Phenotypic Characterization of Insulin-Resistant and Insulin-Sensitive Obesity

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Context: Whereas insulin resistance and obesity coexist, some obese individuals remain insulin sensitive.

Objective: We examined phenotypic and metabolic factors associated with insulin sensitivity in both muscle and liver in obese individuals.

Design and Participants: Sixty-four nondiabetic obese adults (29 males) underwent hyperinsulinemic (15 and 80 mU/m^2 · min)-euglycemic clamps with deuterated glucose. Top tertile subjects for glucose infusion rate during the high-dose insulin clamp were assigned Muscle$_{sen}$ and those in the lower two tertiles were assigned Muscle$_{res}$. Secondarily, top tertile subjects for endogenous glucose production suppression during the low-dose insulin clamp were deemed Liver$_{sen}$ and the remainder Liver$_{res}$.

Main Outcomes Measures: Clinical and laboratory parameters and visceral, subcutaneous, liver, and pancreatic fat were compared.

Results: Muscle$_{sen}$ and Muscle$_{res}$ had similar body mass index and total fat ($P > .16$), but Muscle$_{sen}$ had lower glycated hemoglobin ($P < .001$) and systolic ($P = .01$) and diastolic ($P = .03$) blood pressure (BP). Despite similar sc fat ($P = 1$), Muscle$_{sen}$ had lower visceral ($P < .001$) and liver ($P < .001$) fat. Liver$_{sen}$ had lower visceral ($P < .01$) and liver ($P < .01$) fat and C-reactive protein ($P = .02$) than Liver$_{res}$. When subjects were grouped by both glucose infusion rate during the high-dose insulin clamp and endogenous glucose production suppression, insulin sensitivity at either muscle or liver conferred apparent protection from the adverse metabolic features that characterized subjects insulin resistant at both sites. High-density lipoprotein-cholesterol, 1-hour glucose, systolic BP, and triglycerides explained 54% of the variance in muscle insulin sensitivity.

Conclusions: Obese subjects who were insulin sensitive at muscle and/or liver exhibited favorable metabolic features, including lower BP, liver and visceral adiposity. This study identifies factors associated with, and possibly contributing to, insulin sensitivity in obesity. ***(J Clin Endocrinol Metab* 100: 4082–4091, 2015)**

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Abbreviations: AUC, area under the curve; BMI, body mass index; BP, blood pressure; DXA, dual-energy x-ray absorptiometry; EGP, endogenous glucose production; FABP4, fatty acid-binding protein 4; FFM, fat-free mass; FGF, fibroblast growth factor; GIR$_{HI}$, glucose infusion rate (during high-dose insulin); HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HIRI, hepatic insulin sensitivity index; hsCRP, high-sensitivity C-reactive protein; Liver$_{res}$, liver insulin resistant; Liver$_{sen}$, liver insulin sensitive; MHO, metabolically healthy obesity; MRI, magnetic resonance imaging; Muscle$_{res}$, muscle insulin resistant; Muscle$_{sen}$, muscle insulin sensitive; NEFA, nonesterified fatty acid; OGTT, oral glucose tolerance test; RBP4, retinol-binding protein 4; RQ, respiratory quotient; SBP, systolic BP.

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The prevalence of obesity is rapidly increasing, with more than half a billion adults affected worldwide (1). The associated increase in diabetes, cardiovascular disease, and cancer carries a significant health and financial burden (2). However, not all obese subjects are similarly affected, and some obese individuals are observed to have normal blood pressure (BP), insulin sensitivity, and lipid profile. To target intervention most effectively, it is critical to identify individuals carrying the highest metabolic risks.

Metabolically-healthy obesity (MHO) is a term used to denote obese individuals, who, despite substantial adiposity, remain free from metabolic complications and have relatively normal insulin sensitivity. More than 30 different definitions have been used to identify MHO humans, resulting in variable prevalence rates between 3% and 43% (3). Most studies classify obese subjects as metabolically healthy if they are free of some or all metabolic syndrome criteria, whereas others have classified participants solely or partly based on insulin sensitivity (4).

Insulin resistance is a pivotal component of the metabolic syndrome. It is an obligatory precursor to the development of type 2 diabetes and a likely contributor to cardiovascular disease (5). Given the variability in defining and identifying MHO, whether this phenotype is predictive of lower diabetes and cardiovascular risk cannot be answered. Because insulin resistance is the key unifying factor in the metabolic syndrome, a more pathophysiological definition of MHO may be one based on insulin sensitivity alone.

We hypothesized that insulin sensitivity at muscle or liver (or both) in obese subjects would be associated with a favorable metabolic profile (lower BP, glucose, glycated hemoglobin [HbA1c], and liver and visceral fat). Hence, we examined nondiabetic obese individuals, using a two-step hyperinsulinemic-euglycemic clamp with glucose tracers, and measured clinical and biochemical metabolic parameters, body composition, and abdominal fat distribution. We compared subjects who were insulin sensitive with those who were insulin resistant at muscle and repeated analyses after recategorizing subjects by hepatic insulin sensitivity. In secondary analyses, subjects were segregated into four groups based on the site of insulin resistance (muscle, liver, or both) to investigate whether being insulin sensitive at one site was sufficient in predicting a favorable metabolic phenotype.

Materials and Methods

Subjects

Subjects (n = 184) were recruited by advertisements in newspapers over an 18-month period (2011–2013). The protocol was approved by St Vincent's Hospital Human Research Ethics Committee (Sydney, Australia). Written consent was obtained prior to study commencement. Screening assessed eligibility, including age 18–70 years and body mass index (BMI) > 30 kg/m^2. Exclusion criteria were diabetes; treatment with medications that affect glucose metabolism (glucocorticoids, antipsychotics); alcohol intake greater than 20 g/d or 40 g/d in women and men, respectively; weight change greater than 5% in the 3 months leading up to the study; known renal, cardiac, or liver disease; and current cancer (number excluded, n = 104).

Eligible subjects (n = 80) underwent a 75-g oral glucose tolerance test (OGTT) using American Diabetes Association criteria (6) to exclude subjects with undiagnosed diabetes (n = 2). Fourteen subjects did not proceed to the hyperinsulinemic clamp study due to loss of interest (n = 4), illness (n = 4), and difficult venous access (n = 6). There were no significant differences in age, BMI, BP, waist circumference, or smoking or alcohol status between the final cohort and those 14 subjects (data not shown). Hence, 64 subjects underwent the hyperinsulinemic-euglycemic clamp and body composition studies; 14 had prediabetes (impaired fasting glucose and impaired glucose tolerance, n = 2; impaired fasting glucose alone, n = 1; impaired glucose tolerance alone, n = 11).

All studies were conducted at the Clinical Research Facility at the Garvan Institute of Medical Research (Sydney, Australia) (with the exception of three subjects who underwent an OGTT at their local pathology centers). Subjects were instructed not to perform vigorous exercise, to abstain from alcohol, and to record their diet in the 2 days preceding the clamp study. Subjects attended the Clinical Research Facility after overnight fasting. Premenopausal females had urine β-human chorionic gonadotropin checked to exclude pregnancy. Measurement of BP, waist and hip circumferences, height, and weight were undertaken, and BMI was calculated. Dietary intake was evaluated by 2-day diet diaries that were analyzed using the Australian-based food composition software FoodWorks 7 (Xyris), and the Stanford 7-day activity questionnaires were used to assess physical activity in the preceding 7 days, as described previously (7).

Hyperinsulinemic-euglycemic clamp studies

Subjects underwent a 6-hour, two-step hyperinsulinemic-euglycemic clamp with deuterated glucose tracers (6,6-$^2H_2$; Cambridge Isotope Laboratories). The clamp started with a 2-hour primed (5 mg/kg), continuous (3 mg/kg $\cdot$ h) infusion of [6,6-$^2H_2$]glucose, followed by a 2-hour infusion of low-dose insulin (15 mU/m^2 $\cdot$ min) and a 2-hour infusion of high-dose insulin (80 mU/m^2 $\cdot$ min). The deuterated glucose infusion rate was halved (1.5 mg/kg $\cdot$ h) during, and ceased at the end of, the low-dose insulin infusion. Glucose was infused to maintain whole-blood concentration of 5 mmol/L with variable rate infusion of dextrose (25%, enriched to ~2.5% with deuterated glucose). The low and high glucose infusion rates (GIR_{HI}) were calculated at 90–120 minutes of each clamp stage and normalized for fat-free mass (FFM). Whole-body energy expenditure and respiratory quotient (RQ) were measured at baseline and during the last 30 minutes of each stage of the clamp (Parvo Medics True One).

Deuterated glucose was analyzed by gas chromatography-mass spectrometry (Agilent Technologies) with the correction for natural abundance of ^{13}C, as described previously (8). The between-run and within-run coefficients of variation for unenriched 25% glucose were 0.8% and 2.4%, respectively, as re-

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ported previously (8). Endogenous glucose production (EGP) was estimated using Steele's one-compartment, fixed-volume model (assuming volume of distribution of 20% of body weight and pool fraction of 0.65 [9]), as modified by Finegood et al (10). Systemic glucose appearance and disappearance were estimated using nonsteady-state calculations (9, 10). The hepatic insulin resistance index (HIRI) was calculated as fasting serum insulin * EGP (11). Because EGP was fully suppressed during the high-dose insulin infusion, GIR_{HI} reflects peripheral (mainly muscle) insulin sensitivity. For two subjects, liver insulin sensitivity could not be calculated due to plasma tracer sampling or analysis errors.

Definition of insulin sensitivity in muscle and liver

Tertiles of insulin sensitivity were calculated separately for men and women. Study participants were assigned to the muscle insulin-sensitive ($Muscle_{sen}$) group if GIR_{HI} was in the upper tertile of the cohort and to the insulin-resistant ($Muscle_{res}$) group if GIR_{HI} fell in the lower two tertiles. In separate analyses, subjects were reclassified by the degree of EGP suppression during low-dose insulin. Liver insulin-sensitive subjects ($Liver_{sen}$) were in the upper tertile of EGP suppression and liver insulin-resistant subjects ($Liver_{res}$) were in the lower two tertiles. GIR_{HI} and EGP suppression correlated ($R^2 = 0.14$, $P = .003$).

Dual-energy x-ray absorptiometry (DXA)

Total fat mass, FFM, and central abdominal fat were measured by DXA (Lunar Prodigy; GE-Lunar). In some larger subjects, an analysis was performed by aligning one side of the body on the scanner, with data doubled to achieve overall body composition. Two subjects were too large to undergo DXA scanning; instead, bioimpedance analysis (Tanita body composition analyzer) was used to estimate fat and lean mass. Bioimpedance analysis and DXA-derived measures of body fat mass have previously showed strong correlations ($r = 0.92$, $P < .001$) (12).

Magnetic resonance imaging (MRI)

MRI (3.0 T Philips Achieva) images were acquired by mDIXON software (Edinhoven) to evaluate visceral, sc, liver, and pancreatic fat. Visceral adipose tissue was measured in five slices at L4/L5 intervertebral disc level using Image J software 1.46r (National Institutes of Health, Bethesda, Maryland) and calculated as the difference between total fat and sc fat. Intraorgan fat percentage was evaluated by MRI from three regions of interest (ROI) in the liver (15 × 15 mm) and two ROI in the pancreas (10 × 10 mm at head and tail of pancreas) avoiding blood vessels. ROI were defined and data averaged in a blinded fashion by one observer (D.L.C.) with a radiologist support (B.M.). One female and two males with a BMI range of 45.5–48.5 kg/m^2 could not be scanned due to their size, and their MRI-related data are missing (MRI data, n = 61). Key clinical and metabolic factors, including age, BP, HbA1c, and insulin sensitivity in the muscle and liver for these individuals were within the cohorts' ranges.

Adipocyte size

Periumbilical sc fat biopsy was performed in 53 subjects during the basal clamp stage under sterile conditions using a trocar, as previously described (13). Samples were fixed in Bouin's fluid (Sigma), dehydrated, paraffin embedded, and sectioned (4 μm) and then stained with hematoxylin and eosin. Processed images were acquired by a microscope camera system (Leica DMR, core LAS 4.2). Adipocyte diameter was measured using Image J software 1.46r (National Institutes of Health) by two blinded independent observers. Mean and median adipocyte diameters were calculated from approximately 100 cells per sample.

Measurement of metabolites and hormones

Whole-blood glucose was measured using the YSI 2300 STAT analyzer. Insulin and C-peptide were measured by a RIA (Millipore), lipid profiles by an automated analyzer (Roche), and nonesterified fatty acids (NEFA) by an enzymatic colorimetric assay (Wako, Japan). High-sensitivity C-reactive protein (hsCRP), fibroblast growth factor (FGF)-19, FGF-21, total adiponectin, fatty acid-binding protein 4 (FABP4), lipocalin-2, and retinol binding protein 4 (RBP4) were measured by an ELISA (Antibody and Immunoassay Service, Hong Kong) (14–16). The intra- and interassay coefficients of variation for hsCRP, FGF-19, FGF-21, total adiponectin, FABP4, lipocalin-2, and RBP4 were 4.3% and 5.9%; 4.5% and 5.6%; 4.4% and 9.2%; 5.1% and 6.2%; 4.8% and 5.7%; 3.8% and 5.2%; 4.1% and 7.2%, respectively.

Statistical analysis

Data were expressed as mean ± SD unless otherwise specified. Abnormally distributed data were logarithmically transformed prior to statistical analysis. A Student's *t* test was used to detect differences between the two phenotypes. When four groups were compared, one-way ANOVA with Tukey post hoc analyses detected the differences between groups. Repeated-measures ANOVA assessed the differences in EGP and NEFA suppression from basal to hyperinsulinemia between phenotypes. Pearson's coefficients assessed associations between variables. Stepwise regression analysis was used to assess the contribution of continuous clinical and metabolic variables to muscle or liver insulin sensitivity (GIR_{HI} and EGP suppression, respectively). Variance inflation factors were calculated to avoid potential colinearity. An area under the curve (AUC) for glucose, insulin, and C-peptide responses to the OGTT were calculated using the trapezoidal model. $P < .05$ was considered statistically significant. A statistical analysis was carried out using SPSS version 21.

Results

Metabolic characteristics of insulin-sensitive and insulin-resistant individuals

Characteristics of the cohort categorized separately by GIR ($Muscle_{sen}$ vs $Muscle_{res}$) and liver insulin sensitivity ($Liver_{sen}$ vs $Liver_{res}$) are presented in Table 1. Age and BMI were not different between $Muscle_{sen}$ and $Muscle_{res}$ or between $Liver_{sen}$ and $Liver_{res}$. By design, $Muscle_{sen}$ had higher GIR_{HI} than $Muscle_{res}$ (120 ± 25 vs 76 ± 21 μmol/min · kg FFM). Importantly, GIR_{HI} in $Muscle_{sen}$ was similar to GIR_{HI} measured by our group previously in a group of lean healthy individuals (92 ± 23 μmol/min · kg FFM) (17). EGP suppression during the low-dose insulin infusion was 80% ± 9% and 58% ± 9% in $Liver_{sen}$ and $Liver_{res}$, respectively.

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Table 1. Anthropometric, Clinical and Metabolic Characteristics of Obese Individuals Stratified Based on Muscle ($Muscle_{sen}$ vs $Muscle_{res}$) and Liver ($Liver_{sen}$ vs $Liver_{res}$) Insulin Sensitivity

Characteristics	$Muscle_{sen}$ (M9:F12)	$Muscle_{res}$ (M20:F23)	*P* Value	$Liver_{sen}$ (M9:F12)	$Liver_{res}$ (M18:F23)	*P* Value
Age, y	50 ± 12.6	50 ± 11.0	.97	49 ± 12	51 ± 11	.52
BMI, kg/m^2	35.3 ± 4.2	37.1 ± 4.8	.16	35.6 ± 3.9	36.1 ± 4.1	.65
Waist circumference, cm	107 ± 12	113 ± 14	.08	106 ± 11	114 ± 14	**.046**
Whole body fat, kg[a]	45 ± 11	46 ± 10	.62	45 ± 10	46 ± 10	.64
Central abdominal fat, kg[a]	3.1 ± 0.6	3.6 ± 0.7	**.01**	3.2 ± 0.7	3.5 ± 0.7	.09
Subcutaneous fat, cm^2	514 ± 140	510 ± 132	1.0	496 ± 141	513 ± 128	.51
Visceral fat, cm^2	213 ± 50	289 ± 82	<**.001**	227 ± 51	288 ± 86	**.003**
Pancreatic fat, %	13 ± 7	14 ± 9	.73	12 ± 8	14 ± 9	.14
Liver fat, %	5 ± 5	17 ± 2	<**.001**	8 ± 8	16 ± 12	<**.001**
Mean/median adipocyte size, μm[b]	71 ± 9	75 ± 9	.12	69 ± 7	76 ± 10	**.01**
	70 ± 9	75 ± 10	.11	69 ± 8	76 ± 10	**.01**
Systolic BP, mm Hg[c]	118 ± 8	127 ± 13	**.01**	121 ± 9	126 ± 14	.16
Diastolic BP, mm Hg[c]	78 ± 8	84 ± 10	**.03**	80 ± 12	83 ± 9	.23
Total cholesterol, mmol/L[d]	5.0 ± 0.8	5.0 ± 0.8	.79	5.1 ± 0.9	4.9 ± 0.7	.45
LDL cholesterol, mmol/L[d]	3.1 ± 0.6	3.1 ± 0.7	.96	3.2 ± 0.8	3.1 ± 0.7	.53
HDL cholesterol, mmol/L[d]	1.3 ± 0.3	1.3 ± 0.3	.27	1.3 ± 0.4	1.3 ± 0.3	.54
Triglycerides, mmol/L[d,e]	0.8 (0.6–1.2)	1.1 (0.8–1.4)	**.02**	0.9 (0.6–1.2)	1.1 (0.9–1.4)	**.03**
Fasting glucose, mmol/L	4.7 ± 0.4	4.8 ± 0.5	.12	4.7 ± 0.5	4.9 ± 0.4	.11
OGTT 1-hour blood glucose, mmol/L	6.6 ± 1.4	8.3 ± 2.1	**.001**	6.8 ± 1.8	8.2 ± 2.0	**.01**
OGTT 2-hour blood glucose, mmol/L	5.7 ± 1.5	6.6 ± 1.6	**.04**	5.8 ± 1.6	6.6 ± 1.6	.07
OGTT $AUC_{Glucose}$, mmol/L · 120 min	754 ± 121	887 ± 176	**.003**	763 ± 154	887 ± 168	**.01**
OGTT $AUC_{Insulin}$, mU/L · 120 min[e]	7600 (6323–9854)	11 410 (8287–17 157)	**.002**	7497 (6106–11 353)	11 978 (7941–17 114)	**.004**
OGTT $AUC_{C\text{-}peptide}$, μg/L · 120 min[e]	513 (385–666)	750 (588–1027)	<**.001**	425 (371–612)	746 (586–992)	<**.001**
HbA1c, %	5.2 ± 0.2	5.6 ± 0.3	<**.001**	5.2 ± 0.3	5.6 ± 0.3	<**.001**
Fasting insulin, mU/L[e]	12 (9–16)	18 (13–26)	**.001**	12 (10–16)	18 (13–27)	<**.001**
Fasting NEFA, mmol/L[e]	0.36 (0.29–0.45)	0.37 (0.26–0.46)	.97	0.35 (0.25–0.45)	0.37 (0.28–0.46)	.97
$NEFA_{LO}$, mmol/L[e]	0.04 (0.02–0.05)	0.05 (0.04–0.06)	**.02**	0.03 (0.02–0.05)	0.05 (0.04–0.06)	**.02**
Basal RQ	0.79 ± 0.03	0.80 ± 0.03	.47	0.79 ± 0.02	0.80 ± 0.03	.1
ΔRQ ($RQ_{HI} - RQ_{Baseline}$)	0.19 ± 0.04	0.14 ± 0.05	**.002**	0.18 ± 0.04	0.15 ± 0.05	**.048**
hsCRP, mg/L[e]	2.3 (1.5–4.2)	3.9 (1.8–5.6)	.16	2.1 (1.1–3.7)	4.2 (2.0–5.7)	**.02**
FGF-19, ng/L[e]	128 (69–232)	94 (59–142)	.09	120 (69–187)	96 (56–152)	.12
FGF-21, ng/L[e]	72 (20–109	83 (44–140)	.32	68 (23–105)	91 (46–159)	.09
FABP4, μg/L	60 ± 27	64 ± 27	.63	61 ± 28	65 ± 26	.56
Lipocalin-2, μg/L	40 ± 14	41 ± 14	.67	44 ± 14	40 ± 14	.29
RBP4, mg/L	11 ± 2	11 ± 3	.78	12 ± 2	11 ± 3	.36
Adiponectin, mg/L	17 ± 9	14 ± 7	.08	16 ± 8	14 ± 8	.34

Abbreviation: LDL, low-density lipoprotein.

[a] DXA data were available for 61 participants.

[b] Adipocyte size data were available for 53 participants.

[c] Subjects treated with antihypertensive medications were excluded (included: $Muscle_{sen}$, n = 17, and $Muscle_{res}$, n = 34; $Liver_{sen}$, n = 19, and $Liver_{res}$, n = 30).

[d] Subjects treated with lipid-lowering medications excluded (included: $Muscle_{sen}$, n = 18, and $Muscle_{res}$, n = 37; $Liver_{sen}$, n = 20, and $Liver_{res}$, n = 34).

[e] Data are median (interquartile range).

Data are mean ± SD, unless e above. Bold values signify statistical significance ($P < .05$).

Despite similar total body fat content, $Muscle_{sen}$ had lower central abdominal fat than $Muscle_{res}$ (Table 1). By contrast, no difference in central abdominal fat was observed between $Liver_{sen}$ and $Liver_{res}$. Subcutaneous fat and pancreatic fat were not different between $Muscle_{sen}$ and $Muscle_{res}$ or between $Liver_{sen}$ and $Liver_{res}$ (Table 1). $Muscle_{sen}$ and $Liver_{sen}$ had significantly less abdominal visceral and liver fat compared with their insulin-resistant counterparts. Mean adipocyte size was not different between $Muscle_{sen}$ and $Muscle_{res}$ but was lower in $Liver_{sen}$ compared with $Liver_{res}$ (Table 1).

After exclusion of subjects treated with antihypertensive medications, $Muscle_{sen}$ had lower systolic and diastolic BP than $Muscle_{res}$, but no differences were noted between $Liver_{sen}$ and $Liver_{res}$ (Table 1). After exclusion of subjects treated with lipid-lowering medications, $Muscle_{sen}$ and $Liver_{sen}$ had lower fasting triglycerides compared with their insulin-resistant counterparts. Total cholesterol and high-density lipoprotein (HDL) cholesterol were not different (Table 1).

Fasting blood glucose was not significantly different between groups, but 1-hour blood glucose after a 75-g glucose load, $AUC_{glucose}$, $AUC_{insulin}$, and $AUC_{C\text{-}peptide}$ during the OGTT were significantly lower in $Muscle_{sen}$ and $Liver_{sen}$ compared with their insulin-resistant counterparts (Table 1). Similarly, fasting insulin was significantly lower in $Muscle_{sen}$ and $Liver_{sen}$ compared with $Muscle_{res}$ and $Liver_{res}$, respectively (Table 1). Circulating insulin concentrations during the low- and high-dose insulin clamps were similar between the insulin-sensitive and in-

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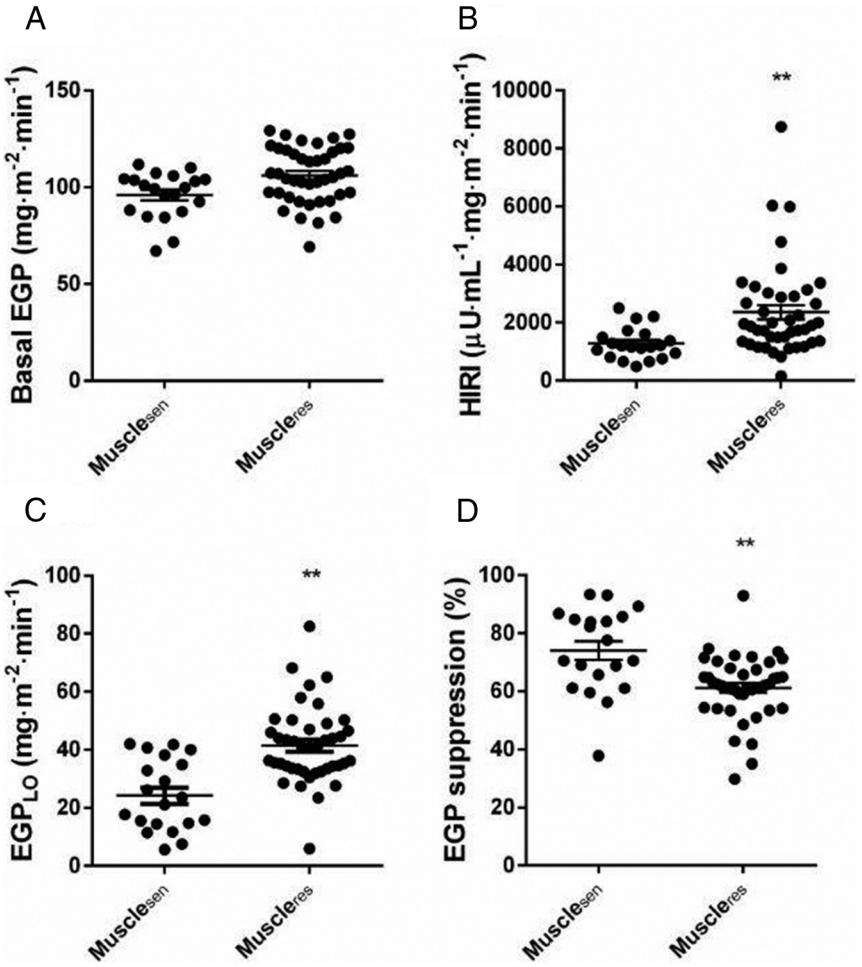

Figure 1. Basal EGP (A), HIRI (B), EGP during the low-dose insulin clamp (EGP_{LO}; C), and EGP suppression during the low-dose clamp (D) in obese individuals stratified based on muscle insulin sensitivity. Differences by a Student's *t* test are noted. **, $P < .01$. EGP suppression = $(EGP_{BL} - EGP_{LO})/EGP_{BL} * 100$.

sulin-resistant groups ($P > .09$). Although fasting serum NEFA concentrations were not different between the insulin-sensitive and insulin-resistant groups, NEFA concentrations during the low-dose insulin infusion were significantly lower in $Muscle_{sen}$ and $Liver_{sen}$ compared with their insulin-resistant counterparts (Table 1), suggesting increased adipose tissue insulin sensitivity.

Basal RQ was not significantly different between groups, but the ΔRQ (RQ during the high dose insulin infusion minus baseline RQ) was significantly higher in $Muscle_{sen}$ and $Liver_{sen}$ compared with their insulin-resistant counterparts (Table 1), suggesting increased metabolic flexibility.

Baseline EGP was not different between $Muscle_{sen}$ and $Muscle_{res}$ (Figure 1A). However, HIRI was significantly lower in $Muscle_{sen}$ (Figure 1B). $Muscle_{sen}$ had lower EGP during the low-dose insulin infusion (Figure 1C) and EGP suppression was significantly blunted in $Muscle_{res}$ (Figure 1D). EGP was not significantly different from zero during the high-dose insulin infusion ($P = .22$).

Circulating hsCRP was lower in $Liver_{sen}$ vs $Liver_{res}$ and FGF-19 and total adiponectin tended to be higher in $Muscle_{sen}$ vs $Muscle_{res}$, whereas other circulating inflammatory markers were not different between the groups (Table 1).

Reported dietary energy and macronutrient intake did not differ between $Muscle_{sen}$ and $Muscle_{res}$, although sugar and saturated fat intake were lower in $Muscle_{sen}$ (Supplemental Table 1). Physical activity level was not different between groups (34 ± 1 and 34 ± 2 metabolic equivalent of task, hours per day in $Muscle_{sen}$ and $Muscle_{res}$, $P = .70$). In the liver groups, there were no differences in energy and macronutrient intake (Supplemental Table 1) or in physical activity level (34 ± 1 and 34 ± 2 metabolic equivalent of task hours per day in $Liver_{sen}$ and $Liver_{res}$, $P = .59$).

Linear regression analyses

In the whole cohort, GIR_{HI} correlated inversely with central abdominal fat, systolic BP, serum triglycerides, fasting glucose, HbA1c, $NEFA_{LO}$, and the OGTT-derived variables $AUC_{glucose}$, $AUC_{insulin}$, and $AUC_{C\text{-}peptide}$ (Supplemental Table 2). Positive associations were noted with serum HDL, ΔRQ, serum FGF-19, and total adiponectin (Supplemental Table 2). All associations remained significant after adjustment for total body fat (Supplemental Table 2). GIR_{HI} correlated inversely with liver and visceral and pancreatic fat (Supplemental Figure 1, A–C) but not with abdominal sc fat (Supplemental Figure 1D).

Liver insulin sensitivity (EGP suppression) was inversely correlated with liver fat (Supplemental Figure 1E) and trended to inversely correlate with visceral fat (Supplemental Figure 1F), but no significant correlation was noted with pancreatic and sc fat (Supplemental Figure 1, G and H, respectively). Moreover, EGP suppression significantly correlated inversely with $AUC_{insulin}$, $AUC_{C\text{-}peptide}$, HbA1c, fasting insulin, $NEFA_{LO}$, and hsCRP (Supplemental Table 2).

Pancreatic fat correlated positively with liver ($r = 0.29$, $P = .02$) and visceral ($r = 0.40$, $P = .001$) fat but not with sc fat ($P = .25$). Pancreatic fat correlated positively with the OGTT-derived measures of β-cell function $AUC_{C\text{-}peptide}$ ($r = 0.33$, $P = .01$) and $AUC_{insulin}$ ($r = 0.33$, $P = .01$).

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Significant associations were noted between mean adipocyte size and GIR_{HI} (r = −0.29, P = .04), ΔRQ (r = −0.37, P = .01), visceral fat (r = 0.30, P = .03), liver fat (r = 0.29, P = .04), fasting insulin (r = 0.39, P = .004), fasting triglycerides (r = 0.47, $P < .001$), HDL cholesterol (r = −0.42, P = .002), and HbA1c (r = 0.36, P = .01). There was a significant inverse correlation between mean adipocyte size and HIRI (r = −0.27, P = .03) and a trend with EGP suppression in the total cohort (r = −0.26, P = .06).

Multiple linear regression analyses

Sixty-four percent of GIR_{HI} variability was explained by ΔRQ, liver fat, HDL cholesterol, and systolic BP (Supplemental Table 3). Because ΔRQ is, to an extent, an alternative measure of insulin sensitivity, an alternative multiple linear regression model was carried out including the same variables but without ΔRQ. Liver fat, HDL, systolic BP (SBP), and HbA1c explained 63% of GIR_{HI} variability. In a clinically applicable multiple linear regression model (including HDL, OGTT 1 h blood glucose, SBP, triglycerides, hsCRP, HbA1c, and waist circumference), HDL, OGTT 1-hour blood glucose, SBP, and serum triglycerides explained 54% of GIR_{HI} variability (Supplemental Table 3).

When HbA1c, hsCRP, triglycerides, SBP, and liver fat were entered into a multiple linear regression model to explain EGP suppression, HbA1c and liver fat explained 22% of the variability and all other variables were not retained (Supplemental Table 3).

Characterization based on both muscle and liver insulin sensitivity

An additional classification of the cohort was carried out based on both GIR_{HI} ($Muscle_{sen}$ and $Muscle_{res}$) and EGP suppression ($Liver_{sen}$ and $Liver_{res}$). As expected, the most significant differences were noted between the two extreme groups (Table 2). Specifically, $Muscle_{sen}Liver_{sen}$ had significantly lower glycemia (ie, $AUC_{glucose}$ and HbA1c), fasting insulin, $AUC_{insulin}$, and $AUC_{C\text{-}peptide}$ during the OGTT compared with $Muscle_{res}Liver_{res}$. $Muscle_{res}Liver_{res}$ had significantly greater visceral adiposity than all other groups and greater liver fat when compared with $Muscle_{sen}Liver_{sen}$ and $Muscle_{sen}Liver_{res}$ (Figure 2, A and B, respectively). Subcutaneous fat and pancreatic fat content were not different between the four groups (Figure 2, C and D, respectively).

Discussion

The definition of metabolic health in obesity is currently unstandardized, leading to inconsistent findings across studies (4). Here we stratified obese nondiabetic individuals to insulin-sensitive or insulin-resistant based on hyperinsulinemic clamps, a method applicable only in relatively small cohort studies. Lower visceral adiposity, liver fat, glycemia, and BP were key features of insulin-sensitive obesity.

Muscle and liver are major insulin target tissues and key players in glucose homeostasis. Visceral fat deposition is associated with metabolic disease and a strong correlation between visceral fat and peripheral insulin resistance is maintained even when BMI is greater than 30 kg/m^2 (18). Strikingly, when our cohort was bidimensionally stratified based on both muscle and liver insulin sensitivity, individuals who were insulin resistant at either muscle or liver were not different in abdominal visceral fat from those who were insulin sensitive in both tissues. Those who were insulin resistant in both muscle and liver had significantly greater visceral adiposity than the group sensitive at both sites.

Liver lipid accumulation is common in obesity and is associated not only with hepatic insulin resistance but also with muscle insulin resistance (19, 20). Our study showed that in obese individuals, liver fat was lower, irrespective of liver insulin sensitivity, if muscle remained insulin sensitive. These findings suggest that the relationships between liver lipid content and hepatic insulin resistance are complex in obesity and that liver lipid aligns better with muscle insulin sensitivity. This is partly supported by other studies in which intrahepatic lipid content predicted muscle insulin resistance (21). The mechanisms behind the association between liver derangements and muscle insulin resistance are intriguing and under investigation in animal models, but a hepatoskeletal muscle endocrine axis is suggested, with molecules originating from the liver modulating insulin sensitivity in muscle. Potential candidates include the insulin sensitizers FGF-19 and FGF-21 (22). Here we found that FGF-19 correlated positively with muscle, but not liver, insulin sensitivity, suggesting a potential endocrine role for FGF-19. C-reactive protein is a proinflammatory hepatokine, and low concentrations have been reported in metabolically healthy obese individuals, defined by either absence of metabolic syndrome features (23) or hyperinsulinemic-euglycemic clamps (24, 25). Unlike FGF-19, hsCRP aligned with liver rather than muscle insulin resistance and may therefore be considered as a hepatic insulin resistance surrogate.

In the multiple linear regression model, ΔRQ strongly predicted muscle insulin sensitivity (GIR_{HI}), in agreement with a previous study in obese adolescents (26). The switch from fat to carbohydrate oxidation during hyperinsulinemia is termed metabolic flexibility (27) and is impaired in insulin-resistant individuals (28). Because ΔRQ

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Table 2. Anthropometric, Clinical, and Metabolic Characteristics of Obese Individuals Categorized as $Muscle_{sen}Liver_{sen}$, $Muscle_{sen}Liver_{res}$, $Muscle_{res}Liver_{sen}$, and $Muscle_{res}Liver_{res}$

Characteristics	$Muscle_{sen}$ $Liver_{sen}$ (n = 12)	$Muscle_{sen}$ $Liver_{res}$ (n = 8)	$Muscle_{res}$ $Liver_{sen}$ (n = 9)	$Muscle_{res}$ $Liver_{res}$ (n = 33)	*P* ANOVA
Age, y	49 ± 4	54 ± 4	50 ± 4	50 ± 2	.76
BMI, kg/m^2	35.4 ± 3.5	34.1 ± 2.9	35.9 ± 4.7	36.6 ± 4.3	.52
Waist circumference, cm	106 ± 12	109 ± 14	107 ± 10	115 ± 14	.16
Whole-body fat, kg[a]	46 ± 11	45 ± 11	44 ± 9	47 ± 10	.90
Central abdominal fat, kg[a]	3.2 ± 0.7	3.0 ± 0.5	3.3 ± 0.7	3.6 ± 0.7	**.04**
Mean/median adipocyte size, μm[b]	68 ± 9	75 ± 9	70 ± 5	76 ± 10	.09
	68 ± 9	74 ± 10	70 ± 6	76 ± 11	.13
Systolic BP, mm Hg[c]	120 ± 8	112 ± 4	122 ± 11	130 ± 14[d]	**.006**
Diastolic BP, mm Hg[c]	78 ± 10	78 ± 7	82 ± 13	85 ± 9	.206
Total cholesterol, mmol/L[e]	5.2 ± 0.8	5.0 ± 0.5	5.0 ± 0.9	4.9 ± 0.7	.87
LDL cholesterol, mmol/L[e]	3.2 ± 0.7	3.1 ± 0.4	3.2 ± 0.9	3.1 ± 0.7	.93
HDL cholesterol, mmol/L[e]	1.4 ± 0.4	1.4 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	.65
Triglycerides, mmol/L[e,f]	0.8 (0.6–1.2)	1.0 (0.3–1.2)	0.9 (0.7–1.3)	1.1 (0.9–1.4)	.19
Fasting glucose, mmol/L	4.5 ± 0.5	4.9 ± 0.2	4.8 ± 0.4	4.8 ± 0.5	.19
OGTT 1-hour blood glucose, mmol/L	6.3 ± 1.5	7.1 ± 1.3	7.4 ± 2.2	8.5 ± 2.0[¥]	**.008**
OGTT 2-hour blood glucose, mmol/L	5.5 ± 1.7	6.2 ± 1.4	6.3 ± 1.3	6.8 ± 1.7	.14
OGTT $AUC_{Glucose}$, mmol/L · 120 min	728 ± 124	806 ± 117	815 ± 187	905 ± 174[g]	**.02**
OGTT $AUC_{Insulin}$, mU/L · 120 min[f]	6827 (5352–8660)	7888 (7341–13 916)	10 076 (6905–11 499)	13 504 (8466–17 188)[g]	**.007**
OGTT $AUC_{C\text{-}peptide}$, μg/L · 120 min[f]	422 (378–557)	533 (460–768)	546 (363–1027)	785 (598–1032)[g]	<**.001**
HbA1c, %	5.1 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.6 ± 0.1[g]	<**.001**
Fasting insulin, mU/L[f]	11 (8–13)	16 (13–23)	15 (11–20)	19 (13–31)[g]	<**.001**
Fasting NEFAs, mmol/L[f]	0.32 (0.26–0.45)	0.38 (0.32–0.46)	0.37 (0.25–0.46)	0.36 (0.27–0.46)	.87
$NEFA_{LO}$, mmol/L[f]	0.03 (0.02–0.05)	0.05 (0.04–0.06)	0.04 (0.02–0.05)	0.05 (0.04–0.06)[g]	**.04**
Basal RQ	0.78 ± 0.02	0.80 ± 0.03	0.79 ± 0.01	0.80 ± 0.04	.36
ΔRQ ($RQ_{HI} - RQ_{Baseline}$)	0.19 ± 0.04	0.17 ± 0.04	0.15 ± 0.04	0.14 ± 0.06[g]	**.03**
hsCRP, mg/L[f]	2.0 (1.2–3.3)	4.3 (2.6–5.7)	3.1 (1.1–4.7)	4.2 (1.9–5.7)	.08
FGF-19, ng/L[f]	130 (76–228	109 (24–338)	90 (66–147)	94 (56–147)	.31
FGF-21, ng/L[f]	80 (29–110)	80 (15–159)	45 (17–94)	91 (51–159)	.14
FABP4, μg/L	56 ± 26	72 ± 24	68 ± 31	63 ± 26	.57
Lipocalin-2, μg/L	40 ± 10	40 ± 21	49 ± 16	39 ± 12	.32
RBP4, mg/L	12 ± 2	10 ± 2	11 ± 2	11 ± 3	.44
Adiponectin, mg/L	17 ± 10	19 ± 8	16 ± 7	12 ± 7	.17

Abbreviation: LDL, low-density lipoprotein. Significance was tested by one-way ANOVA with Tukey post hoc.

[a] DXA data were available for 61 participants.

[b] Adipocyte size data were available for 53 participants.

[c] Subjects treated with antihypertensive medications were excluded (included: $Muscle_{sen}Liver_{sen}$, n = 10; $Muscle_{sen}Liver_{res}$, n = 6; $Muscle_{res}Liver_{sen}$, n = 9; and $Muscle_{res}Liver_{res}$, n = 24).

[d] $P < .01$ between $Muscle_{res}Liver_{res}$ and $Muscle_{sen}Liver_{res}$.

[e] Subjects treated with lipid medications were excluded (included: $Muscle_{sen}Liver_{sen}$, n = 11; $Muscle_{sen}Liver_{res}$, n = 6; $Muscle_{res}Liver_{sen}$, n = 9; and $Muscle_{res}Liver_{res}$, n = 28).

[f] Data are median (interquartile range).

[g] $P < .05$ between $Muscle_{res}Liver_{res}$ and $Muscle_{sen}Liver_{sen}$.

Data are mean ± SD, unless f above. Bold values signify statistical significance ($P < .05$).

is, to an extent, an alternative measure of insulin sensitivity, ΔRQ was excluded in an alternative model, resulting in liver fat and HDL explaining more than half (54%) of the variability in insulin sensitivity, with further, relatively small, contributions from SBP (5%) and HbA1c (4%). A primary aim of our study was to provide clinicians the tools to identify obese individuals at increased risk of metabolic disease, and we have therefore included clinically available markers in a regression analysis. Our findings suggest that HDL cholesterol, OGTT 1-hour blood glucose, SBP, and serum triglycerides explained 54% of the variability in insulin sensitivity. These findings should encourage the use of these simple available tests in identifying obese individuals at a greater metabolic risk.

Mean adipocyte size correlated positively with fasting insulin and inversely with GIR_{HI}, as has been reported by previous cross-sectional studies using hyperinsulinemic-euglycemic clamps (29, 30). The mechanisms linking adipocyte size and insulin resistance are unclear. One theory suggests that enlarged fat cells release more inflammatory cytokines that are implicated in the pathogenesis of insulin resistance (31). The adipocyte overflow hypothesis (32) was also suggested, whereby failure to differentiate leads to larger adipocyte size in obese individuals and consequent ectopic fat deposition in liver and muscle, resulting in peripheral insulin resistance. Further studies are needed to clarify the potential association between insulin sensitivity and adipocyte size.

Evidence relating pancreatic fat to glucose homeostasis is conflicting (33–35). Here pancreatic fat content corre-

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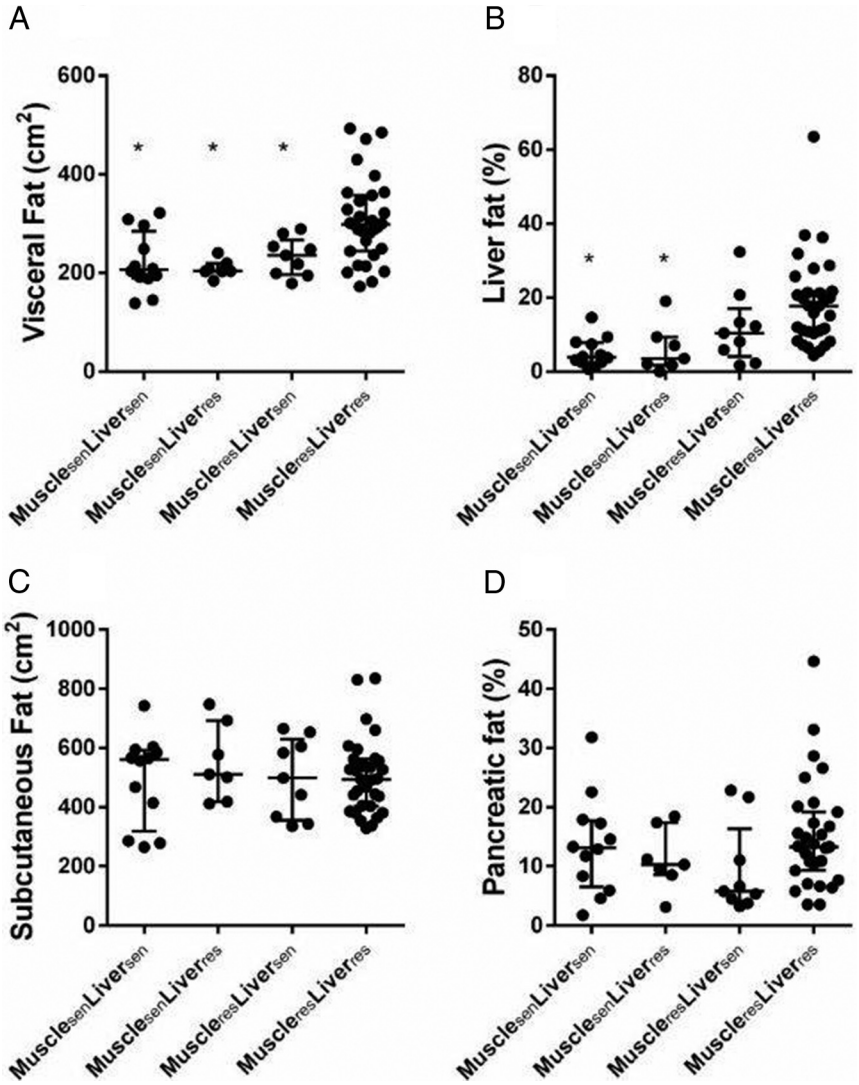

Figure 2. Abdominal visceral (A), liver (B), sc (C), and pancreatic (D) fat in obese individuals stratified based on muscle and liver insulin sensitivity. Differences by one-way ANOVA with Tukey post hoc from $Muscle_{res}Liver_{res}$ as the reference group are depicted. *, $P < .05$. MRI-derived measures were logarithmically transformed prior to the statistical analysis.

lated weakly with muscle, but not liver, insulin resistance, and no differences were observed between subcohorts stratified by either muscle or liver insulin resistance. Pancreatic fat has been reported to inversely relate to β-cell function in prediabetes (33) and type 2 diabetes (34). However, we observed a positive correlation between pancreatic fat and both C-peptide and insulin responses to OGTT. In prediabetes, pancreatic fat content was positively associated with increased pancreatic insulin secretion due to insulin resistance and hyperinsulinemia (36). Because we included only normoglycemic or prediabetic patients in our study, it is not surprising that we have found a positive correlation. Consistent with a previous study (37), pancreatic fat correlated positively with liver and visceral, but not sc, fat.

Dietary intake, macronutrient composition and physical activity habits are difficult to monitor with accuracy in free living individuals. Yet anecdotal data suggest lower saturated fat (38) and alcohol (39) intake and greater physical activity (39) in insulin-sensitive or metabolically healthy obese individuals. In the present study, $Muscle_{sen}$ consumed less sugar and saturated fat, but no significant differences were detected in intake of other macronutrients, energy intake, or physical activity. Future studies with comprehensive physical activity and diet assessment tools, such as pedometers and weighed-food records, are necessary to clarify the involvement of these lifestyle factors in insulin sensitivity in obesity.

Our study has some limitations. First, the sample size was small when the cohort was divided into four groups. Second, one-third of the women were premenopausal, and they were not all assessed during the follicular phase, which could potentially affect insulin sensitivity. Third, dietary and physical activity parameters were self-reported, which could potentially under- or overestimate energy intake and physical activity, respectively. Last, a cohort selection bias may have been introduced by the recruitment through advertisements, which is likely to have attracted a potentially health-conscious obese population. Hence, the findings may not be generalized to the wider obese population.

In conclusion, obese insulin-sensitive subjects are characterized by lower BP and lower visceral and liver fat. Whereas the debate regarding the long-term protective value of insulin sensitivity in obesity persists (40), insulin resistance in both muscle and liver is associated with the poorest cardiometabolic profile and is characterized by visceral and liver fat accumulation. Identification of obese individuals at high risk of metabolic disease is vital for early and effective interventions to minimize disease and health costs. Factors readily measured in clinical practice may serve as early detection tools, guiding targeted intervention.

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Contributions of the authors include the following: D.L.C., J.R.G., D.C., and D.S.-B. designed the studies. D.L.C. performed the clinical studies. D.L.C., D.S.-B., A.P., A.J., and J.Z. performed the laboratory analysis of the data collected. D.L.C., B.M., C.T., and C.L. performed and assisted in measuring the magnetic resonance imaging-related data. D.L.C., J.R.G., D.C., and D.S.-B. interpreted the data. D.L.C., J.R.G., D.C., and D.S.-B. wrote the first draft of the manuscript. All authors edited and approved the final manuscript. J.R.G. and D.S.-B. are the guarantors of this work and have full access to all the data and take full responsibility for the integrity of the data and the accuracy of the data analysis.

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