fully elucidated yet. In addition, no studies evaluated the significance of SS_{SC} levels in Asians. In this study, we studied metabolic parameters reflecting interindividual variations in SS_{SC} among apparently healthy, nonobese Japanese men. We found that subjects in the high SS_{SC} group were characterized by reduced insulin sensitivity in muscle but not in liver and adipose tissue, decreased insulin clearance, hyperinsulinemia during OGTT, and slightly elevated IHL levels. Correlation analysis revealed that all of these factors were significantly correlated with SS_{SC} levels.

Table 3. Results of Univariate Regression Analysis for SS_{SC} in Apparently Healthy Subjects

	SS_{SC}	
	r	***P* Value**
Age	−0.355	**0.012**
DBP	−0.369	**0.009**
Fasting serum insulin	0.565	**<0.001**
Fasting serum C-peptide	0.441	**0.002**
AUC-insulin during OGTT	0.511	**<0.001**
HOMA-IR	0.506	**<0.001**
Matsuda index	−0.371	**0.009**
SFA	0.428	**0.002**
IHL	0.329	**0.027**
SS_{SI} at the second step	0.647	**<0.001**
MCRI at the second step	−0.382	**0.007**
Basal EGP	−0.092	0.529
% Reduction of EGP at the first step	0.192	0.191
% Reduction of EGP/SS_{SI} at the first step	−0.242	0.098
Rd at the second step	−0.409	**0.004**
Rd/SS_{SI} at the second step	−0.565	**<0.001**
% FFA suppression at the first step	−0.207	0.153
% FFA suppression/insulin at the first step	−0.360	**0.011**

Boldface indicates statistical significance ($P < 0.05$).

Subjects with higher SS_{SC} levels were characterized by modestly reduced insulin sensitivity in skeletal muscle. Whereas hyperinsulinemia is generally observed in subjects with insulin resistance, subjects with higher SS_{SC} levels had elevated insulin levels during the 75-g OGTT, despite similar glucose excursions. Our data suggest that higher SS_{SC} levels, along with reduced insulin clearance, cause hyperinsulinemia and successfully compensate for muscle insulin resistance to maintain glycemic levels during OGTT. Likewise, correlation analysis revealed that SS_{SC} levels are significantly correlated with muscle insulin sensitivity and AUC-insulin during OGTT, respectively. Similar observations were generally reported in obese subjects with severe insulin resistance [15]. Thus, these findings suggest that enhanced insulin secretion during hyperinsulinemia, as well as reduced insulin clearance [34], might be involved in compensating for modestly reduced muscle insulin resistance in apparently healthy, nonobese Japanese men.

Subjects in the high SS_{SC} group also had a slightly elevated IHL level. It has been reported that increased liver fat is associated with impaired insulin clearance and hyperinsulinemia [37], whereas chronic hyperinsulinemia is known to promote hepatic *de novo* lipogenesis [38]. On the other hand, muscle insulin resistance has been reported to promote IHL accumulation by altering the pattern of postprandial carbohydrate storage away from muscle glycogen synthesis into hepatic *de novo* lipogenesis [39, 40]. In fact, our previous study has shown that IHL accumulation is closely associated with impaired muscle insulin sensitivity [22, 41]. All of these reasons might explain why IHL accumulation, muscle insulin resistance, impaired insulin clearance, and hyperinsulinemia are observed in the high SS_{SC} group, simultaneously, although the causal relationships among those factors have not been fully understood.

In contrast, previous studies have demonstrated that endogenous insulin secretion during glucose clamp is not associated [20] or positively associated [21] with insulin sensitivity. For example, Anderwald *et al.* [20] showed that SS_{SC} levels during a hyperinsulinemic-isoglycemic clamp study (insulin infusion rate of 40 mU/m^2 per minute) in insulin-resistant whites were similar to those in insulin-sensitive whites. On the other hand, Mari *et al.* [21] showed that an insulin-induced secretory response (percent change of C-peptide during the clamp from basal state) during isoglycemic clamp (insulin infusion rate of 240 $pmol/m^2$ per minute) was positively correlated to insulin sensitivity, whereas this association was substantial in women but moderate in men. Compared with these previous studies, our study only included Japanese men and used a different clamp method [euglycemic clamp, lower insulin infusion rate (20 mU/m^2 per minute), longer duration of insulin infusion (360 minutes)]. Thus, these differences in subject characteristics and clamp protocol may be related to the opposite result from previous reports.

The exact mechanism underlying higher SS_{SC} in nonobese subjects is not known. At least in knockout mice with pancreatic β cells lacking the insulin receptor, basal insulin concentrations are elevated at 6 months of age [42], suggesting that insulin resistance in β cells can contribute to fasting hyperinsulinemia. In addition, circulating FFAs not only stimulate insulin secretion [43] but also induce insulin resistance in pancreatic β cells [44]. In our study, adipose tissue insulin sensitivity (% FFA suppression/insulin at the first step) was negatively correlated with SS_{SC} levels; thus, increases of FFA in these subjects may elicit impaired suppression of insulin release by insulin. On the other hand, a decrease of C-peptide levels secondary to exogenous insulin infusion was not observed in patients with combined pancreas and kidney transplantation in previous studies but was observed in patients with kidney transplantation only [45, 46]. As the main difference between kidney-only transplant patients and pancreas and kidney transplant patients is denervation around the pancreas, these data suggest that endogenous insulin secretion during a hyperinsulinemic state could be neurally mediated. In fact, insulin resistance of the hypothalamus, evaluated by cerebral blood flow using MRI in combination with intranasal insulin administration, was associated with hyperinsulin secretion during OGTT in human. Recent rodent models revealed that a liver–brain–pancreas neuronal relay plays an important role to promote β cell proliferation and insulin secretion [47, 48].

The current study has several limitations. We recruited only Japanese men for this study; thus, our results may not be generalizable to other ethnic groups and females [21, 23, 49]. In addition, as we did not measure C-peptide levels during the 75-g OGTT, it is not certain whether hyperinsulinemia during the 75-g OGTT can be explained by enhanced insulin secretion, decreased insulin clearance, or both. Because we only performed single linear regression analyses as a result of a small number of subjects, it is still unknown whether each parameter is an independent determinant of SS_{SC}. Finally, the current study is cross sectional and thus, cannot address causality.

In conclusion, even some apparently healthy, nonobese Japanese men have higher SS_{SC} levels during hyperinsulinemic-euglycemic clamp. They were characterized by modest muscle insulin resistance, moderate IHL accumulation, hyperinsulinemia during OGTT, and reduced insulin clearance. These data suggest that enhanced insulin secretion during hyperinsulinemia, along with reduced insulin clearance [34], may be an early change to maintain glucose metabolism by the enhancement of insulin secretion in the face of modest muscle insulin resistance in healthy, nonobese Japanese men.

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