Am J Physiol Endocrinol Metab 299: E506–E515, 2010.
First published June 22, 2010; doi:10.1152/ajpendo.00586.2009.

Insulin-sensitive obesity

Nora Klöting,[1] Mathias Fasshauer,[1] Arne Dietrich,[2] Peter Kovacs,[3] Michael R. Schön,[4] Matthias Kern,[1] Michael Stumvoll,[1] and Matthias Blüher[1]

[1]*Department of Medicine,* [2]*Department of Surgery, and* [3]*Junior Research Group N06, Interdisciplinary Centre for Clinical Research, University of Leipzig, Leipzig; and* [4]*Städtisches Klinikum Karlsruhe, Clinic of Visceral Surgery, Karlsruhe, Germany*

Submitted 22 September 2009; accepted in final form 19 June 2010

Klöting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, Stumvoll M, Blüher M. Insulin-sensitive obesity. *Am J Physiol Endocrinol Metab* 299: E506–E515, 2010. First published June 22, 2010; doi:10.1152/ajpendo.00586.2009.—The association between obesity and impaired insulin sensitivity has long been recognized, although a subgroup of obese individuals seems to be protected from insulin resistance. In this study, we systematically studied differences in adipose tissue biology between insulin-sensitive (IS) and insulin-resistant (IR) individuals with morbid obesity. On the basis of glucose infusion rate during euglycemic hyperinsulinemic clamps, 60 individuals with a BMI of 45 ± 1.3 kg/m^2 were divided into an IS and IR group matched for age, sex, and body fat prior to elective surgery. We measured fat distribution, circulating adipokines, and parameters of inflammation, glucose, and lipid metabolism and characterized adipose tissue morphology, function, and mRNA expression in abdominal subcutaneous (sc) and omental fat. IS compared with IR obese individuals have significantly lower visceral fat area (138 ± 27 vs. 316 ± 91 cm^2), number of macrophages in omental adipose tissue (4.9 ± 0.8 vs. 13.2 ± 1.4%), mean omental adipocyte size (528 ± 76 vs. 715 ± 81 pl), circulating C-reactive protein, progranulin, chemerin, and retinol-binding protein-4 (all P values <0.05), and higher serum adiponectin (6.9 ± 3.4 vs. 3.4 ± 1.7 ng/ml) and omental adipocyte insulin sensitivity (all P values <0.01). The strongest predictors of insulin sensitivity by far were macrophage infiltration together with circulating adiponectin ($r^2 = 0.98$, $P < 0.0001$). In conclusion, independently of total body fat mass, increased visceral fat accumulation and adipose tissue dysfunction are associated with IR obesity. This suggests that mechanisms beyond a positive caloric balance such as inflammation and adipokine release determine the pathological metabolic consequences in patients with obesity.

visceral adipose tissue; insulin resistance; inflammation; adipokines; macrophages

OBESITY IS ASSOCIATED WITH AN INCREASED RISK of premature death (2) and represents a fast-growing health problem that is reaching epidemic proportions worldwide (21). Obesity significantly increases the risk of developing type 2 diabetes mellitus, hypertension, coronary heart disease, stroke, and several types of cancer (42). For instance, the risk to develop type 2 diabetes is ninefold higher for obese than for lean men (45). Although there is a strong relationship between obesity and insulin resistance (1, 10, 42), and obese individuals become more insulin sensitive with weight loss (32), not all obese patients are insulin resistant (6, 14).

Data from the European Group for the Study of Insulin Resistance suggest that ~25% of obese individuals [body mass index (BMI) >35 kg/m^2] are insulin sensitive (14).

Address for reprint requests and other correspondence: M. Blüher, Univ. of Leipzig, Dept. of Medicine, Liebigstr. 20, 04103 Leipzig, Germany (e-mail: bluma@medizin.uni-leipzig.de).

It is clinically important to identify insulin-resistant obese persons with an increased risk for developing obesity-associated metabolic and cardiovascular diseases who, therefore, may benefit the most from losing weight (32). Insulin-resistant (IR) and insulin-sensitive (IS) obesity are not clearly defined subgroups but represent the extremes of a continuum, the characterization of which may improve our understanding of the mechanisms linking increased fat accumulation in obesity with impaired insulin sensitivity. Impaired function of adipose tissue caused by the interaction of genetic and environmental factors may represent a causal factor for the association of obesity and insulin resistance in obese IR individuals (3). Visceral and ectopic fat storage belong to adipose tissue dysfunction and are closely related to whole body insulin resistance (31).

Therefore, we sought to identify mechanisms within adipose tissue that may link obesity to its associated metabolic diseases by systematic characterization of paired samples from abdominal subcutaneous (sc) and intra-abdominal omental adipose tissue of IS obese individuals compared with BMI-, age-, and sex-matched IR obese individuals without significant comorbidities, including type 2 diabetes.

RESEARCH DESIGN AND METHODS

Patients. We studied 60 morbidly obese men and women with a BMI = 45 ± 1.3 kg/m^2 who were scheduled to undergo elective cholecystectomy, explorative laparotomy, or gastric sleeve resection. Three-hundred fifty-eight previously described patients were recruited at the Departments of Medicine and Surgery (University of Leipzig) and the Clinic of Visceral Surgery (Karlsruhe Hospital) between May 2005 and October 2008 (24). For the purpose of this study, we selected 221 fat donors for which euglycemic hyperinsulinemic clamp data were available (Fig. 1). On the basis of the glucose infusion rate (GIR) in euglycemic hyperinsulinemic clamp, patients were defined as either IS (GIR > 70 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) or IR (GIR < 60 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (Fig. 1). In the BMI stratum between 42 and 48 kg/m^2, we initially found 32 subjects that could be matched for age, sex, and BMI into IS ($n = 16$) and IR obesity ($n = 16$) subgroups. In this BMI stratum, additional individuals with either IR or IS obesity have been actively recruited. To achieve a clear separation between the two groups, subjects with a GIR between 60 and 70 were excluded (Fig. 1). This resulted in the two experimental groups of IS and IR obesity with 30 subjects each.

All individuals fulfilled the following inclusion criteria: *1*) fasting plasma glucose <7.0 mmol/l; *2*) Hb A_{1c} <6.0%; and *3*) stable weight, defined as the absence of fluctuations of >2% of body weight for ≥3 mo before surgery. In addition, the following exclusion criteria have been defined: *1*) medical and family history of type 1 or type 2 diabetes; *2*) medical history of hypertension or systolic blood pressure (SBP) >140 mmHg and diastolic blood pressure (DBP) >85 mmHg; *3*) any acute or chronic inflammatory disease, as determined by a leucocyte count >8,000 Gpt/l, C-reactive protein (CRP) >5.0 mg/dl,

0193-1849/10 Copyright © 2010 the American Physiological Society

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

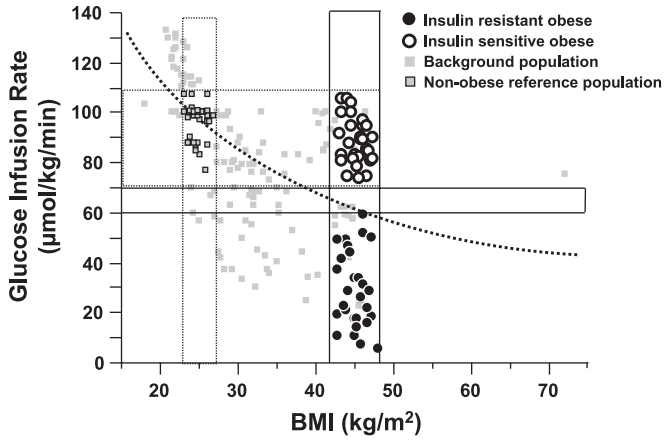


Fig. 1. Subject selection. Subjects were originally taken from a preexisting database (gray squares). Only subjects in the body mass index (BMI) range of 42 to 48 kg/m^2 were included in this study. Additional subjects in this BMI stratum were actively recruited. The dotted hyperbolic line is a regression curve of glucose infusion rate (GIR) during a euglycemic hyperinsulinemic clamp over BMI (GIR = 18.80 + 1,864/BMI; $r^2 = 0.35$, $P < 0.001$). Subjects in the BMI stratum of 42 to 48 kg/m^2 were classified as "insulin-sensitive obese" (GIR >70 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $n = 30$) or "insulin-resistant obese" (GIR < 60 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $n = 30$) according to their GIR during a hyperinsulinemic clamp. To achieve a clear separation between the 2 GIR groups, subjects with a GIR between 60 and 70 were excluded. This resulted in the 2 experimental groups with 30 subjects each. For reference purposes only, a nonobese group in the exact same GIR range as the insulin-sensitive obese group but "right on the hyperbola" (i.e., with the expected BMI) is indicated [32 men, 20 women; GIR 96.6 ± 6.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (means ± SD; range 76–110 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), age 55 ± 17 (24–86 yr)]. The BMI of this group is 24.8 ± 0.9 (23.5–26.9 kg/m^2).

or clinical signs of infection; *4*) clinical evidence of either cardiovascular or peripheral artery disease; *5*) any type of malignant disease; *6*) thyroid dysfunction; *7*) Cushing's disease or hypercortisolism; *8*) alcohol or drug abuse; *9*) pregnancy; *10*) concomitant medication, except contraceptives; and *11*) clinical symptoms of obstructive sleep apnea or Epworth Sleepiness Scale index <10. In both the IS and the IR group, there were five out of 20 postmenopausal women that did not receive any hormone therapy. From the remaining 30 women, 16 (IS group: $n = 7$; IR group: $n = 9$) used combination pills as contraceptive medication. All study protocols were approved by the ethics committee of the University of Leipzig. All participants gave written informed consent before taking part in the study. Anthropometric and demographic data, including age, sex, height, weight, waist, and hip circumferences, body fat mass, fat distribution by computed tomography (CT) or MRI scans, and medical history, as well as baseline blood samples and euglycemic hyperinsulinemic clamp data, were collected 5 ± 4 days prior to elective surgery.

Measurement of body fat content and insulin sensitivity. BMI was calculated as weight divided by squared height. Hip circumference was measured over the buttocks; waist circumference was measured at the midpoint between the lower ribs and iliac crest. Percentage body fat was measured by dual X-ray absorptiometry. In addition, abdominal visceral and sc fat areas were calculated using CT or MRI scans at the level of L4–L5, as described previously (13). In a post hoc analysis of 47 individuals with previous MRI scans, T1-weighted MRI images were used to estimate liver fat content, as described previously (17). Insulin sensitivity was assessed with the euglycemic hyperinsulinemic clamp method, using a previously described protocol (4). Glucose infusion rate (GIR) was calculated from the last 45 min of the clamp, in which GIR could be kept constant to achieve the target plasma glucose concentration of 5.5 (± 5%) mmol/l. Therefore, the duration of the clamp varied between individuals (range 120–200 min). In premenopausal women, clamp studies were performed during the luteal phase of the menstrual cycle.

Analyses of blood samples. All baseline blood samples were collected between 8 and 10 AM after an overnight fast. Plasma insulin was measured with an enzyme immunometric assay for the Immulite automated analyzer (Diagnostic Products, Los Angeles, CA). Serum high-sensitive CRP was measured by immunonephelometry (Dade-Behring, Milan, Italy). Serum total HDL cholesterol, LDL cholesterol, triglycerides, free fatty acids, total adiponectin (ELISA; Linco, St. Charles, MO), leptin, interleukin-6 (IL-6), and retinol-binding protein-4 (RBP4) were measured as described previously (24). Serum monocyte chemoattractant protein-1 (MCP-1) concentrations were measured by immunoassay system (Quantikine human MCP-1 Immunoassay; R & D Systems, Minneapolis, MN). Serum vaspin and progranulin were measured as described previously (49, 50). Serum chemerin and fetuin-A were measured by ELISAs (Biovendor, Heidelberg, Germany).

Characterization of adipose tissue samples. During surgery, adipose tissue samples were taken from the abdominal sc and the intra-abdominal omental fat depots at defined locations. Adipose tissue was analyzed as a whole (immediately frozen in liquid nitrogen after explantation) for histology and mRNA expression analyses. Additional adipose tissue samples were collected in 37°C PBS buffer, and adipocytes were isolated by collagenase (1 mg/ml) digestion. For the determination of glucose transport, isolated adipocytes from the different fat depots were stimulated with 100 nM insulin for 30 min and then incubated for 30 min with 3 μM [U-^{14}C]glucose. Immediately after incubation, adipocytes were fixed with osmic acid and incubated for 48 h at 37°C, and radioactivity was quantified after the cells had been decolorized. Glucose transport data were normalized for cell number. To determine adipocyte number and cell size distribution, 200-μl aliquots of adipocytes were fixed with osmic acid, incubated for 48 h at 37°C, and counted in a Coulter counter (Multisizer III; Beckman Coulter, Krefeld, Germany). Histological analyses and measurement of macrophage count in adipose tissue were performed as described previously (16). In brief, adipose tissue samples were fixed at room temperature in 4% formaldehyde and embedded in paraffin. Five-micrometer sections were mounted on glass slides, deparaffinized in xylol, and stained for CD68 using anti-CD68 monoclonal mouse antihuman antibody (close PGM1 M0876, dilution 1:100; Dako, Glostrup, Denmark). Macrophages were identified in the adipose parenchyma when cytoplasmic staining for CD68 was present along with an identifiable mononuclear nucleus and presented as the number per 100 adipocytes (%macrophages).

Adipose tissue mRNA expression studies. Human *CD68*, *adiponectin*, *leptin*, *IL-6*, *IL-8*, *RBP4*, *chemerin*, *progranulin*, *MCP-1*, *nicotinamide phosphoribosyltransferase* (*Nampt*), *hormone-sensitive lipase* (*HSL*), *tartrate-resistant acid phosphatase* (*TRAP*), *peroxisome proliferator-activated receptor-γ* (*PPARγ*), *hypoxia-inducible factor 1* (*HIF-1*), *macrophage migration inhibitory factor* (*MIF*), and *colony-stimulating factor 1* (*csf-1*) and *sirtuin-1* (*sirt1*) mRNA expression was measured by quantitative real-time RT-PCR in a fluorescent temperature cycler, and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems, Darmstadt, Germany). Total RNA was isolated from paired sc and omental adipose tissue samples using TRIzol (Life Technologies, Grand Island, NY), and 1 μg of RNA was reverse transcribed with standard reagents (Life Technologies). From each RT-PCR, 2 μl was amplified in a 26-μl PCR using the Brilliant SYBR Green QPCR Core Reagent Kit from Stratagene (La Jolla, CA) according to the manufacturer's instructions. Samples were incubated in the ABI PRISM 7000 sequence detector for an initial denaturation at 95°C for 10 min, followed by 40 PCR cycles, each cycle consisting of 95°C for 15 s, 60°C for 1 min, and 72°C for 1 min using specific primers (Supplemental Table S1; Supplemental Material for this article is available at the *AJP-Endocrinology and Metabolism* web site). SYBR Green I fluorescence emissions were monitored after each cycle. mRNA expression for all genes was calculated relative to the mRNA expression of *18S rRNA*, determined by a premixed assay on demand for human *18S rRNA*

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

Table 1. *Basic clinical characteristics of the study groups*

Variable	Insulin-Sensitive Obese (n = 30)	Insulin-Resistant Obese (n = 30)	P Value
Sex (female/male)	20/10	20/10	
Pre/postmenopausal women	15/5	15/5	
Age, yr (range)	44.6 ± 1.9 (42–49)	44.9 ± 2.1 (41–49)	0.61
No. of positive parameters for metabolic syndrome (range) (19)	1.5 ± 0.5 (1–3)	2.3 ± 1.1 (1–3)	
Impaired fasting glucose (n)	0	2	
BMI, kg/m^2 (range)	45.1 ± 1.3 (42.8–47.1)	45.2 ± 1.3 (43.1–47.8)	0.69
Glucose infusion rate, μmol·kg^{-1}·min^{-1}	89.4 ± 9.2	32.9 ± 13.6	<0.001
Clamp, range	74–106	11–60	
Glucose infusion rate, μmol·kg fat-free mass^{-1}·min^{-1} (range)	42.4 ± 3.9 (35–54)	14.2 ± 5.8 (4–32)	<0.001
Fasting plasma glucose, mmol/l (range)	5.23 ± 0.22 (4.97–5.64)	5.69 ± 0.37 (5.16–6.67)	<0.001
%Hb A_{1c} (range)	5.27 ± 0.15 (5.01–5.7)	5.72 ± 0.21 (5.07–6.0)	<0.001
Fasting plasma insulin, pmol/l (range)	29.8 ± 14 (9.8–71.2)	104.7 ± 30 (46.9–165.9)	<0.001
Total cholesterol, mmol/l (range)	4.95 ± 0.9 (3.05–6.76)	5.21 ± 1.0 (3.45–7.02)	0.38
HDL cholesterol, mmol/l (range)	1.41 ± 0.25 (1.1–2.04)	1.0 ± 0.27 (0.57–1.53)	<0.001
LDL cholesterol, mmol/l (range)	2.99 ± 0.9 (1.46–5.17)	3.13 ± 0.9 (1.31–5.12)	0.49
Triglycerides, mmol/l (range)	1.24 ± 0.4 (0.61–2.31)	1.99 ± 1.2 (0.86–6.59)	0.002
Free fatty acids, mmol/l (range)	0.22 ± 0.1 (0.05–0.72)	0.43 ± 0.2 (0.16–0.84)	<0.001
Glutamic-pyruvate transaminase, μkat/l (range)	0.44 ± 0.16 (0.18–0.72)	0.49 ± 0.14 (0.19–0.81)	0.1
Glutamic-oxaloacetic transaminase, μkat/l (range)	0.43 ± 0.15 (0.21–0.71)	0.53 ± 0.16 (0.19–0.77)	0.004
γ-Glutamyltranspeptidase, μkat/l (range)	0.39 ± 0.18 (0.16–0.98)	0.56 ± 0.22 (0.22–1.06)	0.002
Sex hormone-binding globulin, nmol/l (range)	64.3 ± 12.5 (23.5–104)	48.2 ± 16.1 (19.2–85)	<0.05
Testosterone (males), nmol/l (range)	13.8 ± 5.3 (4.2–22.8)	11.6 ± 6.1 (3.5–24.6)	0.4

Data are means ± SD. BMI, body mass index.

(Applied Biosystems). Amplification of specific transcripts was confirmed by melting curve profiles (cooling the sample to 68°C and heating slowly to 95°C with measurement of fluorescence) at the end of each PCR. The specificity of the PCR was verified further by subjecting the amplification products to agarose gel electrophoresis.

Statistical analyses. Data are shown as means ± SD unless stated otherwise. Before statistical analysis, nonnormally distributed parameters were logarithmically transformed to approximate a normal distribution. The following statistical tests were used: paired Student's *t*-test and Pearson's simple correlation. Linear relationships were assessed by least square regression analysis. Statistical analysis was performed using SPSS version 12.0 (SPSS, Chicago, IL). Prediction models for GIR based on clinical parameters and parameters of adipose tissue biology were calculated by multivariate linear regression analysis. P values <0.05 were considered to be statistically significant.

RESULTS

Clinical and metabolic characteristics of IS and IR obesity. Sixty morbidly obese (45 ± 1.3% kg/m^2) but otherwise healthy Caucasian women (n = 40) and men (n = 20) were matched for BMI, percent body fat, age, and sex into groups of IS (n = 30) and IR (n = 30) obesity (Fig. 1 and Table 1). Independently of BMI, body fat mass, age, and sex, we found significantly higher visceral fat area and waist circumference in the IR compared with the IS obese subgroup (Table 2). Only in the IR subgroup did we find a significant negative relationship between visceral fat area and insulin sensitivity (Fig. 2). There were no significant differences in sc fat area (Table 2) or in fat-free mass (IS group: 47.4 ± 1.8%; IR group: 46.3 ± 2.1%; P = 0.7) between the two groups. In a post hoc analysis of

Table 2. *Parameters of fat mass, fat distribution, serum concentrations of adipokines, and mediators of inflammation in individuals with insulin-sensitive and insulin-resistant obesity*

Variable	Insulin-Sensitive Obese	Insulin-Resistant Obese	P Value
%Body fat (range)	50.5 ± 7.0 (40.5–62.3)	51.2 ± 5.8 (42.6–60.2)	0.71
Waist circumference, cm (range)	132 ± 5.2 (122–145)	138 ± 8.1 (125–152)	<0.001
Subcutaneous fat area, cm^2 (range)	935 ± 124 (736–1,233)	890 ± 110 (757–1,134)	0.13
Visceral fat area, cm^2 (range)	138 ± 27 (94–204)	316 ± 91 (167–534)	<0.001
Leptin, ng/ml			
Males (range)	26.8 ± 2.8 (21.6–41.2)	28.3 ± 4.2 (22.2–43)	0.81
Females (range)	48.2 ± 11.5 (27.4–72)	47.6 ± 9.3 (25.6–73)	0.87
Adiponectin, ng/ml			
Males (range)	4.83 ± 2.9 (1.8–11.4)	2.54 ± 1.7 (1.2–6.4)	<0.01
Females (range)	8.87 ± 2.2 (1.5–13.7)	3.87 ± 1.6 (1.6–6.9)	<0.01
C-reactive protein, mg/dl (range)	1.7 ± 1.1 (0.09–4.18)	3.5 ± 1.3 (0.64–5.94)	<0.001
Interleukin-6, pg/ml (range)	1.3 ± 1.7 (0.1–7.4)	2.1 ± 1.7 (0.1–5.6)	0.08
Monocyte chemoattractant protein-1, pg/ml (range)	411 ± 128 (169–678)	453 ± 140 (249–714)	0.23
Progranulin, ng/ml (range)	176 ± 35 (128–277)	261 ± 68 (157–475)	<0.001
Chemerin, ng/ml (range)	194 ± 33 (128–250)	236 ± 35 (176–294)	<0.001
Vaspin, ng/ml (range)	2.07 ± 3.2 (0.27–17.9)	2.14 ± 2.2 (0.49–8.78)	0.9
Fetuin-A, μg/ml (range)	327 ± 74 (165–642)	377 ± 59 (262–538)	0.005
Retinol-binding protein-4, μg/ml (range)	42.7 ± 23 (6.4–92)	88.6 ± 32 (19.1–164)	<0.001

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

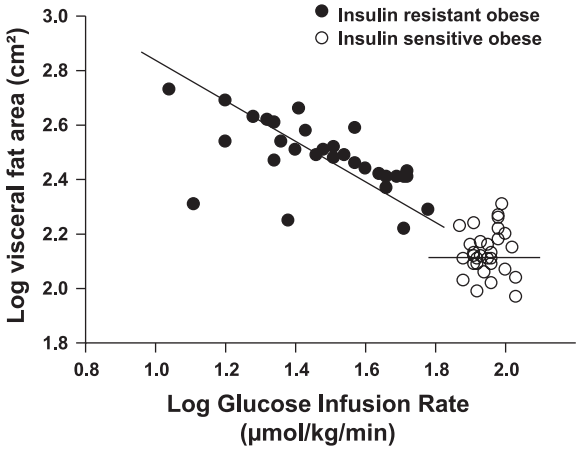


Fig. 2. Relationship between visceral fat area and insulin sensitivity in subgroups of individuals with insulin-sensitive ($n = 30$; $r = -0.64$, $P < 0.001$) and insulin-resistant obesity ($n = 30$; $r = 0.04$, $P = 0.8$). Data are log transformed to achieve normal distribution.

T1-weighted MRI images, we found in individuals where MRI scans were available (IS group: $n = 22$; IR group: $n = 25$) significantly higher liver fat content in IR (9.2 ± 1.8%) compared with IS obese (4.5 ± 1.2%) individuals. However, these data need to be discussed with caution, because measurement of liver fat content was not a primary aim of the study, and therefore, it was not performed by MR spectroscopy. In addition, MRI images were not available for all study participants. IR obesity is further associated with significantly higher fasting plasma glucose, Hb A_{1c}, fasting plasma insulin, triglyceride, and free fatty acid serum concentrations compared with the IS subgroup (Table 1). In addition, circulating parameters of liver function glutamic-oxaloacetic transaminase (GOT) and γ-glutamyltranspeptidase (GGT) were significantly higher the IR obesity group (Table 1). HDL cholesterol serum concentrations are significantly lower in the IR subgroup (Table 1). Noteworthy is that total and LDL cholesterol serum concentrations were not significantly different between the two groups (Table 1). We found significantly higher sex hormone-binding globulin (SHBG) serum concentrations in IS compared with IR individuals (Table 1). Serum testosterone concentrations were not significantly different between the two groups (Table 1).

Circulating adipokines and inflammatory parameters. Adiponectin serum concentration was significantly higher in the IS group, whereas no significant difference between the groups could be found for serum leptin concentrations (Table 2). Serum high-sensitive CRP concentrations were twofold higher in the IR compared with the IS obesity group (Table 2). In addition, there was a tendency for higher IL-6 and MCP-1 serum concentrations in IR obesity; however, these differences were not significant (Table 2). We further asked whether novel circulating markers for the relationship between obesity and insulin resistance were associated with the IR obese phenotype independently of BMI and body fat mass. Among these markers, we found significant differences with twofold higher RBP4, 1.5-fold higher progranulin, and 1.2-fold higher chemerin and fetuin-A serum concentrations in the IR compared with the IS obese group (Table 2). Vaspin serum concentrations were indistinguishable between the groups (Table 2).

Fat mass-independent adipose tissue dysfunction is associated with IR obesity. Our study design allows us to compare morphology and function of adipose tissue between IR and IS obesity independently of total body fat mass, BMI, age, sex, and metabolic diseases as confounding factors. To identify the molecular characteristics of adipose tissue related to insulin resistance, we thoroughly characterized omental and sc adipose tissue samples. We found a strong association between increased visceral fat accumulation and insulin resistance in the IR obese group (Fig. 2), which was associated with an abdominal fat distribution (Fig. 3*A*). We found a striking difference in omental (but not in sc) adipose tissue composition with significantly higher macrophage infiltration in IR obesity (Fig. 3, *B* and *C*). Noteworthy is that, in both IS and IR obesity, macrophage infiltration was significantly higher in omental compared with sc adipose tissue (Fig. 3*C*). These differences were further confirmed by immunohistochemical analysis of CD68 staining (data not shown) and significantly higher *CD68* mRNA expression in omental fat of the IR obese subgroup (Fig. 3*D*). Adipose tissue morphology of IR obese individuals is further characterized by significantly larger adipocyte size, especially in the omental depot (Fig. 3*E*). In the omental fat depot, both mean adipocyte volume (715 ± 81 vs. 528 ± 76 pl, $P < 0.01$) and maximal adipocyte size (1,240 ± 245 vs. 972 ± 165 pl, $P < 0.05$) were significantly higher in the IR group. In parallel, we found in sc adipose tissue samples significantly higher mean adipocyte volume (849 ± 139 vs. 693 ± 115 pl, $P < 0.05$) and maximal adipocyte size (1,419 ± 193 vs. 1,075 ± 231 pl, $P < 0.05$) in the IR compared with the IS obese group. Mean omental adipocyte diameter was 98 ± 22 μm (sc depot: 103 ± 17 μm) in the IS obese group and 136 ± 22 μm (sc depot: 152 ± 37 μm) in the IR obese group ($P < 0.05$, omental diameter distribution curves; Fig. 3*E*). We found significantly decreased insulin-stimulated glucose uptake into isolated adipocytes from omental fat of IR compared with IS obese individuals, suggesting insulin resistance at the level of adipocytes (Fig. 3*F*).

Low macrophage infiltration into omental fat and higher circulating adiponectin almost entirely predict the IS obese phenotype. Using multivariate linear regression analysis, we calculated different models that predict insulin sensitivity as determined by GIR. With the parameters of the International Diabetes Federation (IDF) definition of the metabolic syndrome, i.e., waist circumference, systolic/diastolic blood pressure, fasting plasma glucose, triglycerides, and HDL cholesterol (19), 61% of the variation in GIR can be explained ($r^2 = 0.61$, $P < 0.0001$). Using parameters of liver function [GOT, glutamic pyruvic transaminase (GPT), and GGT], 39% of GIR variation could be predicted ($r^2 = 0.30$, $P < 0.05$). A better prediction of the GIR could be achieved using estimated liver fat content ($r^2 = 0.7$, $P < 0.0001$) and visceral fat area ($r^2 = 0.74$, $P < 0.0001$) or macrophage infiltration into omental adipose tissue ($r^2 = 0.79$, $P < 0.0001$), whereas circulating adiponectin alone predicts GIR with $r^2 = 0.54$ ($P < 0.0001$). Most impressively, among many parameters of adipose tissue function, circulating adiponectin together with percent macrophages in omental adipose tissue almost precisely predict GIR ($r^2 = 0.98$, $P < 0.0001$; Fig. 4). Inclusion of liver function parameters and/or estimated liver fat content from MRI scans

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

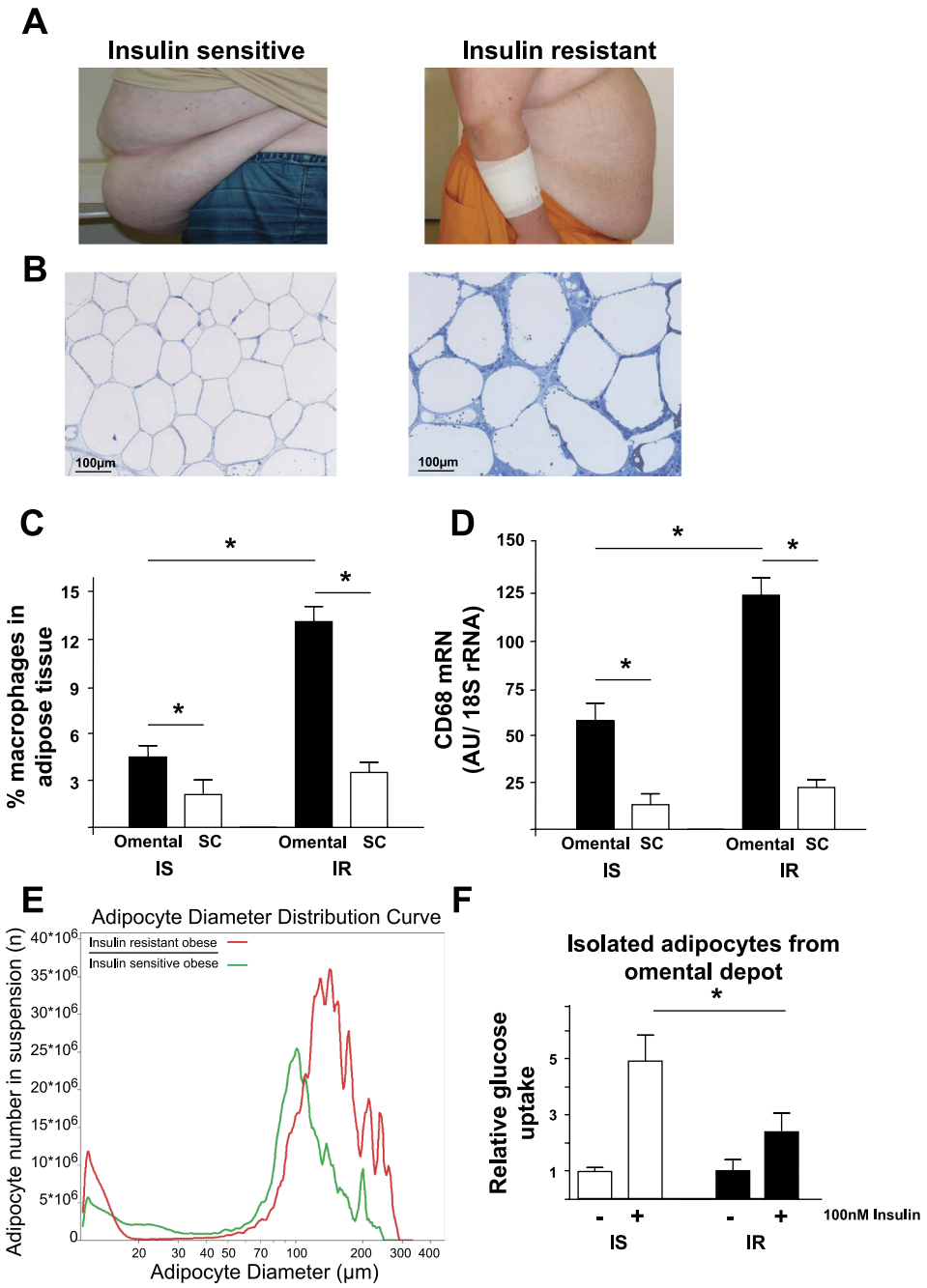


Fig. 3. Representative photographs for the insulin-sensitive (IS) and insulin-resistant (IR) morbidly obese phenotype and increased macrophage infiltration into omental adipose tissue in IR obesity. *A*: the photographs of 1 patient each for the IS and the IR obese subgroups should demonstrate the differences in abdominal fat distribution despite the same BMI of 45.2 kg/m^2. *B*: hematoxylin and eosin staining of omental adipose tissue sections from representative study individuals. Initial magnification, ×20. *C*: increased macrophage infiltration into omental compared with subcutaneous (sc) adipose tissue. *D*: CD68 mRNA expression differences between the fat depots and the IR and IS group. *E*: diameter distribution curves of isolated adipocytes from omental adipose tissue (pooled data from 20 individuals/group). *F*: insulin-stimulated glucose uptake was significantly lower in omental adipocytes from IR compared with IS obese individuals. *$P < 0.05$.

into the model (adiponectin and %macrophages in omental fat) did not significantly improve the prediction of GIR variance ($r^2 = 0.98$, $P < 0.0001$). It has been demonstrated that hormone replacement therapy decreases insulin sensitivity (9). Moreover, it has been argued that insulin-sensitive obesity is mainly a phenotype seen in premenopausal women, who become insulin resistant after menopause. Since 10 women in our study were postmenopausal (5 in each subgroup) and 16 women used contraceptive medication containing progesterone, we included menopausal status, sex hormone serum concentrations, and concomitant contraceptive medication as covariates in multivariate linear regression analysis. There was no significant effect of these covariates beyond the significant associations reported above.

mRNA expression of key adipose tissue genes is altered in IR obesity. To further characterize changes in adipose tissue function related to IR obesity, we analyzed omental and sc mRNA expression of several key mediators of adipose tissue function, including *CD68*, *adiponectin*, *leptin*, *IL-6*, *IL-8*, *RBP4*, *chemerin*, *progranulin*, *MCP-1*, *Nampt*, *HSL*, *TRAP*, *PPARγ*, *HIF-1*, *MIF*, and *csf-1* and *sirt-1*. Since adiponectin serum concentration and macrophage infiltration in omental fat were the best predictors of GIR, we focused on key genes potentially related to these parameters. Indeed, *adiponectin*, *sirt-1*, *TRAP*, *HIF-1*, *MIF*, and *csf-1* mRNA expression was significantly different in adipose tissue of IR compared with IS obese individuals (Fig. 5). In contrast, *PPARγ* and *MCP-1* mRNA expression was not different between the groups. In

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

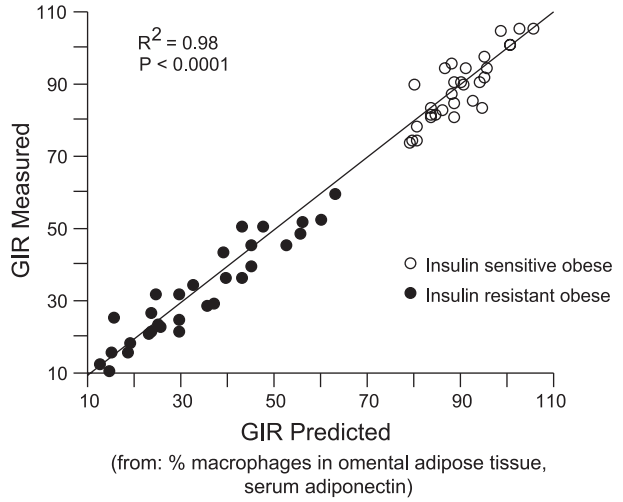


Fig. 4. Strongest prediction model for GIR ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) based on macrophage infiltration in adipose tissue and serum adiponectin. GIR predicted = 100 − 5.2 %macrophages in omental adipose tissue + 1.7 serum adiponectin concentration.

addition, we found significantly altered mRNA expression for several other key adipose tissue genes, including *IL-6*, *IL-8*, *RBP4*, *chemerin*, *progranulin*, *Nampt*, and *HSL* mRNA expression, whereas *leptin* expression was not different between IS and IR groups (Supplemental Fig. S1).

DISCUSSION

This study provides new insight into adipose tissue mechanisms related to insulin resistance in morbidly obese individuals. By strictly matching (for age, sex, BMI, and body fat mass) morbidly obese individuals with and without insulin resistance, we sought to determine mechanisms within adipose tissue that link fat accumulation to insulin resistance. Moreover, with this strict subject selection and tight range of body fat mass, we experimentally (not just statistically) controlled for most of the usual confounding factors.

We found that, independently of BMI and total body fat mass, increased visceral fat area, increased macrophage infiltration into omental adipose tissue, enlarged adipocyte size in both omental and sc fat depots, and omental adipocyte insulin resistance are associated with insulin-resistant obesity. Changes in adipose tissue biology of insulin-resistant obesity are related to dysregulation of circulating adipokines and cytokines, including decreased adiponectin and increased progranulin, chemerin, RBP4, and fetuin-A serum concentrations. In addition, IR obesity is associated with omental but also sc mRNA expression changes of key adipose tissue genes, which may either reflect or contribute to increased omental macrophage infiltration (*CD68*, *HIF-1*, *MIF*, *csf-1*, *progranulin*, *IL-6*, *IL-8*) and/or impaired adipocyte function (*adiponectin*, *RBP4*, *chemerin*, *progranulin*, *TRAP*, *Nampt*, *HSL*, *sirt-1*). Among these parameters of adipose tissue function and mRNA expression, the number of macrophages infiltrating omental adipose tissue and circulating adiponectin are the two single best correlates of insulin sensitivity. Noteworthy is that lower adiponectin serum concentrations in the insulin-resistant obese subgroup might primarily represent significantly lower sc adiponectin expression, since visceral fat represents only ~5–10% of total body fat mass (reviewed in Ref. 44). Therefore, we cannot exclude that (in addition to altered function of visceral fat depots) defects in sc adipose tissue contribute to insulin-resistant obesity. Together, macrophage infiltration and serum adiponectin explain an impressive 98% of the variation in GIR. Interestingly, inclusion of parameters of liver function (GOT, GPT, GGT) or estimated liver fat content did not improve that model. However, prediction of variance in GIR using visceral fat area (r^2 = 0.74) or estimated hepatic steatosis (r^2 = 0.7) alone was similar, suggesting that the association between insulin sensitivity and visceral fat area or liver fat content is exchangeable and that visceral fat area might also be a surrogate parameter for ectopic fat deposition in other sites. In addition, these adipose tissue function parameters are better predictors of insulin sensitivity than established clinical parameters, including parameters of the IDF definition of the metabolic syndrome (19) and visceral fat area, which is a more sophisticated parameter than waist circumference. Taken together, our data support the hypothesis that adipose tissue dysfunction, especially inflammation and decreased adiponectin secretion, is an important contributor to insulin resistance in obesity.

Our data confirm previous findings that visceral fat mass is associated with insulin resistance independent of BMI and support the notion that anatomic distribution of adipose tissue is a strong and independent predictor of adverse health outcomes of obesity (25, 28, 44). Importantly, our data do not provide evidence that omental fat is, independently of other ectopic sites, associated with insulin resistance. The strong association between hepatic steatosis and whole body insulin resistance rather suggests that the different ectopic fat depots are exchangeable with regard to prediction of GIR. We did not

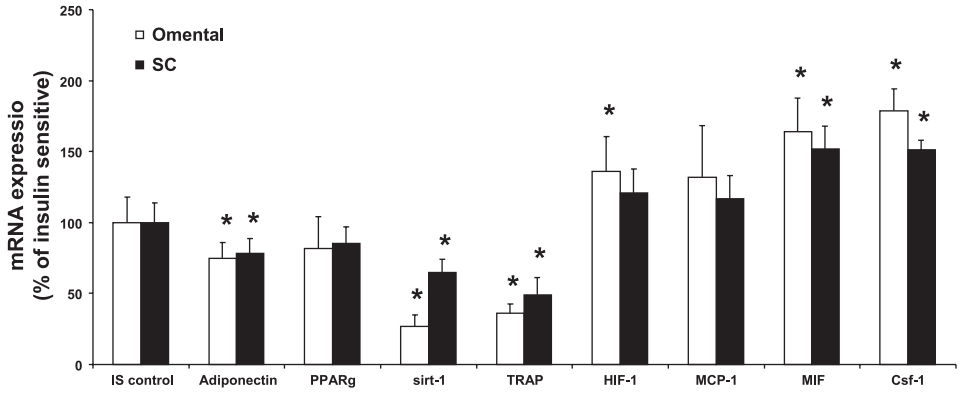


Fig. 5. Differences in omental and sc adipose tissue mRNA expression are associated with IR obese phenotype. *Adiponectin, peroxisome proliferator-activated receptor-γ* (*PPARγ*), *sirtuin-1* (*sirt-1*), *monocyte chemoattractant protein-1* (*MCP-1*), *tartrate-resistant acid phosphatase* (*TRAP*), *hypoxia-inducible factor 1* (*HIF-1*), *macrophage migration inhibitory factor* (*MIF*), and *colony-stimulating factor 1* (*csf-1*) mRNA expression was normalized to the mRNA expression of *18S rRNA* and expressed relative to the expression of the IS obesity group. *$P < 0.05$ for IR vs. IS group.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

investigate whether other ectopic fat depots, including epicardial and intramyocellular fat, are also associated with insulin resistance in our study. However, previous studies have shown that amount of visceral fat correlates with intrahepatic triglyceride, intramyocellular lipid content, and epicardial fat (18, 38, 44), suggesting analogous relationships between other ectopic fat depots and insulin sensitivity, as we observed for visceral fat. Previous studies in lipodystrophic patients have shown profound hepatic and peripheral insulin resistance despite absent visceral fat and normal circulating adipokines supporting the hypothesis that hepatic steatosis might represent the causal mechanism for the development of insulin resistance (30, 33). Moreover, Stefan et al. (38) recently suggested that ectopic fat in the liver may be more important than visceral fat in the determination of insulin-sensitive obesity.

Noteworthy is that variability of GIR was significantly smaller in the insulin-sensitive compared with the insulin-resistant group. These differences in GIR variability may have led to an overestimation of the effect of visceral fat mass on insulin sensitivity in our study. Lower GIR variability in the insulin-sensitive obese group may be due to at least two factors. First, the cutoff for insulin sensitivity, GIR >70 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for insulin-sensitive obesity and <60 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for IR obesity, was arbitrarily chosen and may have offered a wider window for variability in the IR group. Second, the lower GIR variability in the insulin-sensitive group might be due to the absence of "very insulin-sensitive" obese individuals with GIR >110 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, suggesting that in insulin-sensitive obesity GIR is restricted to lower values compared with healthy lean controls within the BMI stratum of 23–25 kg/m^2 (Fig. 1).

In the absence of overt diabetes and dyslipidemia, we found increased fasting plasma glucose and Hb A_{1c} as well as higher triglyceride and lower HDL cholesterol serum concentrations to be associated with insulin-resistant obesity. These characteristics have been described extensively with respect to the insulin-resistant obese phenotype (1, 10, 14, 32) and may simply reflect the group selection difference in whole body insulin sensitivity. Interestingly, the negative correlation between visceral fat area and insulin sensitivity is completely absent in the insulin-sensitive obese subgroup, supporting the extensively reported role of ectopic and visceral fat accumulation in the development of obesity-related insulin resistance (3, 10, 42). In contrast to significant differences in visceral fat mass between insulin-resistant and insulin-sensitive obese individuals, abdominal sc fat area was not significantly different between the groups. Since we did not measure total body sc fat mass, we cannot exclude that differences in sc fat distribution despite indistinguishable total body fat mass contribute to the insulin-resistant phenotype. However, important measurements of adipose tissue dysfunction, including macrophage infiltration and adipocyte-specific insulin resistance, were found specifically in omental but not in sc adipose tissue of insulin-resistant obese individuals. These depot-specific differences may contribute to the independent curvilinear association between visceral adiposity and mortality (25).

Increased macrophage infiltration into omental adipose tissue appears to be the most prominent characteristic, which distinguishes adipose tissue of insulin-resistant from insulin-sensitive obese individuals. However, with our study design, we cannot establish causality for the relationship between omental macrophage infiltration and insulin resistance. Macrophage infiltration could indeed be the consequence, rather than the cause, of adipose tissue dysfunction. In addition, there is still the possibility that insulin-sensitive and -resistant obese groups represent the consequence of two different pathophysiological processes. One could hypothesize that, whereas insulin-resistant obese develop insulin resistance in response to obesity and its associated adipose tissue dysfunction, in the insulin-sensitive obese, persistent insulin sensitivity in the face of caloric surplus may in fact underlie the pathogenesis of obesity itself. Predominant omental macrophage infiltration has been shown to be associated with clinical parameters of obesity-associated comorbidities and hepatic lesions in human morbid obesity (8). Animal studies showed that increased macrophage-specific gene expression in fat precedes a decrease in insulin sensitivity after initiation of high-fat feeding, suggesting that adipose tissue macrophage infiltration is causally linked to insulin resistance (48). Moreover, disruption of MCP-1 action by knockout of either MCP-1 or its receptor CC chemokine receptor 2 is associated with protection against insulin resistance, further supporting the notion that increased adipose tissue macrophage infiltration may causally link obesity with insulin resistance (23, 46). However, it is difficult to establish a causative role of macrophage infiltration in the development of whole body insulin resistance in human obesity. In our study, the observed differences in adipose tissue mRNA expression of factors associated with recruitment of immune cells into adipose tissue, including *MCP-1*, *HIF-1*, *MIF*, *progranulin*, and *csf-1*, could either contribute to or be the result of an increased number of macrophages, especially in omental adipose tissue. However, the signals leading to increased omental macrophage infiltration are not entirely clear. Both genetic and environmental factors could contribute to increased ectopic visceral fat accumulation and hypertrophy of adipocytes (3), and this condition might represent the inability of an obese patient to become more (subcutaneously) obese (40). It has been further suggested that adipose tissue dysregulation is a specific response to relative hypoxia in clusters of adipocytes that become distant from the vasculature as adipose tissue mass expands (29, 41). Increased visceral adipose tissue mass in obesity without an adequate support of vascularization might lead to hypoxia, macrophage infiltration, and inflammation (3). In accord with that, we found significantly higher *HIF-1* mRNA expression in adipose tissue of insulin-resistant obese individuals.

The primary signal causing adipose tissue dysfunction and insulin-resistant obesity could be adipocyte hypertrophy, since enlarged adipocytes are more insulin resistant and produce a proinflammatory adipokine pattern (34, 47). In accord with this, we found enlarged adipocytes in both omental and sc adipose tissue associated with the insulin-resistant obese phenotype. Since the amount of visceral fat is relatively small compared with that of sc fat, significantly larger (and, therefore, more insulin-resistant) adipocytes in the sc fat depot of the insulin-resistant obese group could be the major determinant of impaired whole body insulin resistance in insulin-resistant obesity. As reflected by lower circulating free fatty acids in the insulin-sensitive obese group, our data may suggest that storage capacity of sc fat could be better in the insulin-sensitive group. This condition might represent the inability of the insulin-resistant subgroup to become more obese by ex-

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

panding sc fat depots (40). It is well established that elevated free fatty acid serum concentrations may contribute to insulin resistance (5). Therefore, the insulin-resistant obese subphenotype could at least in part be caused by increased free fatty acids. The higher availability of fatty acids in insulin-resistant obesity could generate an additional metabolic stress within adipose tissue that may contribute to increased immune cell infiltration in omental fat.

It has recently been demonstrated that adipocyte number is a major determinant for fat mass in adults (36). In our study we did not assess a valid measurement of fat cell number, and therefore, we cannot exclude that differences in fat cell number contribute to insulin-sensitive obesity.

Our data confirm previous observations that adipocyte hypertrophy is associated with impaired glucose and lipid metabolism in human obesity (27). Recently, it was demonstrated that overexpression of TRAP, a molecule secreted from adipose tissue macrophages, induces hyperplastic obesity with normal adipocyte lipid metabolism and insulin sensitivity (26). Despite a significantly lower number of macrophages infiltrating omental adipose tissue, we found higher *TRAP* mRNA expression in omental fat of insulin-sensitive obese individuals, suggesting that differences in the macrophage phenotype contribute to insulin-resistant obesity. It has recently been shown that adipose tissue macrophages comprise a particular macrophage subtype that could considerably contribute to the development of insulin resistance (51). Therefore, further studies should characterize macrophage phenotype in insulin-resistant and insulin-sensitive obesity.

Impaired adipogenesis may play a pivotal role leading to hypertrophic obesity, increased ectopic fat deposition, and insulin resistance (12). Differences of adipogenic capacity of progenitor cells from different depots (43) and the loss of adipogenic potential that occurs during the course of obesity (41) might contribute to adipose tissue dysfunction. In obese patients, it was shown that stromal cells from the sc adipose tissue region proliferated faster than those from the omental or visceral fat depots (43). In another recent study, it was demonstrated that the apparent number of preadipocytes in the abdominal sc adipose tissue that can undergo differentiation is reduced in obesity and negatively correlates with fat cell size (20). Taken together, impaired preadipocyte differentiation, particularly in hypertrophic adipose tissue, may be an additional pathogenic factor in the development of insulin-resistant obesity.

Our data support the growing evidence suggesting that adipose tissue inflammation links obesity to insulin resistance. In accord with that, we show here that insulin-sensitive obese individuals are protected against inflammation of visceral adipose tissue. In addition, our study design provides novel mechanistic insight into the relationship between visceral adiposity, inflammation and circulating mediators of insulin resistance, and impaired adipose tissue function. Our data support the association between increased omental adipose tissue *RBP4* mRNA, circulating RBP4, and whole body insulin resistance (4, 15). Increased circulating concentrations of Nampt (35), chemerin (7), progranulin (49), and fetuin-A (37) have been shown to be associated with either insulin resistance, obesity, or both. However, it has been difficult to dissect the effects of obesity and insulin resistance on increased serum concentrations of these molecules. Our data provide new evidence for a significant body fat mass-independent role for Nampt, chemerin, progranulin, and fetuin-A in the development or at least as markers of insulin-resistant obesity.

Low SHBG serum concentrations have previously been shown to significantly predict the risk of type 2 diabetes in both women and men (11). In our study, insulin-sensitive obesity was associated with significantly higher SHBG serum concentrations. Increased circulating SHBG levels in the insulin-sensitive obese group could be due to lower liver fat content, since it has been demonstrated that liver fat independently predicts SHBG serum concentrations (39). In addition, insulin resistance itself and higher insulin levels in the insulin-resistant compared with the insulin-sensitive obese group could explain reduced SHBG levels, because insulin physiologically suppresses serum levels of SHBG (22).

In conclusion, adipose tissue dysfunction characterized by increased visceral fat accumulation (as a surrogate parameter for ectopic fat deposition), increased adipocyte size, and higher macrophage infiltration into omental fat is associated with insulin-resistant morbid obesity. Higher macrophage infiltration into omental fat depot and lower circulating adiponectin are the best predictors of insulin-resistant obesity, suggesting that adipose tissue dysfunction may play a causal role in human obesity-associated insulin resistance. Noteworthy is that macrophage infiltration could also be the result rather than the cause of adipose tissue dysfunction. There is still the possibility that insulin-sensitive and -resistant obese groups represent the consequence of two different pathophysiological processes; however, longitudinal data from the preobese to morbidly obese state are required to address this question. As a consequence, impaired adipose tissue function contributes to a proinflammatory, atherogenic, and diabetogenic state and may be mechanistically linked to the development of obesity-associated disorders. Therefore, targeting not only caloric imbalance but also adipose tissue dysfunction in obesity may represent a novel strategy to prevent obesity-related diseases.

GRANTS

This work was supported by the Kompetenznetz Adipositas (Competence Network for Obesity) funded by the Federal Ministry of Education and Research (FKZ 01GI0829), the NGFNplus (FKZ 01GI0827), and grants from Deutsche Forschungsgemeinschaft, the clinical research group "Atherobesity" (KFO 152, project BL 833/1-1; M. Blüher), and project FA 476/4-1 (M. Fasshauer).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

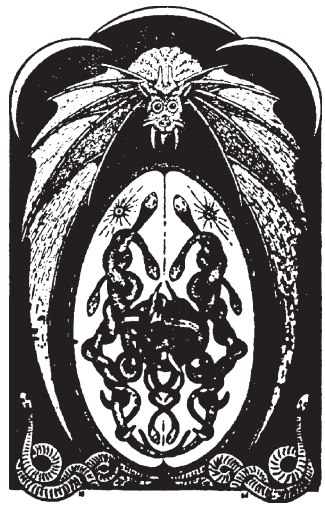
1. **Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM.** Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest* 96: 88–98, 1995.
2. **Adams KF, Schatzkin A, Harris TB, Kipnis V, Mouw T, Ballard-Barbash R, Hollenbeck A, Leitzmann MF.** Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. *N Engl J Med* 355: 763–778, 2006.
3. **Blüher M.** Adipose tissue dysfunction in obesity. *Exp Clin Endocrinol Diabetes* 117: 241–250, 2009.
4. **Blüher M, Unger R, Rassoul F, Richter V, Paschke R.** Relation between glycaemic control, hyperinsulinaemia and plasma concentrations of soluble adhesion molecules in patients with impaired glucose tolerance or type II diabetes. *Diabetologia* 45: 210–216, 2002.
5. **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* 32: 14–23, 2002.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

6. **Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G.** Relationship between degree of obesity and in vivo insulin action in man. *Am J Physiol Endocrinol Metab* 248: E286–E291, 1985.
7. **Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, Walder K, Segal D.** Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology* 148: 4687–4694, 2007.
8. **Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, Coussieu C, Basdevant A, Bar Hen A, Bedossa P, Guerre-Millo M, Clément K.** Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. *Diabetes* 55: 1554–1561, 2006.
9. **Cooper BC, Burger NZ, Toth MJ, Cushman M, Sites CK.** Insulin resistance with hormone replacement therapy: associations with markers of inflammation and adiposity. *Am J Obstet Gynecol* 196: 123.e1–123.e7, 2007.
10. **Després JP, Lemieux I.** Abdominal obesity and metabolic syndrome. *Nature* 444: 881–887, 2006.
11. **Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, Buring JE, Gaziano JM, Liu S.** Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med* 361: 1152–1163, 2009.
12. **Dubois SG, Heilbronn LK, Smith SR, Albu JB, Kelley DE, Ravussin E; Look AHEAD Adipose Research Group.** Decreased expression of adipogenic genes in obese subjects with type 2 diabetes. *Obesity (Silver Spring)* 14: 1543–1552, 2006.
13. **Ferland M, Després JP, Tremblay A, Pinault S, Nadeau A, Moorjani S, Lupien PJ, Thériault G, Bouchard C.** Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Br J Nutr* 61: 139–148, 1989.
14. **Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G.** Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). *J Clin Invest* 100: 1166–1173, 1997.
15. **Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, Kahn BB.** Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 354: 2552–2563, 2006.
16. **Harman-Boehm I, Blüher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, Shai I, Klöting N, Stumvoll M, Bashan N, Rudich A.** Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *J Clin Endocrinol Metab* 92: 2240–2247, 2007.
17. **Hussain HK, Chenevert TL, Londy FJ, Gulani V, Swanson SD, McKenna BJ, Appelman HD, Adusumilli S, Greenson JK, Conjeevaram HS.** Hepatic fat fraction: MR imaging for quantitative measurement and display—early experience. *Radiology* 237: 1048–1055, 2005.
18. **Hwang JH, Stein DT, Barzilai N, Cui MH, Tonelli J, Kishore P, Hawkins M.** Increased intrahepatic triglyceride is associated with peripheral insulin resistance: in vivo MR imaging and spectroscopy studies. *Am J Physiol Endocrinol Metab* 293: E1663–E1669, 2007.
19. **International Diabetes Federation.** *Worldwide Definition of the Metabolic Syndrome* (Online). http://www.idf.org/metabolic-syndrome [2005].
20. **Isakson P, Hammarstedt A, Gustafson B, Smith U.** Impaired preadipocyte differentiation in human abdominal obesity: role of Wnt, tumor necrosis factor-alpha, and inflammation. *Diabetes* 58: 1550–1557, 2009.
21. **James PT, Rigby N, Leach R; International Obesity Task Force.** The obesity epidemic, metabolic syndrome and future prevention strategies. *Eur J Cardiovasc Prev Rehabil* 11: 3–8, 2004.
22. **Jayagopal V, Kilpatrick ES, Jennings PE, Hepburn DA, Atkin SL.** The biological variation of testosterone and sex hormone-binding globulin (SHBG) in polycystic ovarian syndrome: implications for SHBG as a surrogate marker of insulin resistance. *J Clin Endocrinol Metab* 88: 1528–1533, 2003.
23. **Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M.** MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 116: 1494–1505, 2006.
24. **Klöting N, Graham TE, Berndt J, Kralisch S, Kovacs P, Wason CJ, Fasshauer M, Schön MR, Stumvoll M, Blüher M, Kahn BB.** Serum retinol-binding protein is more highly expressed in visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. *Cell Metab* 6: 79–87, 2007.
25. **Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R.** Visceral fat is an independent predictor of all-cause mortality in men. *Obesity (Silver Spring)* 14: 336–341, 2006.
26. **La˚ng P, van Harmelen V, Rydén M, Kaaman M, Parini P, Carneheim C, Cassady AI, Hume DA, Andersson G, Arner P.** Monomeric tartrate resistant acid phosphatase induces insulin sensitive obesity. *PLoS One* 3: e1713, 2008.
27. **Leonhardt W, Haller H, Hanefeld M.** The adipocyte volume in human adipose tissue: II. Observations in diabetes mellitus, primary hyperlipoproteinemia and weight reduction. *Int J Obes* 2: 429–439, 1978.
28. **Montague CT, O'Rahilly S.** The perils of portliness: causes and consequences of visceral adiposity. *Diabetes* 49: 883–888, 2000.
29. **Pasarica M, Sereda OR, Redman LM, Albarado DC, Hymel DT, Roan LE, Rood JC, Burk DH, Smith SR.** Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. *Diabetes* 58: 718–725, 2009.
30. **Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, Cline GW, DePaoli AM, Taylor SI, Gorden P, Shulman GI.** Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest* 109: 1345–1350, 2002.
31. **Rasouli N, Molavi B, Elbein SC, Kern PA.** Ectopic fat accumulation and metabolic syndrome. *Diabetes Obes Metab* 9: 1–10, 2007.
32. **Reaven GM.** Importance of identifying the overweight patient who will benefit the most by losing weight. *Ann Intern Med* 138: 420–423, 2003.
33. **Shulman GI.** Cellular mechanisms of insulin resistance. *J Clin Invest* 106: 171–176, 2000.
34. **Skurk T, Alberti-Huber C, Herder C, Hauner H.** Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab* 92: 1023–1033, 2007.
35. **Sommer G, Garten A, Petzold S, Beck-Sickinger AG, Blüher M, Stumvoll M, Fasshauer M.** Visfatin/PBEF/Nampt: structure, regulation and potential function of a novel adipokine. *Clin Sci (Lond)* 115: 13–23, 2008.
36. **Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T, Concha H, Hassan M, Rydén M, Frisén J, Arner P.** Dynamics of fat cell turnover in humans. *Nature* 453: 783–787, 2008.
37. **Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Kröber SM, Machicao F, Fritsche A, Häring HU.** Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. *Diabetes Care* 29: 853–857, 2006.
38. **Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, Balletshofer B, Machicao F, Fritsche A, Häring HU.** Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med* 168: 1609–1616, 2008.
39. **Stefan N, Schick F, Häring HU.** Sex hormone-binding globulin and risk of type 2 diabetes. *N Engl J Med* 361: 2675–2676, 2009.
40. **Tan CY, Vidal-Puig A.** Adipose tissue expandability: the metabolic problems of obesity may arise from the inability to become more obese. *Biochem Soc Trans* 36: 935–940, 2008.
41. **Trayhurn P, Wang B, Wood IS.** Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? *Br J Nutr* 100: 227–235, 2008.
42. **Van Gaal LF, Mertens IL, De Block CE.** Mechanisms linking obesity with cardiovascular disease. *Nature* 444: 875–880, 2006.
43. **Van Harmelen V, Röhrig K, Hauner H.** Comparison of proliferation and differentiation capacity of human adipocyte precursor cells from the omental and subcutaneous adipose tissue depot of obese subjects. *Metabolism* 53: 632–637, 2004.
44. **Wajchenberg BL.** Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 21: 697–738, 2000.
45. **Weinstein AR, Sesso HD, Lee IM, Cook NR, Manson JE, Buring JE, Gaziano JM.** Relationship of physical activity vs body mass index with type 2 diabetes in women. *JAMA* 292: 1188–1194, 2004.
46. **Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, Charo I, Leibel RL, Ferrante AW Jr.** CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* 116: 115–124, 2006.
47. **Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE.** Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia* 43: 1498–1506, 2000.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017

48. **Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H.** Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112: 1821–1830, 2003.
49. **Youn BS, Bang SI, Klöting N, Park JW, Lee N, Oh JE, Pi KB, Lee TH, Ruschke K, Fasshauer M, Stumvoll M, Blüher M.** Serum progranulin concentrations may be associated with macrophage infiltration into omental adipose tissue. *Diabetes* 58: 627–636, 2009.
50. **Youn BS, Klöting N, Kratzsch J, Lee N, Park JW, Song ES, Ruschke K, Oberbach A, Fasshauer M, Stumvoll M, Blüher M.** Serum vaspin concentrations in human obesity and type 2 diabetes. *Diabetes* 57: 372–377, 2008.
51. **Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Györi G, Zlabinger GJ, Stulnig TM.** Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. *Int J Obes (Lond)* 31: 1420–1428, 2007.



Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on May 30, 2017