Relationship between visceral adiposity and intramyocellular lipid content in two rat models of insulin resistance

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INSULIN RESISTANCE is characterized by abnormalities in carbohydrate and lipid metabolism in the development of type 2 diabetes. Skeletal muscle tissue accounts for the majority of insulin-stimulated glucose utilization (15). Its failure to respond to the insulin signal is a key feature of the prediabetic state. There is now evidence to suggest that an increase in intramyocellular lipids (IMCL), as opposed to the more metabolically inert extramyocellular lipids (EMCL), is associated with insulin resistance in skeletal muscle (23, 29, 36, 45).

Central adiposity is well accepted as a predisposing factor for insulin resistance (24, 28). Accumulation of visceral rather than subcutaneous (SC) fat is thought to constitute the link between glucose intolerance and IMCL stores (43), with circulating free fatty acids (FFAs) playing a pivotal role (5, 9, 11, 12). Arner (3) has proposed a model that requires the release and transport of FFAs from visceral fat to the liver through the portal vein. This in turn would affect liver function and especially lead to dyslipidemia and increased triglyceride (TG) storage within the muscle cell. Several elements support this hypothesis: 1) visceral fat is associated with a high rate of lipid oxidation (10, 36); 2) alteration of glucocorticoid metabolism in the omental fat, as described in type 2 diabetes (44), triggers a high lipolytic response of visceral fat to stress (3); and 3) removal of visceral fat prevents insulin resistance from occurring during the aging process (19), which intuitively suggests a mass effect of adipose tissue on glucose intolerance. Yet little is known about whether visceral fat can modulate IMCL deposition.

The mechanism by which muscle fat inhibits glucose utilization still remains to be determined. However, there is some evidence suggesting that the accumulation of long-chain acyl-CoA within the muscle cell potentially alters the insulin signaling cascade through chronic activation of protein kinase C and the ultimate impairment of GLUT4 translocation (17, 22). The extent to which genetic background exerts control on visceral adiposity, IMCL storage, and insulin sensitivity is also not clear. The discovery of the ob gene and analysis of the biology of its gene product revealed that leptin is an adipocyte-derived satiety factor involved not only in the regulation of food intake but also energy expenditure. Animals in which the ob gene is defective or that lack leptin receptors, such as the homozygous fa/fa Zucker rat, develop an obesity-like syndrome (34). Because of this, the fa/fa Zucker rat is now widely used as a relevant animal model for insulin resistance.

The primary aim of the present study was to characterize the nature of the relationship between visceral adiposity and IMCL accumulation in two animal models of insulin resistance. In a first set of experiments (study A), both in vivo magnetic resonance imaging (MRI) and localized proton magnetic resonance spectroscopy (1H MRS) were used to measure regional adiposity and IMCL content, respectively, in lean and fatty (fa/fa) Zucker rats. To cover a wide range of physiological conditions, measurements were carried out in the context of dietary manipulations. Secondarily, the role of the genetic component of insulin resistance in the control of visceral adiposity and IMCL levels was also assessed. To further elucidate the possible interaction between visceral fat and muscle fat depots (i.e., IMCL), fa/fa Zucker rats were challenged with repeated injections of dexamethasone, an estab-
lished model for hyperglycemia (39). This second set of experiments (study B) was designed 1) on the basis that glucocorticoid excess increases the basal lipolytic rate in primary cultured rat adipocytes (13) and 2) on the assumption that adipocyte-specific defects can lead to peripheral insulin resistance through IMCL accumulation. Finally, a more in-depth characterization of dexamethasone effects on carbohydrate and lipid metabolism was obtained from an ex vivo metabolomic analysis of plasma samples and muscle extracts.

METHODS

Animals

For the diet-induced model (study A), 8-wk-old male fa/fa Zucker rats (Z; 324 ± 4 g, n = 14) and lean Zucker rats (ZL; 281 ± 2 g, n = 14) were investigated over a 4-wk period. Each group was divided into two subgroups, such that rats were fed either a normal chow diet (Z-N and ZL-N, both n = 6) (12% fat cal; Harlan Teklad, Madison, WI; rodent diet no. 8604) or a high-fat diet (Z-HF and ZL-HF, both n = 8) (54% fat cal; Research Diets, New Brunswick, NJ; diet no. 590080201). Food consumption, corrected for spillage, was measured periodically over the course of the 4-wk observation period. For the glucocorticoid-induced model (study B), 16 8-wk-old male fa/fa Zucker rats were divided into 2 groups. The first group of 8 animals was given 3 × 11 mg/kg ip doses of dexamethasone (Dexa) over the course of 11 days (day 0, day 2, and day 11), whereas the second group was dosed with saline. Animals had free access to a normal chow diet (12% fat cal; Harlan Teklad rodent diet no. 8604) throughout this second study. Plasma was collected from these animals 1 day subsequent to the first Dexa treatment. Given the relatively short half-life of Dexa in plasma (~180 min), we assumed that the hyperglycemic response, as measured 24 h later under fasting conditions, would result more from a cascade of metabolic events implying long-lasting changes in muscle metabolism than from an acute effect of the hormone treatment. Finally, on day 12, animals were killed and tissues collected for metabolomic evaluation.

Experimental Design

For both animal models, in vivo MRI and MRS measurements were made under 1.8–2% isoflurane in O2 anesthesia. Protocol timelines are summarized in Fig. 1. A and B. For ex vivo analysis, fat distribution, IMCL, and body weight were measured in every rat within 3 days before and after the 4-wk diet period. Oral glucose tolerance tests (OGTTs) were performed on day 0 and day 28. For study B, fat distribution, IMCL, body weight gain, plasma metabolites, and hormone fasting levels were determined on day 0 (before injection) and 2 days after the second injection of Dexa. OGTTs were performed on day −10, and day 5. On day 12, animals were anesthetized with pentobarbital sodium (75 mg/kg ip), and muscle tissues (fast-twitch muscle, left epitrochlearis; slow-twitch muscle, left soleus) were dissected out for the measurement of glucose transport using an in vitro 2-deoxy-D-glucose (2-DG) technique and metabolonomic evaluation [right tibialis anterior (TA) muscle only].

OGTT and Analytic Procedures

After a 16-h fast, rats were administered a 1.0 g/kg glucose bolus orally, and blood samples were obtained via tail nick at 0, 15, 30, 45, 60, and 120 min after glucose administration. Blood samples (200 μl) were collected in heparinized microcentrifugation tubes (Brinkmann Instruments, Westbury, NY). Samples were immediately centrifuged (10,000 rpm at 4°C for 5 min) and measured for plasma glucose and lactate concentrations with the use of a YSI 2700 dual-channel biochemistry analyzer (Yellow Springs Instrument, Yellow Springs, OH). Fasting plasma FFA and TG concentrations were measured by use of a standard kit (Wako, Osaka, Japan). The rest of the plasma samples were stored at −20°C for insulin measurement with the use of an ELISA assay kit (American Laboratory Products, Windham, NH).

In Vivo MRI and Localized 1H-MRS

All in vivo magnetic resonance (MR) measurements were performed on a Bruker Avance 3.0 T/60 cm wide-bore instrument (Bruker Medical, Billerica, MA) equipped with a 12-cm internal diameter (ID) actively shielded gradient insert and a B-S20 shim system. For the measurement of whole body adiposity, a birdcage

Fig. 1. Protocol timelines. A: study A. B: study B. OGTT, oral glucose tolerance test; FFA, free fatty acid; TG, triglyceride; IMCL, intramyocellular lipids; Dexa, dexamethasone; Glc, glucose; D, day.
resonator (72-mm ID; Bruker Medical) or a Litz resonator (85-mm ID; Doty Scientific, Columbia, SC) was used as a radiofrequency transmitter/receiver, depending on the rat size. The rat was laid on a support and positioned in the center of the coil. After slice positioning by use of an orthogonal scout scan, a total of ~100 contiguous 2-mm-thick transversal slices covering the rat body were recorded with a turbo-spin echo technique, with 32 echoes/excitation and 128 phase-encoding steps. The imaging sequence was optimized for short echo time (TE 6 ms) and short repetition time (TR 250 ms) to allow signal suppression from tissues other than fat. The spatial resolution in plane varied with the rat size from (625 µm)² to (859 µm)². Usually, 2-mm-thick slices were obtained in three blocks (40 slices/block) to cover the whole body. Maximal signal intensity was reached with sufficient signal averaging (8 averages), resulting in an ~45-min total acquisition time/rat.

Image analysis was carried out with Paravision 2.1 image analysis software (Bruker, Karlsruhe, Germany). The abdominal region between the inguinal area region and the diaphragm was first selected from the two-dimensional image series. Fat distribution was then determined by manually outlining the visceral fat along the well-defined parietal peritoneum in each slice within the abdominal region. As a result, regional changes in fat were assessed on the basis of total, intra-abdominal, or visceral and SC fat depots. The abdominal fat is the sum of visceral and SC fat within the abdominal region. MRI-visible visceral fat comprises omental, retroperitoneal, and mesenteric fat depots. Two-dimensional image series were then imported into IDL software (Boulder, CO) for pixel counting-based determination of fat volumes. A signal threshold was used after applying a Gauss filter, a maximum likelihood, and a class select interaction to exclude all nonfat tissues in each slice. A density factor of 0.9 g/ml was used to convert fat volumes (in ml) into fat mass (in g). The MRI technique for determining total body fat mass has been validated by comparison of data (linear regression, slope 0.98) from both the MRI and the tritiated water dilution technique in lean and obese Zucker rats, as previously described (14). Lean weight was calculated by subtracting total fat from body weight.

IMCL data were obtained from the TA muscle (IMCLTA) by use of both the 72-mm birdcage resonator (transmitter) and a 2.0-cm surface coil (receiver) in a cross-coil fashion. Anesthetized rats were positioned prone with the left leg placed on top of the surface coil within the isocenter of the magnet. Transverse, sagittal, and coronal scout images were acquired to carefully position the 2 × 2 × 2-mm³ volume of interest in the left TA muscle, avoiding blood vessels and gross adipose tissue deposits. Localized ¹H-MR spectra were obtained by use of a point-resolved spectroscopy sequence (TE 18 ms, TR 2 s, 4,096 data points over a 6-kHz spectral width, chemical shift selective suppression of water, 1,000 scans). Before the data acquisition, the magnetic field was shimmed on water signal within the voxel of interest by use of Fastmap (40) to achieve typical line widths of ~11 Hz. Spectra were processed using the Nuts-PPC software package (AcornNMR, Fremont, CA). Once spectra were line broadened, phased, and baseline corrected, peak areas for total creatine [tCr; 3.02 parts per million (ppm)], EMCL content in the TA (EMCLTA; methylene peak at 1.5 ppm), and IMCLTA (methylene peak at 1.3 ppm) were determined with the use of a line-fitting procedure. IMCLTA content was then expressed as a percentage of tCr content.

**Metabonomics**

**Sample preparation for high-resolution MRS.** Plasma samples were prepared by the addition of 100 µl of neat D₂O to 100 µl of plasma for a total sample volume (plasma plus D₂O) of 200 µl. Muscle tissue extracts were prepared by first grinding the tissue into a fine powder under liquid nitrogen and then lyophilizing the ground tissue to a constant weight. The resulting powder was extracted with 7% perchloric acid for 2–6 h at 4°C. Centrifugation (1,840 g for 12 min at room temperature) was used to separate the supernatant from the insoluble material. The resulting pellet was set aside and stored at −20°C until further use. The perchloric acid supernatants were neutralized with 2 M K₂CO₃, centrifuged for 10 min to remove salts, and then lyophilized to a white powder of constant weight. The lyophilates were then dissolved in 590 µl of D₂O containing 1.5 mg/ml of sodium 3-trimethylsilyl-(2,2,3,3-D₄)-propionate (TMSP). Ten microliters of 0.1 M deuterated ethylenediamine tetra-acetic acid in D₂O (EDTA-d₄, pH 7.4) was added to chelate any residual paramagnetic species. Supernates were then centrifuged to remove any particulate matter, and the supernatants were transferred to 5-mm NMR tubes. The pellets generated from the perchloric acid extractions were transferred to glass containers and extracted overnight with CHCl₃ at 4°C. After this, the CHCl₃ layer was carefully removed and transferred in a fresh glass flask. The CHCl₃ was then removed by evaporation under nitrogen gas until an even and apparently dry film was formed. These samples were put under vacuum overnight to remove residual CHCl₃. The dried residue was dissolved in CDC1₃ and transferred to a 5 mM NMR tube.

High-resolution ¹H-MR spectra of plasma samples were acquired at 300 ± 1 K with the use of a Bruker DMX500 spectrometer operating at an ¹H frequency of 499.87 MHz. For all plasma samples, ¹H-MR spectra were collected with a Carr-Purcell-Meiboom-Gill spin-echo pulse sequence, [D – 90° – (t – 180° – t₀) – acquire], where time (t) is 1 ms and no. of repetitions (n) is 40, for a fixed spin-spin relaxation delay of 80 ms. Plasma spectra were collected with 128 free induction decays (FIDs), 65,536 complex data points, a spectral width of 8 kHz, and a relaxation delay (D) of 1.8 s. The water signal was irradiated during the relaxation delay. ¹H-MR spectra of muscle tissue extracts were acquired at 300 ± 1 K with a Bruker DMX400 spectrometer operating at an ¹H frequency of 400.13 MHz. All perchloric acid muscle extract data were acquired using a (D – 90° – t₁ – 90° – t₀ – 90° – acquire) pulse sequence, where the evolution period (t₁) was 3 µs and mixing time (t₀) was 150 ms. ¹H-MR spectra of TA muscle extracts were acquired with 256 FIDs, 32,768 complex data points, a spectral width of 6 kHz, and a relaxation delay of 2 s. The water signal was irradiated during t₀ and the relaxation delay. All muscle lipid extract spectra were acquired using a (D – 90° – acquire) pulse sequence. Muscle extract spectra were acquired with 16 FIDs, 65,536 complex data points, a spectral width of 4.4 kHz, and a relaxation delay of 2 s. All spectra were processed by multiplying the FID by an exponential weighting function corresponding to a line broadening of 0.3 Hz before Fourier transformation. Metabolite assignments were made on the basis of data from previous literature (18, 33, 48) and in certain cases confirmed by spiking. The mean fatty acid chain length (FACL) was determined from the intensities (F) of all ¹H-MR fatty acid resonances according to the equation (50)

\[
\text{FACL} = \left( \frac{2/3 \left( F(\omega CH_3) + F(\alpha CH_3) + F(\beta CH_2) + F(CH_3)_0 \right) + F(\Delta CH_2,\Delta) + F(\Delta - 1CH_2) + 2F(2CH_2)}{2/3 \left[ F(\text{CH}_3) \right]} \right)
\]

In this equation, the sum of the intensities (F) of all ¹H-MR fatty acid resonances, which reflects the total number of nonquaternary carbons in the fatty acid chain, is divided by the intensity of the ωCH₃ resonance, which is taken to represent the number of fatty acid molecules, as it is assumed that there is a single methyl group per fatty acid molecule. For consistency, the intensities of the ωCH₃ resonance and the ΔCH resonance [F(C₃H₆O)] and F(CH₃)] are normalized to be equivalent to those of the methylene resonances.

**Data reduction and principal component analysis.** Spectra were Fourier transformed and phase and baseline corrected using XWINNMR (Bruker, Karlsruhe, Germany). Chemical shifts were referenced to TMSP at a proton chemical shift of 0.0 ppm. Spectra were reduced to 256 integrated regions of equal width (0.04 ppm) by use of AMIX (Analysis of MIXtures) software package version 2.5. For the plasma and perchloric acid muscle extract data, the spectral region corre-
Table 1. Effects of 4-wk high-fat diet on plasma metabolites, insulin, and whole body glucose tolerance in fa/fa and lean Zucker rats

<table>
<thead>
<tr>
<th></th>
<th>High Fat</th>
<th>Normal Chow</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>fa/fa</td>
<td>Lean</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>4.2±0.1</td>
<td>0.7±0.0</td>
</tr>
<tr>
<td>FFA, mEq/l</td>
<td>3.2±0.8</td>
<td>1.0±0.1*</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>3.2±0.3</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>Baseline</td>
<td>3.0±0.3</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
<td>2.7±0.4</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>Baseline</td>
<td>93.0±10.2</td>
<td>2.6±0.8</td>
</tr>
<tr>
<td>4 Wk</td>
<td>122.7±10.2</td>
<td>21.3±9.8*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>149.1±5.3</td>
<td>99.4±3.5†</td>
</tr>
<tr>
<td>Baseline</td>
<td>178.9±16.8</td>
<td>126.7±6.2†</td>
</tr>
<tr>
<td>Insulin × insulin, AUC</td>
<td>77±0</td>
<td>21±3‡</td>
</tr>
<tr>
<td>Baseline</td>
<td>449±23†</td>
<td>167±44‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. TG, triglyceride; FFA, free fatty acid; AUC, area under the curve. *P < 0.05 and †P < 0.01, 4 wk vs. baseline; ‡P < 0.01, fa/fa vs. lean Zucker rats; §P < 0.01, high-fat vs. normal diet. Responding to a proton chemical shift of 5.0–6.4 ppm was set to zero integral to remove effects of variation in the suppression of the residual H2O resonance. The reduced data set was collected into a single Excel (Microsoft Excel 2002, SP-2) data table such that each row contained the integral descriptors for an individual 1H-MR spectrum.

Glucose Transport

Tissue levels of the glucose analog 2-DG were measured in epitrochlearis (fast-twitch) and soleus (slow-twitch) muscles to further identify ex vivo the barriers to insulin-stimulated glucose uptake that are expected in the Dexta-treated group. Once transported into the cell and phosphorylated by hexokinase, 2-DG is minimally metabolized, hence making it a good marker for glucose transport (21). In this study, mannitol was also used in the preparation to determine the extracellular space and 2-DG intracellular concentration. Mannitol has a molecular weight similar to 2-DG but cannot enter the cell because of the lack of a transport system to cross the cell membrane.

Measurement of 2-DG transport was performed in the morning after a 4- to 6-h fast. Rats were anesthetized with 5 mg/100 g body wt of pentobarbital sodium injected intraperitoneally, and both epitrochlearis and soleus muscles were dissected out. Muscles were individually placed in 25-ml Erlenmeyer flasks containing 2 ml of Krebs-Henseleit buffer with 2 mM sodium pyruvate, 36 mM mannitol, and 0.1% bovine plasma albumin (Sigma Chemical, St. Louis, MO). The flasks were placed in a Dubnoff shaking water bath maintained at 30°C and gassed continuously with 95% O2-5% CO2 throughout the experiment. This initial incubation, which lasted for 30 min, allowed muscles to recover from the dissection. Glucose transport was then measured according to Hansen et al. (21).

Statistical Analysis

All values are expressed as means ± SE. Intergroup comparisons and time course variations were analyzed with the Student’s t-test and a two-way ANOVA for repeated measurements when appropriate. Correlations were assessed by linear and nonlinear regression analysis. Principal component analysis was performed with SIMCA-P version 10 (Umetrics, Umeå, Sweden). Two-dimensional principal component score plots were constructed to establish the presence of any treatment-related patterns or clusters in the data. P < 0.05 was considered statistically significant.

RESULTS

Study A: Effects of the High-Fat Diet on Fat Distribution, IMCL Content, Plasma Lipid Profile, and Whole Body Insulin Resistance

At the onset of the study, the 8-wk-old fa/fa Zucker rats were heavier, mildly hyperglycemic, hyperinsulinemic, hyperlipidemic, and insulin resistant compared with their age-matched lean counterparts (Table 1). Enriching the animal diet from 12.2 to 54.4% fat calories resulted in a loss of appetite in both fa/fa (−20%; P < 0.001 vs. normal Chow diet) and lean Zucker rats (−13%; P < 0.01 vs. normal Chow diet), with the result that the average daily caloric intake remained steady in animals of the same genetic background (Table 2). Despite this, both obese and lean rats given the high-fat (HF) diet showed a marked increase (P < 0.01) in body weight gain (Table 2).

The increased body weight gain of the animals on the HF diet could be attributed to higher food efficiency, which can be calculated from the total weight gain divided by the total food intake consumed during the 4-wk diet period (Z-HF, 0.70 ± 0.05; ZL-HF, 0.46 ± 0.04; Z-N, 0.36 ± 0.01; and ZL-N, 0.25 ± 0.02 g/g; P < 0.01 ANOVA). The HF diet resulted in a marked impairment of glucose tolerance, especially in obese rats. The incremental change in the glucose excursion after the oral glucose load, when factored in for the insulin production (e.g., the product glucose × insulin, Table 1), was 414, 91, and 342% higher in Z-HF (P < 0.01), ZL-HF (P = 0.06), and Z-N rats (P < 0.01), respectively, than in lean control rats (ZL-N). Surprisingly, circulating FFA levels decreased for animals given the HF diet (P < 0.05). Plasma FFAs were not significantly changed for rats given normal Chow. However, an increase in plasma TGs was measured in Z-N rats (Table 1). Whole body adiposity, as determined from the total body fat mass/body weight ratio, increased more in fa/fa [Z-HF 0.60 ± 0.03 (Δ = 0.24 ± 0.04) vs. Z-N 0.48 ± 0.02 (Δ = 0.13 ± 0.02); P < 0.05] and lean [ZL-HF 0.15 ± 0.01 (Δ = 0.09 ± 0.01) vs. ZL-N 0.09 ± 0.01 (Δ = 0.02 ± 0.01); P < 0.01] Zucker rats given the HF diet compared with animals given a normal Chow diet. For the fa/fa Zucker rats, the greatest accumulation of fat occurred in the SC region (Fig. 2), whereas for the lean Zucker rats, fat accumulation in the SC and visceral areas was comparable.

After the 4-wk diet period, IMCLTA content was markedly increased in both Z-HF and ZL-HF rats [Δ(IMCLTA/tCr),
2.5 ± 1.0 and +1.1 ± 0.3, respectively; \(P < 0.01\), whereas no change or a slight decrease was measured in ZL-N [\(\Delta \text{IMCL}\text{TA}/\text{tCr}\), 0.2 ± 0.4, not significant (NS)] and Z-N rats [\(\Delta \text{IMCL}\text{TA}/\text{tCr}\), −0.9 ± 0.4, NS] (Fig. 3). In keeping with glucose and insulin excursion data (i.e., OGTT), linear regression analysis indicated that whole body glucose intolerance was highly correlated with total fat content (\(r = 0.90, P < 0.001\)) and IMCL\text{TA} (\(r = 0.81, P < 0.001\)) accumulation. A positive linear relationship was also detected between visceral fat accumulation and the change in the IMCL\text{TA}/\text{tCr} ratio when analyzing data by groups (\(fa/\text{fa}\) Zucker, \(r = 0.91, P < 0.001\); lean Zuckers, \(r = 0.82, P < 0.01\)). The slope of this relationship was approximately threefold greater (\(P < 0.05\)) in \(fa/\text{fa}\) Zucker (slope 0.25) than in lean Zucker rats (slope 0.08) (Fig. 4). This is likely indicative of the major influence that genetic background has on regional fat distribution. Compared with visceral fat, a much weaker correlation between SC fat accumulation and \(\text{IMCL}_{\text{TA}}/\text{tCr}\) was found for the \(fa/\text{fa}\) Zucker rats (\(r = 0.65, P < 0.01\)) and lean Zucker rats (\(r = 0.50, P = 0.08\)), and the slope of the relationship was similar for each group (e.g., a slope of 0.04 for \(fa/\text{fa}\) Zucker rats and 0.035 for lean Zucker rats).

Study B: Effects of Dexa Treatment on Fat Distribution, IMCL Content, Plasma Lipid Profile, Insulin Resistance, and Metabonomics

Body weight gain in Dexa-treated rats was reduced by almost 45% (\(P < 0.001\) compared with control rats) (Table 3). Dexa treatment did not increase further the level of hyperinsulinemia, as normally encountered in fatty Zucker rats. However, the treatment was clearly diabetogenic, as Dexa-treated rats became hyperglycemic (\(P < 0.01\) vs. saline) (Table 3).
The reduced body weight gain of the Dexa group could primarily be attributed to a lower accumulation of SC fat, especially in the abdominal region (Dexa +36% vs. saline +69%, \( P < 0.03 \); Fig. 5). Dexa had a minimal effect on visceral fat accumulation (Fig. 5) and on food intake (Dexa 30.0 ± 2.7 vs. saline 31.2 ± 1.7 g·day\(^{-1}\)·rat\(^{-1}\), as measured over 5 treatment days). At the cellular level, IMCL content decreased by ~33% in the saline group (IMCL\(_{TA/TCr}\) = 3.0 ± 0.6 at day 12, \( P < 0.05 \), vs. baseline = 4.5 ± 1.3) but remained unchanged in the Dexa group (~6%; IMCL\(_{TA/TCr}\) = 3.6 ± 0.7 at day 12, \( P > 0.05 \), vs. baseline = 3.8 ± 0.6). A similar decrease in IMCL was also observed in a parallel study using fatty Zucker rats of a similar age under a normal chow diet (data not published).

OGTT results are presented in Fig. 6. Plasma glucose \( \times \) insulin excursion data (i.e., area under the curve) indicated an ~70% impairment in the whole body insulin sensitivity of Dexa rats (\( P < 0.01 \) vs. saline). Muscle glucose transport data obtained from both the epitrochlearis and the soleus muscles are presented in Fig. 7. In the absence of insulin, basal glucose transport, although occurring at a low rate, was approximately fourfold greater in soleus than in epitrochlearis muscles of saline groups. Although not significant, an ~41% diminution in basal glucose transport was detected in Dexa-treated epitrochlearis muscles. When a submaximal physiological dose of insulin was added to the incubation medium, a 2.3- and 1.7-fold elevation in tissue 2-DG was observed in fast-twitch and slow-twitch muscles of saline groups, respectively. A reduction of insulin action on muscle glucose transport by Dexa was detected in both muscle types investigated (i.e., epitrochlearis ~39% and soleus ~53%). However, this effect was significant only in the soleus preparation. Altogether, these data indicate that muscle glucose transport in fatty Zucker rats was further impaired by Dexa treatment.

The biochemical effects of Dexa were characterized by the combined use of ex vivo high-resolution \(^1\)H-MR and principal component analysis. The \(^1\)H-MR spectra of plasma from both Dexa-treated and saline groups were dominated by lipid signals (Fig. 8). Principal component analysis of the plasma \(^1\)H-MR spectra showed that Dexa induced significant changes in plasma lipid profile as indicated by the separation of the Dexa and saline groups in the principal component score plot (Fig. 9A). This separation resulted primarily from an increase in the intensity of the fatty acid (CH\(_2\))n resonance observed at 1.34 ppm. Dexa increased the mean chain length of the serum lipids (+11%, \( P < 0.05 \)) and increased the fatty acid (CH\(_2\))/6(CH\(_3\)) ratio (+15%, \( P < 0.05 \)), indicating an increase in the saturated fatty acid content of the circulating lipids.

### Table 3. Effects of Dexa on body wt gain, plasma lipid profiles, and whole body insulin resistance in fa/fa Zucker rats

<table>
<thead>
<tr>
<th></th>
<th>Baseline Saline</th>
<th>Baseline Dexa</th>
<th>After Saline</th>
<th>After Dexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>289±12</td>
<td>293±10</td>
<td>(120±8)</td>
<td>(63±8)*</td>
</tr>
<tr>
<td>Fasting lactate, mM</td>
<td>2.8±0.3</td>
<td>2.5±0.1</td>
<td>3.7±0.4</td>
<td>5.4±0.2†</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>165±6</td>
<td>164±4</td>
<td>164±7</td>
<td>348±54†</td>
</tr>
<tr>
<td>Fasting insulin, µU/ml</td>
<td>207±36</td>
<td>206±36</td>
<td>170±26</td>
<td>203±32</td>
</tr>
<tr>
<td>Fasting Glc × Ins, AU</td>
<td>34,756±6,721</td>
<td>34,052±6,171</td>
<td>27,574±3,948</td>
<td>65,448±10,410†</td>
</tr>
<tr>
<td>FFA, mEq/l</td>
<td>10.07±2.13</td>
<td>7.85±0.78</td>
<td>2.85±0.21</td>
<td>2.97±0.11</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>6.171±0.21</td>
<td>3.726±0.034</td>
<td>3.948±0.10</td>
<td>6,721±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values in parentheses are Δbody wt (g). Glc, glucose; Ins, insulin; AU, arbitrary units. *\( P < 0.05 \) and †\( P < 0.01 \), dexamethasone (Dexa) vs. saline.
Figure 9B shows the principal component score plot generated from 1H-MR spectra of the water-soluble fraction (i.e., perchloric acid) of the TA muscle extracts. Dexa-treated animals clearly separate from those of the saline group, indicating that Dexa treatment significantly altered the metabolic profile of the TA muscle. Analysis of the principal component loadings indicated that the predominant differences between the metabolite profiles of the two groups were as follows: 1) the presence of glycogen and 2) a reduction in myocellular glutamate, glutamine, and arginine for the Dexa-treated rats, the latter observation being consistent with a Dexa-induced efflux of these amino acids (2, 39). The presence of glycogen in the TA muscle of Dexa-treated animals was confirmed by assay (26). Despite the presence of muscle glycogen in only the Dexa-treated animals, muscle glucose levels were not significantly different between the two groups.

Finally, analysis of the lipid fraction of the TA muscle extracts (by 1H-MR) showed that Dexa had a minimal effect on muscle lipid composition. In particular, no significant differences in the fatty acid (CH₂)ₙ/ωCH₃ ratio or mean fatty acid chain length were observed between the two groups. It should be noted that this finding indicates that no correction factor for the in vivo IMCL measurements is needed, as would be the case if the lipid composition of the TA had been significantly altered by Dexa treatment.

DISCUSSION

The primary purpose of this work was to assess whether visceral adiposity, a known predisposing factor for the development of type 2 diabetes (11), might influence the accumulation of intramyocellular triglycerides. To do this, we used two rat models of insulin resistance. For both fa/fa and lean Zucker rats in ex vivo experiments, the 4-wk high-fat diet resulted in an increased accumulation of SC fat, IMCL in the fast-twitch TA muscle, and, to a lesser extent, visceral fat. In this diet-induced model of insulin resistance, IMCL contents were found to be highly correlated with visceral adiposity, whereas the correlation between IMCL content and SC fat deposition was comparatively weak. Furthermore, the increase in IMCL content relative to the accumulation of visceral adiposity was approximately threefold greater in fa/fa Zucker rats compared with lean Zucker rats. This latter finding is of particular interest, since it illustrates how the genetic background may in part be responsible for abnormalities of lipid metabolism, an implicit defect in the pathogenesis of type 2 diabetes. Conversely, our data obtained from the dexamethasone-induced model of insulin resistance (i.e., study B) have failed to demonstrate any variation in the visceral fat mass, despite greater IMCL contents, on the diabetogenic treatment. The disparity observed between these two models of insulin resistance illustrates how various mechanisms may all factor into the differences reported on fat accumulation.

Obesity-induced alterations in glucose/lipid metabolism have been explained with the assumption that the increase in visceral adipose tissue is accompanied by a rise of plasma FFAs in the portal vein (7). Under fasting conditions, we did not observe a diet-induced increase in circulating fat that would support such a model. In fact, the selective increase in FFA mobilization to the portal vein, which is thought to contribute to hepatic insulin resistance and dyslipidemia (3), was not measured here. However, our data cannot rule out that extensive fat cycling might have nonetheless occurred between lipid...
storage and lipolysis even though high-fat feeding resulted in a net increase in visceral adiposity.

Chronic increased availability of circulating FFAs may also, concomitantly and without any further connection, stimulate direct absorption in both adipose and muscle tissues. We observed that, for a similar increase in visceral fat, IMCL accumulation was significantly greater in leptin-deficient than in non-leptin-deficient animals. This could be explained by factors modulating fat infiltration such as adipocyte-secreted hormones (e.g., adiponectin, leptin, resistin, IGF-I, angiotensin, and so forth) from visceral fat, all molecules well known to impact fat oxidation and glucose homeostasis. Thus the impaired postabsorptive fat oxidation in the muscle, as it has been described in human obesity (24, 51) and was mimicked in a relevant animal model using prolonged inhibition of fatty acid oxidation (16), can lead dietary fat to accumulate in muscle tissues. The ability of the fatty Zucker rats in the present study to maintain plasma lipid levels relatively unchanged while in excess of dietary fat (vs. baseline) would support such a mechanism. During the enlargement of fat cells, dietary fat could conceivably be diverted to muscle cells, resulting in greater IMCL accumulation. Considering that the endocrine function of large adipocytes is impaired in obesity (49), the risk of developing insulin resistance may then further increase through a negative feedback mechanism.

Still, the question arises as to whether IMCL accumulation can also result from a change in muscle lipid metabolism. Initially, study B was designed on the assumption that, visceral fat being more responsive to stress (6, 8), glucocorticoid stimulation might exacerbate the shift of lipids from the portal vein to ectopic regions such as the skeletal muscle. In agreement with this hypothesis, IMCL increased along with an impairment of glucose transport (e.g., 2-DG data) after the diabetogenic treatment with dexamethasone. However, our study has failed to demonstrate any variation in visceral fat accumulation on the dexamethasone treatment. Instead, the decrease in body weight gain could be attributed almost exclusively to a lower accumulation of fat in the SC region of the abdomen. This result was unexpected, given the high density of glucocorticoid receptors normally present in visceral fat tissue (35, 37). Nevertheless, this finding agrees with recent data showing that both SC abdominal and visceral fat can be closely associated with insulin resistance in humans (25, 28). Because the high levels of IMCL cannot be explained on the basis of the portal vein mechanism alone, pathogenic factors associated with the endocrine function of large SC fat pads and/or dexamethasone-induced changes in carbohydrate and lipid metabolism may also be considered as key modulators of peripheral insulin resistance. In support of this hypothesis, first, the ex vivo metabonomic analysis of muscle extracts demonstrated similar intracellular glucose levels for both control and treated animals but increased glycogen in dexamethasone-treated rats. This latter result likely reflects an inhibitory effect of dexamethasone on muscle glycogen phosphorylase, as described...
controls previously in the liver (32). Second, our high-resolution 
$^1$H-MR data obtained from plasma samples showed that, although plasma FFA and triglyceride concentrations remained unchanged, the mean chain length and saturated fatty acid content of the serum lipids significantly increased upon treatment. This indicates that lipolytic products were drained from the adipose SC tissue and/or the liver into the systemic circulation. Of note, the “dexamethasone stimulation model” of type 2 diabetes in this respect differs from the lipodystrophic syndrome, for which insufficient adipose tissue leads to excess IMCL content and insulin resistance (38). Third, recent data have shown that, after reaching a peak level at 10 wk of age, IMCL contents decrease in aging Zucker rats, independent of insulin resistance (30). We observed similar trends both in study A (−21%, $P = 0.1$; fa/fa Zucker rats on a normal chow diet, from 8 to 13 wk of age) and in study B (−41%, $P < 0.05$; control fa/fa Zucker rats, from 8 to 12.5 wk of age). However, IMCL$_{TA}$ levels were kept elevated in dexamethasone-treated animals. The continued elevation of IMCL$_{TA}$ may result from an accumulation of lipid molecules or may reflect a decrease in lipid oxidation. Our ex vivo analysis showed that, in contrast to the increased aliphatic content observed for the plasma lipids, dexamethasone did not alter the lipid composition in the TA muscle, suggesting that the primary effect of dexamethasone was to inhibit muscle fatty acid oxidation.

Glucocorticoids and their synthetic analogs are known to modulate a wide range of physiological processes through a variety of mechanisms, involving for example the endocrine, renal, immune, and vascular systems, but also exert a direct impact on substrate utilization. In our study, the potent effects of dexamethasone on lipid and glucose metabolism may be summarized as follows. In hyperinsulinemic Zucker rats, dexamethasone is obviously able to counteract the antilipolytic effects of insulin (4) by contributing to the release of fatty acids from adipose tissue. At the same time, proliferation of new adipocytes may be limited and their endocrine function impaired. Muscle glucose uptake as measured by the 2-DG method is affected through an inhibition of transport across the membrane. Glycolytic flux may be lowered as well, since intramyocellular glucose levels remain steady. Glycogen stores are increased through inhibition of glycogen breakdown, and anaerobic catabolism is diverted towards lactate production. Because glucocorticoids stimulate muscle mitochondrial activity (46), the energy supply to the muscle cell is ensured through utilization of either dietary fat or lipolytic products from adipose tissue, while intracellular lipids essentially remain unused. At this point, muscle glucose uptake may be further impaired through a mechanism involving high intramyocellular lipid content. Whether the lipid droplet size, shape, or location and/or the existence of a high diacylglycerol concentration in the cytosol interferes directly or indirectly with the insulin signaling pathway still remains to be determined.

In summary, our results showed that the high-fat diet-induced increase in IMCL was strongly related to an accumulation of fat in the abdominal area and greater insulin resistance. Even though both fasting plasma FFA and triglyceride concentrations remained unchanged, the lipolytic effect of dexamethasone may be reflected by an increase in mean fatty acid chain length and in the saturated fatty acid content of circulating lipids. Because such a change was not observed from muscle extracts, high levels of IMCL measured in response to dexamethasone treatment may result more from an inhibition of lipid utilization than the uptake and/or production of longer fatty acid chains.

Although a causal relationship has not been established yet, visceral adiposity may influence IMCL accumulation under dietary manipulations. However, there are inherent limitations to using visceral fat as a true marker of insulin resistance that eventually lead to reconsideration of the portal vein hypothesis. Excess dietary fat can cause adipocyte enlargement and fat storage in ectopic sites such as skeletal muscles (41). This in itself may be sufficient to explain the strong correlation found between visceral adiposity and IMCL deposition. Such a correlation is not surprising, considering the recent findings on IMCL and intra-abdominal lipid accumulation both being present early in the development of peripheral insulin resistance (47). A cause-effect association may in fact not play such a prevalent role as several other elements would support. As mentioned earlier, in lipodystrophic patients (42) and transgenic fatless mice (27, 35), insulin resistance can be predicted by an increase in muscle triglycerides independently of visceral adiposity. In addition, a selective depletion of IMCL may lead
to reversal of insulin resistance in obese patients regardless of total body fat content (20). Finally, SC fat that does not drain into the portal vein has been correlated with insulin resistance in men with type 2 diabetes (1). Our data suggest that a diabetogenic treatment using repeated injections of dexamethasone can have a more direct effect on muscle lipid metabolism, e.g., can lower fat oxidation through acyl-CoA dehydrogenase inhibition (31), independently of visceral adiposity. Given the fact that dexamethasone- and high-fat diet-induced insulin resistances are probably operating under different mechanisms, normalizing IMCL stores may then be considered as a preferential pathway to treat insulin resistance, even though the molecular link between elevated IMCL and impaired insulin signaling still remains to be determined. For pharmacological investigations using animal models, IMCL should be viewed as a more relevant biomarker of insulin resistance than visceral fat.

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