Sclerostin and Insulin Resistance in Prediabetes: Evidence of a Cross Talk Between Bone and Glucose Metabolism

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PATHOPHYSIOLOGY/COMPLICATIONS

OBJECTIVE

A gene mutation of the Wnt/β-catenin signaling cascade is present in rare patients with the insulin resistance syndrome. Sclerostin is a circulating peptide inhibiting Wnt/β-catenin signaling. Our aims were to evaluate serum sclerostin in subjects with prediabetes and to analyze its relationship with insulin resistance and β-cell function.

RESEARCH DESIGN AND METHODS

We performed a cross-sectional study including 43 healthy normal glucose-tolerant (NGT) individuals and 79 individuals with impaired glucose regulation (IGR), which included subjects with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and combined IFG-IGT, undergoing oral glucose tolerance test (OGTT) and dual-energy X-ray absorptiometry. A subgroup of 18 with NGT and 30 with IGR also underwent a euglycemic–hyperinsulinemic clamp with tracer.

RESULTS

Sclerostin levels were higher in IGR compared with NGT (50.8 ± 2.4 vs. 38.7 ± 2.3 pmol/L; $P = 0.01$), positively correlated with HOMA-insulin resistance (IR) ($r = 0.62$; $P < 0.001$), and negatively correlated with insulin-mediated total body glucose disposal ($r = -0.40$; $P < 0.001$). Fasting endogenous glucose production (EGP) and hepatic and adipose tissue insulin resistance indexes were positively correlated with sclerostin levels ($r = 0.48$, $r = 0.62$, and $r = 0.61$, respectively; $P < 0.001$). Fasting and OGTT insulin clearance were inversely correlated with sclerostin serum levels ($r = -0.52$ and $r = -0.44$, respectively; both $P < 0.001$). Sclerostin levels were not correlated with β-cell function parameters. In multiple linear regression analysis, the addition of sclerostin levels to the traditional risk factors for insulin resistance improved the r^2 associated with HOMA-IR (r^2 change: 0.055; F change: 28.893; $P = 0.001$) and insulin-mediated total body glucose disposal (r^2 change: 0.059; F change: 4.938; $P = 0.033$).

CONCLUSIONS

Sclerostin levels are increased in individuals with prediabetes and correlated with insulin resistance in skeletal muscle, liver, and adipose tissue. The correlation between sclerostin and insulin clearance at fasting state and during OGTT is novel; thus, studies are needed to explore the potential causal relationship.

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Type 2 diabetes mellitus (T2DM) is a worldwide epidemic, importantly reducing life expectancy and quality of life because of chronic complications such as cardiovascular diseases, renal failure, and blindness (1). Impaired glucose regulation (IGR), a condition that includes impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), precedes the onset of T2DM (2).

IGR, also defined as prediabetes, is characterized by insulin resistance and altered insulin secretion, two key pathophysiological features preceding the development of T2DM (3). The components of the insulin resistance syndrome, such as obesity, IGR, hypertension, dyslipidemia, endothelial dysfunction, and hyperinsulinemia, are associated with a higher risk of developing cardiovascular diseases (4–6). Furthermore, 10–15% of individuals with prediabetes already have evidence of diabetic microvascular complications, suggesting that the mechanisms responsible for diabetes complications are already active when T2DM is not yet clinically manifested (7).

Bone has recently emerged as a new important organ in the regulation of glucose metabolism (8). Sclerostin, a 190-amino acid glycoprotein secreted mainly by osteocytes, is a circulating hormone that antagonizes the Wnt (Wingless-type mouse mammary tumor virus integration side) pathway, thus suppressing osteoblast activity and downregulating bone turnover (9). Interestingly, a gene mutation in the Wnt signaling pathway has been associated with hyperlipidemia, hypertension, and early coronary artery disease in patients with metabolic syndrome (10,11).

The role of sclerostin in insulin resistance in humans has not been explored. The aim of this study was to evaluate the relationship between sclerostin and whole-body glucose metabolism in individuals with prediabetes compared with normal glucose-tolerant (NGT) subjects.

RESEARCH DESIGN AND METHODS

The cross-sectional study included 43 healthy NGT individuals and 79 individuals with IGR, which included individuals with IFG, IGT, and combined IFG-IGT. According to the American Diabetes Association, the diagnosis of prediabetes is made on the basis of one of the following clinical biochemistry criteria: *1*) 2-h plasma glucose (PG) during an oral glucose tolerance test (OGTT) of 140–199 mg/dL (IGT); *2*) fasting plasma glucose (FPG) of 100–125 mg/dL (IFG); or *3*) HbA_{1c} of 5.8–6.4% (12).

Exclusion criteria were *1*) diagnosis of T2DM; *2*) blood pressure $>$140/90 mmHg; *3*) serum creatinine $>$1.6 mg/dL; *4*) hematocrit $<$35%; *5*) evidence of major organ system disease, including cancer, as determined by medical history, physical examination, and routine screening blood chemistries; and *6*) use of medications known to affect glucose metabolism. All study subjects had a stable body weight for at least 3 months and had not participated in strenuous exercise before enrollment. All subjects underwent an OGTT (75 g), dual-energy X-ray absorptiometry (DXA) whole-body scan, and determination of plasma sclerostin levels. A subgroup of 18 with NGT and 30 with IGR also underwent a euglycemic–hyperinsulinemic clamp combined with tritiated glucose to directly measure hepatic glucose production and suppression after insulin infusion.

The study protocol was approved by the institutional review board of the University of Texas Health Science Center and the South Texas Veterans Healthcare System, Audie Murphy Hospital at San Antonio, TX. The study protocol was conducted in accordance with the guidelines of the Declaration of Helsinki. Written and oral informed consent was obtained from all participants enrolled in this study.

Study Procedures

All metabolic studies were done in the morning at The Bartter Research Unit of the South Texas Veterans Healthcare System after an overnight fast of 10–12 h.

OGTT: A catheter was placed in an antecubital vein and blood samples were collected at -30, -15, 0, 30, 60, 90, and 120 min for determination of PG, C-peptide, insulin, glucagon, and free fatty acid (FFA) concentrations.

Hyperinsulinemic–euglycemic clamp: At 0600 h (-180 min), after a 10-h overnight fast, a prime (25 mCi $\times$ FPG/100) continuous (0.25 μCi/min) infusion of [3-^{3}H]-glucose was started via a catheter placed into an antecubital vein and continued throughout the study. A second catheter was placed retrogradely into a vein on the dorsum of the hand, which was then placed in a heated box (60°C). Baseline arterialized venous blood samples for determination of plasma [3-^{3}H]-glucose radioactivity, PG, insulin, C-peptide, glucagon, FFA, and sclerostin concentrations were drawn at -30, -20, -10, -5, and 0 min. At time 0, insulin was infused at 60 mU/kg · min. Arterialized blood samples were collected every 5 min for PG determination, and a 20% glucose infusion was adjusted to maintain the PG concentration at 100 mg/dL. Throughout the insulin clamp, blood samples for determination of PG concentration were drawn every 10–15 min for determination of plasma insulin concentration and [3-^{3}H]-glucose specific activity.

DXA: DXA whole-body scan was performed to determine fat and lean body mass and whole-body minus head bone mineral density (Hologic, Waltham, MA).

Calculations

β-Cell function was assessed from the OGTT using a model describing the relationship between insulin secretion (ISR; expressed in pmol · min^{-1} · m^{-2}) and glucose concentration as the sum of two components (13,14). The first component represents the dependence of ISR on glucose concentration through a dose-response function relating the two variables. From the dose-response, β-cell glucose sensitivity (the slope) and ISR at a fixed glucose concentration of 5.2 mmol/L for NGT and 5.8 mmol/L for IGR were calculated. The dose-response is modulated by a potentiation factor, accounting for various mechanisms (prolonged hyperglycemia, nonglucose substrates, gastrointestinal hormones, neural modulation). The potentiation factor averages 1 during the test and expresses relative potentiation or inhibition of ISR; its excursion is quantified by the ratio between the 2-h and the baseline value (potentiation ratio). The second ISR component represents the dependence of ISR on the rate of change of glucose concentration and is determined by a single parameter (rate sensitivity), which is related to early insulin release. The model parameters were estimated from glucose and C-peptide concentrations (using C-peptide deconvolution [15]), as previously described (14). The integral of ISR during the whole

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test (total ISR) was also calculated. Because a frequently sampled OGTT was performed, a dynamic index of insulin sensitivity (SI) derived from minimal model analysis was calculated (16).

Basal insulin clearance was calculated as the ratio of insulin secretion and insulin concentration in the basal period; insulin clearance during the OGTT was determined as the ratio of mean insulin secretion and insulin concentration during the OGTT. Under steady-state postabsorptive conditions, the rate of endogenous glucose appearance was calculated as the [3-^{3}H]-glucose infusion rate (DPM/min) divided by the steady-state plasma [3-^{3}H]-glucose specific activity (DPM/mg). During the euglycemic insulin clamp, the rate of glucose appearance was calculated with the Steele equation, using a distribution volume of 250 mL/kg. Endogenous (primarily reflects hepatic) glucose production (EGP) was calculated by subtracting the exogenous glucose infusion rate from the rate of glucose appearance. The rate of insulin-mediated total-body glucose disposal (TGD/SSPI) was determined by adding the rate of residual EGP to the exogenous glucose infusion rate and dividing it for the steady state of plasma insulin during the last 30 min of the clamp (17). Hepatic insulin resistance index (HIRI) was the product of fasting EGP and plasma insulin concentration. The adipose tissue IRI (ATIRI) was the product of fasting plasma FFA and fasting plasma insulin levels.

Biochemical and Hormone Analyses

Circulating sclerostin was measured in duplicate using a commercially available ELISA (Biomedica, Vienna, Austria) according to the manufacturer's instructions. The intra-assay and interassay variability was <5%. Sclerostin measurements were reported in picomoles per liter, and the lower limit of detection was <10 pmol/L. Plasma glucose levels were measured using the glucose oxidase method (GM9; Analox Instruments, London, U.K.). Plasma insulin and C-peptide levels were measured by radioimmunoassay method (Siemens Medical Solutions Diagnostics, Tarrytown, NY). Plasma glucagon levels were measured by a radioimmunoassay method (Linco Research). Plasma [3-^{3}H]-glucose radioactivity was measured in Somogyi serum precipitates.

Statistical Analysis

Values were calculated as mean ± SEM or as median (interquartile range) for variables with a skewed distribution. The difference between means in NGT and IGR was compared with the two-sided unpaired *t* test and repeated-measures ANOVA, with time and group as factors. To further explore the association between sclerostin and glucose metabolism alterations, we used ANOVA and post hoc analysis to also perform a subgroup analysis of IFG, IGT, and combined IFG-IGT subjects. Variables that were not normally distributed were log-transformed before analysis. A P value of <0.05 (two-tailed analysis) was considered to be statistically significant. Pearson correlation (normal distribution) and Spearman correlation analyses (nonnormal distribution) were used to assess the correlations between serum sclerostin and other continuous parameters, and we used partial correlations to correct the possible influence of age, sex, BMI, and body fat on sclerostin values. Multivariate analyses were performed to evaluate the contribution of sclerostin in the association with insulin resistance, insulin sensitivity, and insulin clearance. Data were analyzed using SPSS 20 software (IBM Corp, Armonk, NY).

RESULTS

Baseline Characteristics of the Study Population

The clinical, biochemical, metabolic, and densitometric data of NGT and IGR subjects are summarized in Table 1. The two study groups were comparable in age, sex, BMI, fat mass, lean body mass, and bone density. IGR subjects had higher FPG and 2-h PG, fasting insulin, and HbA_{1c} (all $P < 0.05$). IGR subjects were insulin resistant in skeletal muscle, liver, and adipose tissue and had lower insulin clearance at baseline and during OGTT compared with NGT subjects (all $P < 0.05$). Also, as expected, IGR subjects showed a higher insulin secretion rate despite higher PG during the OGTT and a reduction of the glucose dose-response compared with NGT subjects (Supplementary Fig. 1*A*–*C*). A subgroup analysis showed that subjects with isolated IFG or IGT or with combined IFG-IGT had similar age, BMI, fat content, and bone density although they manifested typical glucose metabolism alterations associated with isolated IFG or IGT or combined IFG-IGT (Supplementary Table 1). Subjects with isolated IGT and combined IFG-IGT had a higher insulin secretion rate ($P = 0.02$), whereas β-cell glucose sensitivity was consistently reduced throughout the three subgroups (Supplementary Table 1). Sclerostin serum levels were higher in IGR than in NGT (50.8 ± 2.4 vs. 38.7 ± 2.3 pmol/L; $P = 0.01$). However, when subjects were divided according to glucose tolerance status, isolated IFG and combined IFG-IGT manifested significantly higher sclerostin serum levels compared with NGT (55.8 ± 2.9 and 50.8 ± 5.1 vs. 38.7 ± 2.3 pmol/L; $P = 0.02$, respectively), but no difference was found in sclerostin serum levels between isolated IGT and NGT. When subjects were further divided according to sex or ethnicity, serum sclerostin levels were similar in men and women in both the NGT and IGR groups ($P = 0.54$ and $P = 0.45$, respectively; data not shown), and no differences were found in subgroup analysis.

Relationship of Serum Sclerostin Levels With Insulin Sensitivity in Muscle, Liver, and Adipose Tissue

Serum sclerostin levels were positively correlated with HOMA-insulin resistance (IR) ($r = 0.62$; $P < 0.001$) (Fig. 1*A*) and negatively with insulin-mediated total body glucose disposal as measured by the hyperinsulinemic–euglycemic clamp (Ln TGD/SSPI: $r = -0.40$; $P < 0.001$) (Fig. 1*B*). Multiple linear regression modeling was performed using HOMA-IR, SI, and TGD/SSPI as dependent variables (Table 2). In model 1, independent variables were BMI, body fat, and HbA_{1c}. In model 2, sclerostin was added as an independent variable to model 1 to assess the association of sclerostin with insulin resistance. In model 1, BMI and HbA_{1c} were independently associated with HOMA-IR ($r^2 = 0.192$; $P < 0.001$). In model 2, sclerostin was independently associated with HOMA-IR and increased the r^2 compared with model 1 (r^2 change: 0.055; F change: 28.893; $P = 0.001$). Moreover, the SI index was separately used as a dependent variable (model 1) to evaluate the association of sclerostin with insulin sensitivity. In model 1, BMI and 2-h PG were independently associated with SI ($r^2 = 0.293$). In model 2, sclerostin was independently associated with SI and increased the r^2

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Table 1—Characteristics of study participants in the whole population and the clamp subgroup

Characteristic	Whole population: NGT (N = 43)	Whole population: IGR (N = 79)	P value
Age (years)	44.0 ± 1.9	46.0 ± 1.4	0.42
Sex			0.54
Men (n)	11	21	
Women (n)	32	58	
Weight (kg)	82.5 ± 3.6	84.5 ± 3.2	0.61
BMI (kg/m^2)	31.1 ± 1.1	31.9 ± 1.2	0.55
Fat content (%)	35.7 ± 1.5	38.0 ± 1.5	0.22
Lean mass (%)	64.3 ± 1.5	62.0 ± 1.6	0.21
Bone density (g/m^2)	0.976 ± 0.02	0.951 ± 0.01	0.23
FPG (mg/dL)	94 ± 1	105 ± 1	<0.001
2-h PG (mg/dL)	109 ± 1	150 ± 4	<0.001
HbA$_{1c}$ (%) (mmol/mol)	5.60 ± 0.04 (38 ± 0.4)	5.70 ± 0.04 (39 ± 0.4)	0.03
Fasting plasma insulin (mU/L)	5.5 ± 0.6	7.8 ± 0.6	0.01
FFA (mmol/L)	0.49 ± 0.04	0.52 ± 0.04	0.59
Matsuda Index (μmol · min^{-1} · kg^{-1})	8.8 ± 1.3	4.5 ± 1.3	<0.001
OGIS (mL · min^{-1} · m^{-2})	402 ± 8	346 ± 5	<0.001
HOMA-IR (mmol/L · mU/L)	1.3 ± 0.1	2.0 ± 0.2	<0.001
Fasting insulin clearance (L · min^{-1} · m^{-2})	4.8 ± 0.7	3.5 ± 0.8	0.02
OGTT insulin clearance (L · min^{-1} · m^{-2})	2.5 ± 0.5	1.7 ± 0.5	0.04
ATIRI (mmol/L · mU/L)	2.6 ± 0.3	4.4 ± 0.3	<0.001
Sclerostin (pmol/L)	38.7 ± 2.3	50.8 ± 2.4	0.01
	Clamp subgroup analysis		
	NGT (n= 18)	IGR (n= 30)	P value
Age (years)	43.2 ± 2.8	47.1 ± 2.2	0.29
Sex			0.56
Men (n)	6	9	
Women (n)	12	21	
Weight (kg)	80.5 ± 6.2	89.5 ± 2.9	0.15
BMI (kg/m^2)	29.6 ± 1.4	32.4 ± 1.0	0.11
Fat content (%)	36.9 ± 2.2	36.8 ± 1.6	0.95
Lean mass (%)	63.0 ± 2.1	63.2 ± 1.6	0.94
FPG (mg/dL)	94 ± 1	106 ± 1	<0.001
2-h PG (mg/dL)	112 ± 4	145 ± 5	<0.001
HbA$_{1c}$ (%) (mmol/mol)	5.5 ± 0.1 (37 ± 1.1)	5.7 ± 0.1 (39 ± 1.1)	0.19
Fasting plasma insulin (mU/L)	4.7 ± 0.6	8.6 ± 1.0	0.01
FFA (mmol/L)	0.49 ± 0.04	0.52 ± 0.04	0.59
HOMA-IR (mmol/L · mU/L)	1.1 ± 0.1	2.3 ± 0.3	<0.001
TGD/SSPI (mg · kg^{-1} · min^{-1}/mU · L^{-1})	8.4 ± 0.9	5.9 ± 0.6	0.02
Fasting EGP (mg · kg^{-1} · min^{-1})	2.0 ± 0.1	2.1 ± 0.2	0.72
Clamp EGP (mg · kg^{-1} · min^{-1})	0.4 ± 0.2	1.1 ± 0.5	0.03
HIRI [(mg · kg^{-1} · min^{-1}) · (mU/L)]	9.1 ± 1.1	14.9 ± 1.1	<0.001
Sclerostin (pmol/L)	39.2 ± 3.7	58.1 ± 6.1	0.03

Data are expressed as mean ± SEM unless indicated otherwise.

(r^2 change: 0.032; F change: 4.766; P = 0.031).

Similarly, a multiple linear regression analysis was performed using TGD/SSPI, a direct measure of insulin sensitivity in humans, as a dependent variable. In model 1, independent variables were BMI, body fat, HbA$_{1c}$, FPG, 2-h PG, and fasting plasma insulin. In model 2, sclerostin was added as an independent variable to model 1 to assess the contribution of sclerostin to the association with insulin sensitivity. BMI and fasting plasma insulin were independent predictors of insulin sensitivity (r^2 = 0.518). Consistently, in model 2, sclerostin was strongly independently associated with insulin sensitivity and increased the r^2 (r^2 change: 0.059; F change: 4.938; P = 0.033). Fasting EGP and the HIRI were positively correlated with sclerostin serum levels (r = 0.48 and r = 0.62, respectively; $P < 0.001$ after correction for age, sex, BMI, body fat, and FPG) (Fig. 1C and D). Moreover, the ATIRI was positively correlated with serum sclerostin levels (r = 0.61; $P < 0.001$) (Supplementary Fig. 2C).

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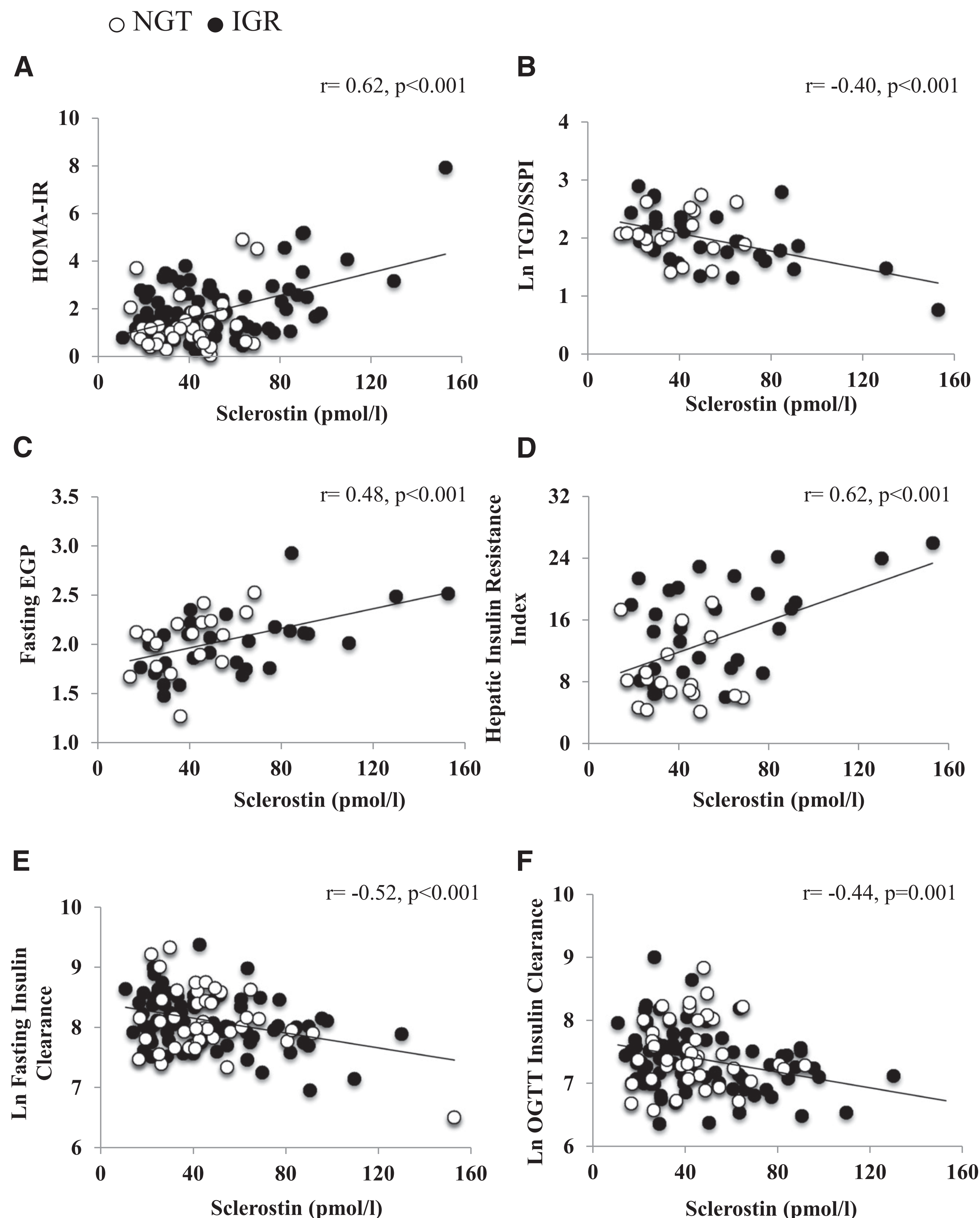

Figure 1—Partial correlation between serum sclerostin levels controlled for age, sex, BMI, body fat percentage, and HOMA-IR (*A*), LnTGD/SSPI (*B*), fasting EGP (*C*), hepatic insulin resistance (*D*), Ln fasting insulin clearance (*E*), and Ln OGTT insulin clearance (*F*).

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Insulin clearances at baseline (Ln fasting insulin clearance) and during OGTT (Ln OGTT insulin clearance) were inversely correlated with sclerostin serum levels ($r = -0.52$ and $r = -0.44$, respectively; both $P < 0.001$ after correction for age, sex, BMI, and TGD/SSPI) (Fig. 1*E* and *F*). To further examine the correlation between sclerostin serum levels and insulin

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Table 2—Multivariate analysis with HOMA-IR (whole population), SI (whole population), and TGD/SSPI (clamp subgroup) as dependent variables

Multivariate analysis	r^2 (*P* value)	r^2 change	*F* (*P* value)	*F* change (*P* value)	β	*P* value
HOMA-IR (mmol/L · mU/L)						
Independent variables						
Model 1	0.192 (<0.001)		7.430 (<0.001)			
Body fat (%)					0.041	0.643
BMI (kg/m^2)					0.374	<0.001
2-h PG (mg/dL)					0.065	0.491
HbA_{1c} (%)					0.208	0.026
Model 2	0.247 (<0.001)	0.055	13.291 (<0.001)	28.893 (0.001)		
Sclerostin (pmol/L)					0.423	<0.001
SI (mL · min^{-1} · kg^{-1}/pmol · L^{-1})						
Independent variables						
Model 1	0.293 (<0.001)		9.810 (<0.001)			
Body fat (%)					−0.148	0.084
BMI (kg/m^2)					−0.290	0.001
2-h PG (mg/dL)					−0.321	0.001
HbA_{1c} (%)					−0.103	0.249
Independent variable						
Model 2	0.325 (<0.001)	0.032	10.681 (<0.001)	4.766 (0.031)		
Sclerostin (pmol/L)					−0.182	0.031
TGD/SSPI (mg · kg^{-1} · min^{-1}/mU · L^{-1})						
Independent variables						
Model 1	0.518 (<0.001)		6.447 (<0.001)			
Body fat (%)					−0.193	0.126
BMI (kg/m^2)					−0.367	0.010
FPG (mg/dL)					−0.480	0.746
2-h PG (mg/dL)					−0.129	0.352
FPI (mU/L)					−0.318	0.022
HbA_{1c} (%)					−0.039	0.765
Model 2	0.577 (<0.001)	0.059	6.830 (<0.001)	4.938 (0.033)		
Sclerostin (pmol/L)					0.320	0.030

clearance independently from whole-body insulin sensitivity, we performed a multiple linear regression analysis in a model including age, sex, BMI, and TGD/SSPI as independent variables (Supplementary Table 2). When fasting insulin clearance was used as a dependent variable, TGD/SSPI remained independently associated with fasting insulin clearance (model 1) (r^2 = 0.245).

In model 2, sclerostin serum levels were independently associated with fasting insulin clearance and increased r^2 (r^2 change: 0.183; *F* change: 13.477; *P* = 0.001). Similarly, when OGTT insulin clearance was used as a dependent variable, TGD/SSPI remained independently associated with OGTT insulin clearance (model 1), and in model 2, sclerostin serum levels were independently associated with OGTT insulin clearance and increased the r^2 (r^2 change: 0.120; *F* change: 7.445; *P* = 0.009). Sclerostin levels were also negatively correlated with IRIs derived from OGTT as OGIS and the Ln Matsuda Index, and positively with ATIRI (r = −0.38, r = −0.42, and r = 0.61, respectively; all $P < 0.001$) (Supplementary Fig. 2*A*–*C*).

Serum sclerostin levels were positively correlated with the fasting insulin secretory rate (fasting ISR) (r = 0.19; *P* = 0.04), although no correlation was observed with the total ISR, β-cell glucose sensitivity, potentiation factor, and rate sensitivity (data not shown). Interestingly, when subjects were further divided according to their glucose tolerance status, those with isolated IFG were mostly accountable for the correlations between serum sclerostin levels and insulin sensitivity in skeletal muscle (r = 0.63; $P < 0.01$ for HOMA-IR; r = −0.55; *P* = 0.03 for Ln Matsuda Index; r = −0.64; $P < 0.01$ for OGIS; r = −0.57; *P* = 0.05 for Ln TGD/SSPI), liver (r = 0.61; *P* = 0.03 for fasting EGP), and adipose tissue (r = 0.71; $P < 0.01$ for ATIRI) (Supplementary Table 3). Similarly, in subjects with isolated IFG, serum sclerostin levels were strongly correlated with fasting insulin clearance (r = −0.44; *P* = 0.02), and a significant correlation between serum sclerostin levels and OGTT insulin clearance was found in subjects with combined IFG-IGT (r = −0.37; *P* = 0.03) (Supplementary Table 3).

Relationship of Serum Sclerostin Levels With Glucose Levels

Serum sclerostin levels were positively correlated with FPG (r = 0.22; *P* = 0.01) (Fig. 2*A*), fasting and 2-h plasma insulin (r = 0.63 and r = 0.45, respectively; $P < 0.001$) (Fig. 2*B* and *C*), and HbA_{1c} (r = 0.18; *P* = 0.04) (Fig. 2*D*). No correlations were found between serum sclerostin levels and plasma glucagon levels at fasting and during OGTT (*P* = 0.56 and *P* = 0.67, respectively; data not shown). When subjects were further divided according to the glucose tolerance status, subjects with isolated IFG and combined IFG-IGT were accountable for the correlation between serum sclerostin levels and fasting plasma insulin (r = 0.6; $P < 0.01$ and r = 0.52; $P < 0.01$, respectively) and 2-h plasma insulin (r = 0.72; $P < 0.01$

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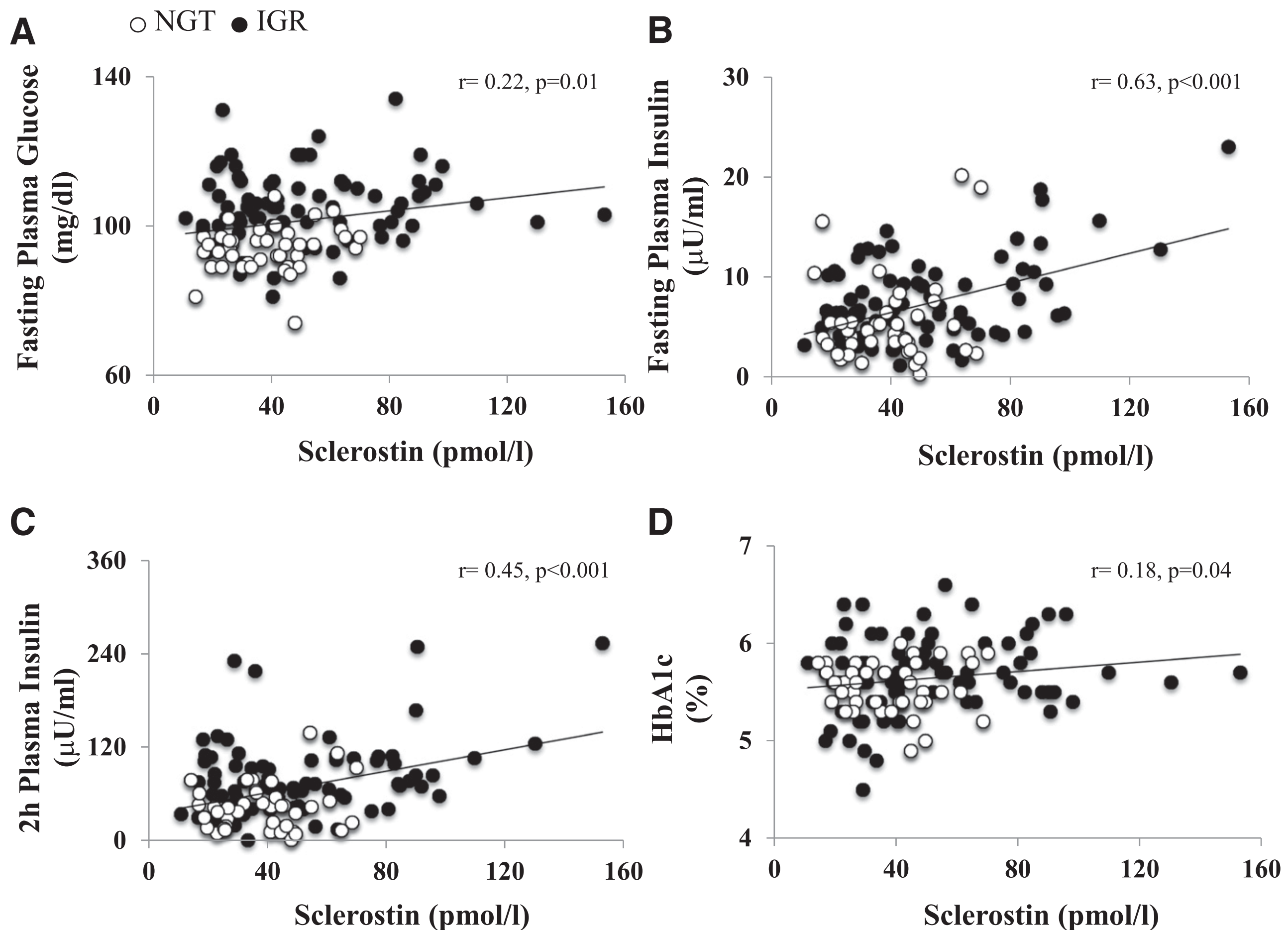

Figure 2—Partial correlation between serum sclerostin levels controlled for age, sex, BMI, body fat percentage, and FPG (*A*), fasting plasma insulin (*B*), 2-h plasma insulin (*C*), and HbA_{1c} (*D*) in NGT and IGR.

and *r* = 0.29; *P* = 0.08, respectively) (Supplementary Table 3).

CONCLUSIONS

This study shows that serum sclerostin levels are increased in subjects with prediabetes and strongly correlated with plasma insulin and insulin resistance derived from OGTT and euglycemic–hyperinsulinemic clamp. Our results suggest, although do not prove a direct causality, that in early stages of glucose intolerance and insulin resistance, sclerostin might play a role in insulin action and clearance in humans. Sclerostin might have a role in Wnt signaling in hepatic glucose metabolism by acting through the same pathways. Interestingly, we show that fasting EGP and the HIRI are strongly correlated with serum sclerostin levels. A recent study demonstrated that β-catenin, the downstream mediator of canonical Wnt signaling, modulates hepatic insulin signaling and glucose production (18). Also, a cross-sectional study including patients with liver cirrhosis found serum sclerostin levels were increased compared with control subjects and correlated with markers of liver dysfunction (19), suggesting a possible role of sclerostin in liver metabolism.

Interestingly, we observed a strong correlation between serum sclerostin levels and insulin clearance at fasting and during the OGTT, which was decreased in IGR individuals. This is a rather novel observation obtained by using an established method for measuring insulin clearance in humans (20,21).

Insulin clearance by the liver is an important component of the insulin degradation pathway, which has been recently suggested as a potential target for T2DM treatment (22). The liver removes ∼80% of endogenous insulin, and the remainder is cleared by the kidneys and muscles (23). However, clearance rates for insulin decrease in glucose intolerance (24), obesity, abdominal obesity in particular (25), hypertension (26), hepatic cirrhosis, and nonalcoholic fatty liver disease (27), implicating a possible link between insulin resistance and reduced insulin clearance (28). Reduced insulin clearance has important implications in the pathophysiology of T2DM and IGR. For example, animal models have shown that decreased insulin clearance serves as a compensatory mechanism to preserve β-cell function and maintain peripheral insulin levels in the states of insulin resistance (29). It is tempting to hypothesize that sclerostin might have a role in modulating insulin clearance by acting on the Wnt signaling pathway in the liver.

IFG and IGT subjects have distinct pathophysiological abnormalities. On one hand, IFG subjects manifest hepatic insulin resistance associated with normal insulin-mediated glucose disposal

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(reflecting mainly skeletal muscle glucose uptake). On the other hand, IGT subjects conversely manifest almost normal hepatic insulin sensitivity with reduced insulin-mediated glucose disposal (3). This is the potential involvement in the modulation of the insulin clearance, which occurs prevalently although not exclusively in the liver, further reinforced by the findings that sclerostin levels are significantly higher in the subgroups of patients with IFG and combined IFG-IGT in which insulin resistance in the liver is present.

Serum sclerostin levels positively correlated with FPG and 2-h PG and HbA_{1c}. In a small recent study evaluating a group of healthy offspring of patients with T2DM, serum sclerostin levels were consistently correlated with FPG, although they were not correlated with 2-h PG (30). Another study demonstrated that compared with healthy controls, circulating levels of sclerostin were increased in patients with T2DM, positively associated with duration of T2DM and glycated hemoglobin, and inversely related to bone turnover markers (31,32). Similarly, serum sclerostin levels were elevated in subjects with type 1 diabetes compared with normal control subjects and correlated with HbA_{1c} (33). Hyperglycemia has a detrimental effect on bone health, acting directly on bone cells and indirectly through the formation of advanced glycation end products that have been shown to reduce bone strength and increase the risk of falls and fractures. Moreover, hyperglycemia is directly involved in T2DM microvascular complications and accelerated atherosclerosis. Circulating sclerostin is further increased in patients with T2DM with atherosclerotic lesions, such as abnormal intima-media thickness, carotid plaques, and aortic calcification, compared with patients with T2DM without atherosclerotic lesions (34). Therefore, our results suggest that a possible detrimental role of sclerostin on bone metabolism and atherosclerosis might begin early in the pathogenesis of glucose metabolism alterations, at the stage of IGR. A recent study showed that serum sclerostin levels were correlated with HOMA-IR but not with HOMA–β-cell function in healthy offspring of T2DM patients (30). In patients with T2DM, the increase of serum sclerostin is associated with a significant decrease in the β-catenin serum concentration, suggesting, indeed, that sclerostin signaling is modulated in diabetes (31). On one hand, this gives support to the hypothesis that bone, which produces almost all the available sclerostin, may play a role also in glucose metabolism and insulin resistance (8,32,35). On the other hand, it can be speculated that anti-sclerostin antibody treatment, which is a promising option for severe osteoporosis and surgical implant fixation, might have a role in T2DM treatment (36).

In subcutaneous and visceral white adipose tissue from lean and obese subjects, the Wnt pathway was altered in obese subjects, and its inhibition was associated with insulin resistance (37). Therefore, sclerostin-mediated inhibition of Wnt signaling might directly cause adipogenesis and adipose tissue insulin resistance, which are present in T2DM and in prediabetes (38). Given that obesity is a major risk factor for developing T2DM, changes in components of the Wnt signaling pathway could conceivably contribute to the increased risk of diabetes development by affecting adipogenesis.

Although a strong correlation between serum sclerostin levels, bone density, and body fat was previously demonstrated in healthy subjects and in patients with T2DM (31,39,40), our results could not confirm these findings. Our study is limited by the sole measurement of whole-body minus head bone density and not the individual sites such as the spine, hip, and forearm. Our study has other limitations. First, the cross-sectional design does not allow the study of potential causes of changes in sclerostin levels during the development of IGR, thus providing a stronger link between insulin resistance and increased sclerostin levels. Second, the sample size is relatively small and might affect the statistical power, although the study groups were well matched for age, sex, BMI, and body composition, including bone density.

Strengths of our study are the evaluation for the first time of circulating serum sclerostin in subjects with NGT and IGR and the extensive and detailed evaluation of biochemical and clinical parameters of glucose metabolism, derived from OGTT and the gold standard, euglycemic clamp with glucose tracer to evaluate hepatic glucose production.

In summary, sclerostin circulates in higher amounts in individuals with prediabetes, particularly in IFG and IFG-IGT, and is strongly correlated with whole-body insulin sensitivity and EGP as well as insulin clearance, supporting the hypothesis that sclerostin action is important not only in the regulation of bone formation and resorption but also in the regulation of glucose metabolism in humans. Further studies are needed to clarify the mechanisms by which sclerostin could affect insulin action in vivo and to evaluate the role of sclerostin in the natural history of glucose intolerance from prediabetes to T2DM.

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