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 (SSSC) 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 SSSC levels are present; however, the characteristics of subjects with high SSSC levels in those populations have not been fully elucidated.

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

Design and Participants: We studied 49 nonobese (BMI < 25 kg/m²), healthy Japanese men. We evaluated SSSC 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 SSSC groups based on the median SSSC value and compared their clinical parameters. Compared with the low SSSC group, the high SSSC 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 SSSC.

Conclusions: In healthy, nonobese men, higher SSSC 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; 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; SBF, systolic blood pressure; SFA, subcutaneous fat area; SSSC, steady-state serum C-peptide; SSIG, 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.

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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 (SSSC) levels during hyperinsulinemic-euglycemic clamp, obese subjects with insulin resistance had higher SSSC 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, SSSC levels during clamp are highly variable, even in healthy controls, suggesting the presence of interindividual variations in SSSC. Indeed, at least previous three reports failed to detect substantial differences in SSSC 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 SSSC 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 SSSC levels during glucose clamp. Finally, although Asians secrete much less insulin than other ethnicities [23], no studies evaluated the significance of SSSC levels in Asians.

In this context, the current study was designed to examine SSSC levels during hyperinsulinemic-euglycemic clamp in nonobese, healthy Japanese individuals and clarify the association between SSSC levels and metabolic characteristics. We measured tissue-specific insulin resistance as well as SSSC levels in apparently healthy, nonobese [body mass index (BMI) < 25 kg/m²] Japanese men using a two-step hyperinsulinemic-euglycemic clamp.

1. Research Design and Methods

A. Study Subjects

SSSC 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 SSSC in apparently healthy, nonobese men, we selected those with a BMI of 21.0 to <25.0 kg/m² who did not have cardiometabolic risk factors, such as elevated fasting plasma glucose (FPG) ≥ 110 mg/dL, dyslipidemia (triglycerides (TG) ≥ 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 1H-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/m² body surface area (BSA)] of [6,6-2H₂]glucose (Cambridge Isotope Laboratories, Tewksbury, MA) was injected intravenously, followed by constant infusion of 2 mg/m² 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/m² per minute for 5 minutes, followed by 20 mU/m² per minute for 5 minutes) and continuous insulin infusion at 10 mU/m² per minute for 3 hours (0 to 180 minutes). In the second step of the clamp, after a priming insulin infusion (80 mU/m² per minute for 5 minutes, followed by 40 mU/m² per minute for 5 minutes), insulin was infused continuously at 20 mU/m² per minute for 3 hours (180 to 360 minutes). The infusion of [6,6-2H₂]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% [6,6-2H₂]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 [6,6-2H₂]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 (SSSI) and used it as an index of hepatic insulin sensitivity [31]. Likewise, Rd at the second step was divided by SSSI 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]: $MCRI = \frac{IIR}{SSSI - (BSI \times SSSC/BSC)}$, where $IIR$ = insulin infusion rate, $SSSI$ = SSSI during glucose clamp, $BSI$ = basal serum insulin, $SSSC$ = SSSC during glucose clamp, and $BSC$ = basal serum C-peptide.

D. 1H-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 $\times 100/(H_2O + S$-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 SSSC 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. SSSI levels during the second step of the glucose clamp in all subjects reached 36.4 ± 5.2 μU/mL (Table 2). The mean SSSC level during the second step was 0.87 ± 0.39 ng/mL (Table 2). We then divided the subjects into the high SSSC group (n = 24) and low SSSC group (n = 25), based on the median value of SSSC (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 SSSC group had a mean SSSC level that was 2.1 times higher than the level in the low SSSC 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 SSSC group and the high SSSC group, except for DBP. Whereas fasting C-peptide levels were comparable between the groups, fasting serum insulin levels
were significantly higher in the high SS\textsuperscript{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\textsuperscript{SC} group compared with the low SS\textsuperscript{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\textsuperscript{SC} group compared with the low SS\textsuperscript{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\textsuperscript{SC} group had a significantly higher area under the curve of insulin (AUC-insulin) than the low SS\textsuperscript{SC} group (Table 1 and Fig. 1). Thus, the Matsuda index, an index of insulin sensitivity, was lower in the high SS\textsuperscript{SC} group compared with the low SS\textsuperscript{SC} group (Table 1). These data suggest that the high SS\textsuperscript{SC} group was characterized by impaired insulin sensitivity, hyper-insulinemia, 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\textsuperscript{SC} group had significantly higher SS\textsubscript{SI} levels than the low SS\textsuperscript{SC} group during both steps of the glucose clamp. In theory, higher endogenous insulin secretion during a hyperinsulinemic-euglycemic state in the high SS\textsuperscript{SC} group contributed to this difference (Table 2). On the other hand, insulin clearance was decreased in the high SS\textsuperscript{SC} group, which also contributed to elevated insulin levels during the clamp study. Based on the calculated

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**Table 1. Clinical Characteristics of the Low SS\textsuperscript{SC} and High SS\textsuperscript{SC} Groups**

|                      | Overall | Low SS\textsuperscript{SC} | High SS\textsuperscript{SC} | P Value |
|----------------------|---------|-----------------------------|-----------------------------|---------|
| n                    | 49      | 25                          | 24                          | 0.078   |
| 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\textsuperscript{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\textsuperscript{3} | 21.3 ± 2.9 | 21.0 ± 2.2 | 21.6 ± 3.5 | 0.497   |
| 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\textsuperscript{2} | 75.3 ± 28.0 | 73.1 ± 30.6 | 77.6 ± 25.6 | 0.582   |
| Abdominal SFA, cm\textsuperscript{2} | 106 ± 40 | 96.4 ± 42.7 | 116.9 ± 34.8 | 0.072   |
| VO\textsubscript{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\textsubscript{2peak}, peak oxygen uptake.
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/SSSI at the second step) was significantly lower in the

![Figure 1](image-url)  
**Figure 1.** (A) Glucose and (B) insulin levels during OGTT in the high and low SSSC groups.
high SSSC group compared with the low SSSC 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 SSSC 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\textsubscript{SSC} at the Second Step and Other Parameters

To investigate further the association between SS\textsubscript{SSC} 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\textsubscript{SSC} was negatively correlated with age and DBP. In addition, SS\textsubscript{SSC} was positively correlated with SFA and IHL. SS\textsubscript{SSC} 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\textsubscript{SSC} was negatively correlated with muscle insulin sensitivity (Rd/SSSI 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/SSSI at the first step).

3. Discussion

Interindivudal variations in SS\textsubscript{SSC} exist even in healthy subjects; however, the characteristics of individuals with higher and lower SS\textsubscript{SSC} levels have not been fully elucidated yet. In addition, no studies evaluated the significance of SS\textsubscript{SSC} levels in Asians. In this study, we studied metabolic parameters reflecting interindividual variations in SS\textsubscript{SSC} among apparently healthy, nonobese Japanese men. We found that subjects in the high SS\textsubscript{SSC} 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\textsubscript{SSC} levels.

| Table 3. Results of Univariate Regression Analysis for SS\textsubscript{SSC} in Apparently Healthy Subjects |
|----------------|----------------|
|                | SS\textsubscript{SSC} |
|                | \( r \)     | \( P \) 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         |
| SSS\textsubscript{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\textsubscript{SI} at the first step | -0.242       | 0.098         |
| Rd at the second step | -0.409       | 0.004         |
| Rd/SS\textsubscript{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 SSSC levels were characterized by modestly reduced insulin sensitivity in skeletal muscle. Whereas hyperinsulinemia is generally observed in subjects with insulin resistance, subjects with higher SSSC levels had elevated insulin levels during the 75-g OGTT, despite similar glucose excursions. Our data suggest that higher SSSC 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 SSSC 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 SSSC 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 SSSC 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 SSSC levels during a hyperinsulinemic-isoglycemic clamp study (insulin infusion rate of 40 mU/m² 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/m² 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/m² 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 SSSC 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 SSSC 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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Additional Information

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References and Notes

1. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988;37(12):1595–1607.
2. Meistas MT, Rendell M, Margolis S, Kowarski AA. Estimation of the secretion rate of insulin from the urinary excretion rate of C-peptide. Study in obese and diabetic subjects. Diabetes. 1982;31(5):449–453.
3. DeFronzo RA. Insulin secretion, insulin resistance, and obesity. Int J Obes. 1982;6(Suppl 1):73–82.
4. Faber OK, Christensen K, Kehlet H, Madsbad S, Binder C. Decreased insulin removal contributes to hyperinsulinemia in obesity. J Clin Endocrinol Metab. 1981;53(3):618–621.
5. Savage PJ, Flock EV, Mako ME, Blix PM, Rubenstein AH, Bennett PH. C-Peptide and insulin secretion in Pima Indians and Caucasians: constant fractional hepatic extraction over a wide range of insulin concentrations and in obesity. J Clin Endocrinol Metab. 1979;48(4):594–598.
6. Rossell R, Gomis R, Casamitjana R, Segura R, Vilardell E, Rivera F. Reduced hepatic insulin extraction in obesity: relationship with plasma insulin levels. J Clin Endocrinol Metab. 1983;56(3):608–611.
7. Mizgier ML, Casas M, Contreras-Ferrat A, Llanos P, Galgani JE. Potential role of skeletal muscle glucose metabolism on the regulation of insulin secretion. Obes Rev. 2014;15(7):587–597.
8. Porte D, Jr, Pupo AA. Insulin responses to glucose: evidence for a two pool system in man. J Clin Invest. 1969;48(12):2309–2319.
9. Chen M, Porte D, Jr. The effect of rate and dose of glucose infusion on the acute insulin response in man. J Clin Endocrinol Metab. 1976;42(6):1168–1175.
10. Ward WK, Beard JC, Halter JB, Pfeifer MA, Porte D, Jr. Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus. *Diabetes Care*. 1984;7(5):491–502.
11. Liljenquist JE, Horwitz DL, Jennings AS, Chiasson JL, Keller U, Rubenstein AH. Inhibition of insulin secretion by exogenous insulin in normal man as demonstrated by C-peptide assay. *Diabetes*. 1978;27(5):563–570.
12. Elahi D, Nagulesparan M, Hershcopf RJ, Muller DC, Tobin JD, Blix PM, Rubenstein AH, Unger RH, Andres R. Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. *N Engl J Med*. 1982;306(20):1196–1202.
13. Cavallo-Perin P, Bruno A, Scaglione L, Gruden G, Cassader M, Pagano G. Feedback inhibition of insulin and glucagon secretion by insulin is altered in abdominal obesity with normal or impaired glucose tolerance. *Acta Diabetol*. 1993;30(3):154–158.
14. Pincelli AI, Brunani A, Caumo A, Scacchi M, Pasqualinotto L, Tibaldi A, Dubini A, Bonadonna S, Cavagnini F. Hyperinsulinemia in the physiologic range is not superior to short-term fasting in suppressing insulin secretion in obese men. *Metabolism*. 2001;50(1):107–111.
15. Muscelli E, Pereira JA, Lazarin MA, da Silva CA, Pareja JC, Saad MJ. Lack of insulin inhibition on insulin secretion in non-diabetic morbidly obese patients. *Int J Obes Relat Metab Disord*. 2001;25(6):798–804.
16. Waldhäuser WK, Gasić S, Bratusch-Marrain P, Korn A, Nowotny P. Feedback inhibition by biosynthetic human insulin of insulin release in healthy human subjects. *Am J Physiol*. 1982;243(6):E476–E482.
17. Argoud GM, Schade DS, Eaton RP. Insulin suppresses its own secretion in vivo. *Diabetes*. 1987;36(8):959–962.
18. Baynes C, Anyaoku V, Johnston DG, Elkeles RS. Feedback inhibition of insulin secretion in type 2 diabetes. *Clin Sci (Lond)*. 1991;81(5):685–690.
19. Peiris AN, Stagner JJ, Vogel RL, Samols E, Nakagawa A. Lack of insulin feedback inhibition in non-obese and obese men. *Metabolism*. 1993;42(3):371–375.
20. Anderwald C, Tura A, Grassi A, Krebs M, Szendroedi J, Roden M, Bischof MG, Luger A, Pacini G. Insulin infusion during normoglycemia modulates insulin secretion according to whole-body insulin sensitivity. *Diabetes Care*. 2011;34(2):437–441.
21. Mari A, Tura A, Natali A, Anderwald C, Balkau B, Lalic N, Walker M, Ferrannini E, Investigators R; RISC Investigators. Influence of hyperinsulinemia and insulin resistance on in vivo β-cell function: their role in human β-cell dysfunction. *Diabetes*. 2011;60(12):3141–3147.
22. Takeno K, Tamura Y, Kagawuchi 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/m². *J Clin Endocrinol Metab*. 2016;101(10):3676–3684.
23. Møller JB, Dalla Man C, Overgaard RV, Ingwersen SH, Tornæe CW, Pedersen M, Tanaka H, Ohsugi M, Ueki K, Lyne J, Vasconcelos NM, Pedersen BK, Kadokawa T, Cobelli C. Ethnic differences in insulin sensitivity, β-cell function, and hepatic extraction between Japanese and Caucasians: a minimal model analysis. *J Clin Endocrinol Metab*. 2014;99(11):4273–4280.
24. Matsuzawa Y. Metabolic syndrome—definition and diagnostic criteria in Japan. *J Atheroscler Thromb*. 2005;12(6):301.
25. Nishitani M, Shimada K, Sunayama S, Masaki Y, Kume A, Fukao K, Sai E, Yamashita H, Ohmura H, Onishi T, Shiroya M, Sato H, Shimada A, Yamamoto T, Amano A, Daida H. Impact of diabetes on muscle mass, muscle strength, and exercise tolerance in patients after coronary artery bypass grafting. *J Cardioil*. 2011;58(1):173–180.
26. Shafer KJ, Siders WA, Johnson LK, Laikaski HC. Validity of segmental multiple-frequency bioelectrical impedance analysis to estimate body composition of adults across a range of body mass indexes. *Nutrition*. 2009;25(1):25–32.
27. 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.
28. Kelley DE, McKolanis TM, Hegazi RA, Kuller LH, Kalhan SC. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol Endocrinol Metab*. 2003;285(4):E906–E916.
29. Lindegaard B, Freisig C, Petersen AM, Plomgaard P, Ditlevsen S, Middendorf B, Van Hall G, Wojtaszewski JF, Pedersen BK. Inhibition of lipolysis stimulates peripheral glucose uptake but has no effect on endogenous glucose production in HIV lipodystrophy. *Diabetes*. 2007;56(8):2070–2077.
30. Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann NY Acad Sci*. 1959;82(2):420–430.
31. Kotronen A, Juurinen L, Tiikkainen M, Vehkavaara S, Yki-Järvinen H. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. Gastroenterology. 2008;135(1):122–130.

32. Abdul-Ghani M, DeFronzo RA. Fasting hyperglycemia impairs glucose—but not insulin-mediated suppression of glucagon secretion. J Clin Endocrinol Metab. 2007;92(5):1778–1784.

33. 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. 2017;41(8):1288–1294.

34. Kaga H, Tamura Y, Takeno K, Kakehi S, Funayama T, Furukawa Y, Nishitani-Yokoyama M, Shimada K, Daida H, Aoki S, Giacca A, Kanazawa A, Kawamori R, Watada H. Correlates of insulin clearance in apparently healthy non-obese Japanese men. Sci Rep. 2017;7(1):1462.

35. Tamura Y, Tanaka Y, Sato F, Choi JB, Watada H, Niwa M, Kinoshita J, Ooka A, Kumashiro N, Igarashi Y, Kyogoku S, Maehara T, Hirose T, Kawamori R. Effects of diet and exercise on muscle and liver intracellular lipid contents and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab. 2005;90(6):3191–3196.

36. Sato F, Tamura Y, Watada H, Kumashiro N, Igarashi Y, Uchino H, Maehara T, Kyogoku S, Sunayama S, Sato H, Hirose T, Tanaka Y, Kawamori R. Effects of diet-induced moderate weight reduction on intrahepatic and intramyocellular triglycerides and glucose metabolism in obese subjects. J Clin Endocrinol Metab. 2007;92(8):3326–3329.

37. Kotronen A, Vehkavaara S, Seppälä-Lindroos A, Bergholm R, Yki-Järvinen H. Effect of liver fat on insulin clearance. Am J Physiol Endocrinol Metab. 2007;293(6):E1709–E1715.

38. Najjar SM, Perdomo G. Hepatic insulin clearance: mechanism and physiology. Physiology (Bethesda). 2019;34(3):198–215.

39. Flannery C, Dufour S, Rabel R, Shulman GI, Petersen KF. Skeletal muscle insulin resistance promotes increased hepatic de novo lipogenesis, hyperlipidemia, and hepatic steatosis in the elderly. Diabetes. 2012;61(11):2711–2717.

40. Rabel R, Petersen KF, Dufour S, Flannery C, Shulman GI. Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. Proc Natl Acad Sci USA. 2011;108(33):13705–13709.

41. Furukawa Y, Tamura Y, Takeno K, Funayama T, Kaga H, Suzuki R, Watanabe T, Kakehi S, Kanazawa A, Kawamori R, Watada H. Impaired peripheral insulin sensitivity in non-obese Japanese patients with type 2 diabetes mellitus and fatty liver. J Diabetes Investig. 2018;9(3):529–535.

42. Kulkarni RN, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell. 1999;96(3):329–339.

43. Dobbins RL, Chester MW, Stevenson BE, Daniels MB, Stein DT, McGarry JD. A fatty acid-dependent step is critically important for both glucose- and non-glucose-stimulated insulin secretion. J Clin Invest. 1998;101(11):2370–2376.

44. Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. Eur J Clin Invest. 2002;32(s3):14–23.

45. Boden G, Chen X, DeSantis R, Kolaczynski J, Morris M. Evidence that suppression of insulin secretion by insulin itself is neurally mediated. Metabolism. 1993;42(6):786–789.

46. Luzi L, Battezzati A, Perseghin G, Bianchi E, Vergani S, Secchi A, La Rocca E, Spotti D, Ferrari G, Di Carlo V, Pozza G. Lack of feedback inhibition of insulin secretion in denervated human pancreas. Diabetes. 1992;41(12):1632–1639.

47. Imai J, Katagiri H, Yamada T, Ishigaki Y, Suzuki T, Kudo H, Uno K, Hasegawa Y, Gao J, Kaneko K, Ishihara H, Niijima A, Nakazato M, Asano T, Minokoshi Y, Oka Y. Regulation of pancreatic beta cell mass by neuronal signals from the liver. Science. 2008;322(5905):1250–1254.

48. Yamamoto J, Imai J, Izumi T, Takahashi H, Kawana Y, Takahashi K, Kodama S, Kaneko K, Gao J, Uno K, Sawada S, Asano T, Kalimchenko VV, Susaki EA, Kanazaki M, Ueda HR, Ishigaki Y, Yamada T, Katagiri H. Neuronal signals regulate obesity induced β-cell proliferation by FoxM1 dependent mechanism. Nat Commun. 2017;8(1):1930.

49. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G; European Group for the Study of Insulin Resistance (EGIR). Insulin resistance and hypersecretion in obesity. J Clin Invest. 1997;100(5):1166–1173.