Prolonged exposure to elevated glucocorticoid levels is known to produce insulin resistance (IR), a hallmark of diabetes mellitus. Although not fully elucidated, the underlying molecular mechanisms by which glucocorticoids induce IR may provide potential targets for pharmacological interventions. Here we characterized muscle lipid metabolism in a dexamethasone-aggravated diet-induced obesity murine model of IR. Male C57BL/6 mice on a high-fat diet for 2 months when challenged with dexamethasone showed elevated food consumption and weight gain relative to age and diet-matched animals dosed with saline only. Dexamethasone treatment impaired glucose tolerance and significantly increased the intramyocellular lipid content in the tibialis anterior muscle (TA). A good correlation ($r = 0.76, P < 0.01$) was found between accumulation in intramyocellular lipid content in the TA and visceral adiposity. The linoleic acid (18:2) to polyunsaturated acid ratio was increased in the dexamethasone-treated animals (+29%; $P < 0.01$), suggesting a possible increase in stearoyl-CoA desaturase 2 activity, as reported in Sertoli cells. The treatment was also accompanied by a reduction in the percent fraction of o-3 and long-chain polyunsaturated fatty acids in the TA. Analysis of the low-molecular-weight metabolites from muscle extracts showed that there was no dysregulation of muscle amino acids, as has been associated with dexamethasone-induced muscle proteolysis. In conclusion, dexamethasone-induced insulin resistance in diet-induced obese mice is associated with a profound perturbation of lipid metabolism. This is particularly true in the muscle, in which an increased uptake of circulating lipids along with a conversion into diabetogenic lipids can be observed. (Endocrinology 149: 758–766, 2008)
increased lipoprotein lipase activity in the adipose tissue (26), and an impairment of endothelium-dependent vasodilation (27), which itself is an important determinant of insulin sensitivity.

A major advantage of using dexamethasone is that the insulin-resistant state can be generated in a relatively short period of time (28). Typically 8–12 wk on a high-fat diet are required for a C57BL/6J male mouse to become obese and mildly hyperglycemic and develop a progressive impairment of glucose tolerance (29). This can be compared with the dexamethasone-aggravated model in which insulin resistance can be generated within a week of dosing. Because the molecular mechanisms by which dexamethasone induces insulin resistance are not yet fully understood, the metabolic characterization of such models is important for drug testing.

As an extension of our previous work with dexamethasone-challenged rats (19), the objective of this study was to characterize the effect of dexamethasone on lipid metabolism in a glucocorticoid-aggravated diet-induced obesity (DIO) mouse model of insulin resistance. We found that dexamethasone significantly increased IMCL in the mouse, and this was associated with increased glucose intolerance. Ex vivo analysis has also shown that dexamethasone induced significant alterations to the muscle lipid profile.

Materials and Methods

Animals

Twenty-seven 8-wk-old male mice were divided into two groups and placed on a high-fat diet (54% fat calories; Research Diets Inc., New Brunswick, NJ; no. 99080201) for 2 months before the study. A cohort of 14 animals was given three ip doses of 11 mg/kg dexamethasone (dexa) over the course of 5 d (d0, 2, and 4). The remaining animals (i.e. the second cohort) were dosed with saline. In rats, dexamethasone has a plasma half-life of between 2 and 3 h (30). It is metabolized predominantly by the liver (>70%) and the metabolites are excreted via the kidney. All animals had free access to water and the high-fat diet throughout the study. Time-course changes in whole-body glucose disposal were measured at baseline (6 d before the initiation of the dexamethasone treatment at d0) and from d9 to 11 after dexamethasone treatment using the oral glucose tolerance test (OGTT). IMCL was measured 5 to 3 d before the initial dexamethasone dosing (baseline) and on d 8 (i.e. d 4 after the final administration of dexamethasone). Finally on d 12, animals were killed and tissues were collected for metabonomic evaluation. Lipid droplets were also quantified for their size and number in both the soleus and the tibialis anterior (TA) muscle using electron microscopy as described below. All experimental procedures were carried out in compliance with the guidelines of the Novartis Institutional Animal Care and Use Committee.

OGTT

After a 6-h fasting period, mice were given an oral bolus of glucose (1.0 g/kg), and blood samples were obtained via a tail nick 0, 30, 60, and 120 min after glucose administration. Blood samples (20 μl) were collected in heparinized microcentrifugation tubes (Brinkmann Instruments, Inc., Westbury, NY) and were immediately centrifuged (10,000 rpm at 4 C for 5 min). Plasma glucose concentrations were then measured using a YSI 2700 dual-channel biochemistry analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Plasma insulin levels were measured using an ELISA kit (American Laboratory Products Co., Windham, NH). The homeostasis model assessment (HOMA) index was also calculated as a surrogate measure of in vivo insulin sensitivity using the following formula:

In vivo nuclear magnetic resonance (NMR)

All NMR data were obtained on a DMX400 (9.4T) magnet (Bruker, Billerica, MA) equipped with a microimaging gradient system (3.4 cm diameter bore size, 250 mT/m maximal gradient strength). With this equipment, magnetic resonance spectroscopy (MRS) was used to assess intramyocellular lipid levels, whereas magnetic resonance imaging (MRI) was used to assess fat distribution (i.e. sc fat and visceral fat amount). For in vivo MRS experiments, a home-built Helmholtz coil with a loop diameter set at 10 mm to accommodate for the mouse leg was used. A restrainer was added to the coil to keep the mouse in an upright position. The animal holder was covered with a cone to allow for distribution of the anesthesia gas mixture (2% isoflurane) from top to bottom in the chamber.

Muscle fat by MRS

The left mouse leg was placed within the Helmholtz coil such that the knee joint was approximately 2 mm atop the isocenter of the magnet. Scout images were acquired to verify placement of the leg and to guide the 1 × 1 × 1 mm3 volume of interest in the left TA muscle, avoiding

FIG. 1. In vivo measurement of IMCL content in a 16-wk-old mouse after 2 months on a fat-enriched diet. A, Sagittal, transverse, and coronal sections through a mouse leg. The white square centered on the TA muscle indicates the 1 mm3 voxel from which in vivo spectrum (B) was obtained. The major components of each spectrum were line fitted for lipid quantification. IMCL and EMCL levels were determined from the IMCLCH2 (1.3 ppm) and EMCLCH2 (1.5 ppm) resonances relative to the total creatine peak (3.02 ppm) as described in the text.

HOMA = fasting [insulin (microinternational units per milliliter)]
fasting [glucose (millimoles per liter)]/22.5

FIG. 2. Effects of dexamethasone on food consumption (A) and body weight (B). Day 0 corresponds to the onset of dexamethasone treatment. Data are presented as mean ± SEM. * , P < 0.05.
blood vessels and gross adipose tissue deposits (Fig. 1). Localized 1H-MR spectra were obtained using a point-resolved-spectroscopy sequence [echo time of 25 msec, repetition time of 2 sec, 90°/180° hermite pulses of 500 msec each, 4096 data points over a 10 kHz spectral width, class of chemical-shift-selective water suppression (6 msec sinc pulse), 512 scans, and a gradient spoiling time of 1.0 msec at 8% absolute power]. Before the acquisition, the magnetic field was shimmed to achieve typical line widths of approximately 15 Hz. Spectra as seen in Fig. 1 were processed using the Nuts-PPC software package (AcornNMR, Inc., Free- mont, CA). Once spectra were line broadened, phased, and baseline corrected, peak areas for total creatine (tCr; 3.02 ppm), extramyocellular lipids (EMCL; methylene peak at 1.5 ppm), and IMCL (methylene peak at 1.3 ppm) were determined using a line-fitting procedure. IMCL content was then expressed as a percentage of tCr content. In skeletal muscle, tCr has been documented as a good reference for quantification of IMCL (31).

Fat distribution by MRI

Assessment of body fat was performed using an Alderman-Grant resonator (inner diameter 30 mm; Bruker Medical Inc., Billerica, MA). Sixty contiguous 1-mm-thick transversal slices were obtained along the mouse body using a turbospin echo technique with 32 echoes per excitation and 128 phase-encoding steps. An echo time (2.6 msec) and repetition time (2 sec) were used for the suppression of nonfat tissues (19). The spatial resolution was (270 μm)² and eight averages were collected, resulting in an approximately 20-min total acquisition time per mouse.

Fat distribution was determined by manually outlining the visceral fat along the well-defined parietal peritoneum in each slice within the abdominal region, as described earlier (19). Regional changes in fat were thereby assessed from image segmentation that led to total, intraabdominal, or visceral and sc fat depots. MRI visible fat comprises omental, retroperitoneal, and mesenteric fat depots. The resulting segmented two-dimensional image series were then imported into IDL software (ITT, Visual Information Solutions, Boulder, CO) for pixel counting-based determination of fat volumes. A signal threshold was used after applying a Gauss filter, a maximum likelihood estimator, 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 (milliliters) into fat mass (grams).

Light and electron microscope analysis

Soleus and TA muscles were placed in modified Karnovsky’s fixative. Five animals from each of the dexamethasone and saline control groups were randomly selected for electron microscopy processing using the microwave-assisted technique (32–34). After primary fixation, samples were rinsed in cacodylate buffer and postfixed in 0.1 M phosphate-buffered 1% osmium tetroxide. Tissues were dehydrated through an upgraded acetone series. Samples were embedded in EMbed (Epic Microscopy, Hatfield, PA) 812 in flat silicon molds. Semi thin and ultrathin sections were cut with a Leica Ultracut Ultramicrotome. Thin sections were stained with toluidine blue-basic fuchsin for light microscopic evaluation. Ultrathin sections cut for transmission electron microscopy TEM survey were double stained with uranyl acetate and lead citrate. Grids were examined using a Zeiss (LEO, Thorn- wood NY) EM-902A transmission electron microscope integrated with a Metavision Pro software (Micros, Bellevue, WA). At the light microscope level, a qualitative assessment of the overall cell morphology and number of lipid droplets present was recorded. At the ultrastructural level, a quantitative assessment of average lipid droplet size (square nanometers), average total lipid area (square nanometers), and the percentage of lipid per fiber area (square nanometers) per square nanometer were recorded. In addition, a qualitative assessment of the plasma, nuclear and mitochondrial membranes plus lipid droplet morphology and integrity was recorded.

Sample preparation for metabonomic analysis

Muscle tissue extracts were prepared as previously described (19). Whole TA muscles were ground into a fine powder under liquid nitrogen and lyophilized to constant weight. The powdered tissue was extracted with 10 ml of 7% perchloric acid and CHCl₃ overnight at 4°C. The perchloric acid extract was neutralized with 2 M K₂CO₃ (pH 7.1–7.4). The insoluble salts were removed by centrifugation and the supernatant was lyophilized. The resulting powder was reconstituted in 600 μl of D₂O containing 0.15 mg/ml sodium 3-trimethylsilyl (2,2,3,3-D₄) propionate. The CHCl₃ layer was evaporated under nitrogen gas and the residual CHCl₃ was removed under vacuum overnight. The dried residue was dissolved in 600 μl CDCl₃.

High-resolution NMR spectroscopy

High-resolution 1H-MR spectra of perchloric acid extracts of the muscle samples were acquired at 300 ± 1 K using a Bruker DMX500 spectrometer operating at 1H frequency of 499.87 MHz. Spectra of the perchloric acid muscle extracts were acquired using a (D-90°-acquire) pulse sequence, where tᵢ was 3 msec and tᵣ was 80 msec. Each spectrum was acquired with 128 free induction decay (FID), 65,536 complex data points, a spectral width of 6 kHz, and a relaxation delay of 1.8 sec. The water signal was irradiated during tᵣ and the relaxation delay. All muscle lipid extract spectra were collected using a (D-90°-acquire) pulse sequence with 1,024 FIDs, 32,768 complex data points, a spectral width of 6.0 kHz, and a relaxation delay of 2 sec. 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. Spectra of the perchloric acid extracts and chloroform extracts were referenced to 3-(trimethylsilyl)propionic-2,2,3,3-D₄-acid at 8H 0.0 ppm and the residual chloroform resonance at 8H 7.27 ppm, respectively. Metabolite assignments were made on the basis of previous literature data (35–37) and in certain cases confirmed by spiking.

Metabonomic data analysis

Spectra were reduced by integrating regions of equal width (0.04 ppm) using AMIX version 2.5 (Bruker, Karlsruhe, Germany). For the spectra of the perchloric acid extracts, the spectral region corresponding to 8H 5.0 to 4.6 ppm was set to zero integral to remove effects of

### TABLE 1. Effects of dexamethasone on plasma hormones and metabolites as well as whole-body insulin sensitivity as calculated from the HOMA index in high-fat fed mice

<table>
<thead>
<tr>
<th></th>
<th>Baseline saline</th>
<th>Baseline dexamethasone</th>
<th>After saline</th>
<th>After dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma lactate (mM)</td>
<td>8.5 ± 0.6</td>
<td>8.3 ± 0.4</td>
<td>7.2 ± 0.6</td>
<td>7.2 ± 0.3</td>
</tr>
<tr>
<td>Fasting plasma glucose (mM)</td>
<td>14.8 ± 0.6</td>
<td>15.3 ± 0.6</td>
<td>15.6 ± 0.5</td>
<td>16.1 ± 0.5</td>
</tr>
<tr>
<td>Fasting plasma adiponectin (ng/ml)</td>
<td>9644 ± 772</td>
<td>9052 ± 1067</td>
<td>9036 ± 478</td>
<td>9427 ± 708</td>
</tr>
<tr>
<td>Fasting plasma insulin (μIU/ml)</td>
<td>7.3 ± 1.0</td>
<td>7.8 ± 0.6</td>
<td>7.6 ± 0.9</td>
<td>9.9 ± 1.1</td>
</tr>
<tr>
<td>HOMA index (mM/liter/μU/liter)</td>
<td>4.7 ± 0.7</td>
<td>5.5 ± 0.6</td>
<td>5.3 ± 0.7</td>
<td>7.2 ± 0.9</td>
</tr>
</tbody>
</table>

Similar to the OGTT, samples were taken 6 d prior to the initiation of the dexamethasone treatment at d 0 and from d 9 to 11 after dexamethasone. Data are presented as mean ± SEM.
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. Triglyceride and fatty acid resonances were integrated using ACD 7.0 (Advanced Chemistry Development, Inc., Toronto, Ontario, Canada).

**Multivariate analysis**

Principal component analysis and partial least squares discriminant analysis (PLS-DA) were performed using SIMCA-P version 10 (Umetrics AB, Umeå, Sweden). Two-dimensional principal component analysis or PLS-DA score plots were constructed to establish the presence of any treatment-related patterns or clusters in the data. Spectral features that contributed to the separation of the two groups were integrated using ACD version 6.0 (Advanced Chemistry Development Inc., Toronto, Canada).

**Statistics**

Results are expressed as mean ± SEM. Intergroup comparisons were made using appropriate ANOVA and simultaneous multiple comparison procedures (with Bonferroni correction). P < 0.05 was considered statistically significant.

**Results**

**Glucose tolerance, body weight, and fat distribution**

Dexamethasone induced a significant increase (14%) in food consumption during the 5-d treatment period (Fig. 2). Food consumption for the dexamethasone-treated group returned to pretreatment levels once dexamethasone treatment ceased. Consistent with this, dexamethasone-treated animals showed a higher body weight gain (i.e., \( \Delta \text{body weight}_{\text{dexamethasone}} \))

![Fig. 3. Dexamethasone-induced changes in whole-body glucose tolerance as measured during an oral glucose challenge. Depicted data show actual plasma glucose (A) and insulin (B) excursion values as well as respective changes (C and D) observed throughout the treatment period. The test was performed 6–7 d before the initiation of the dexamethasone treatment and from d 9 to 11 after dexamethasone treatment using the OGTT. A glucose load of 1.0 g/kg was given orally to each animal at time 0. Note in both A (i.e. saline (post) vs. dexamethasone (post)) and C that glucocorticoid treatment resulted in a significant increase in glucose excursion values relative to the saline group (\( P < 0.05 \) for the group \( \times \) time interaction using repeated measure two-way ANOVA), whereas insulin excursion values remained similar for both groups. Data are presented as mean ± SEM. *\( P < 0.0125 \), dexamethasone (post) vs. saline (post); §\( P < 0.0125 \), dexamethasone vs. saline.

![Fig. 4. Fat distribution in dexamethasone- vs. saline-treated mice. Data are presented as mean ± SEM. *\( P < 0.05 \) vs. saline.](image)
Fig. 5. Dexamethasone-induced increase in IMCL levels in the TA muscle of mice fed with a high-fat diet for 2 months (left panel). IMCL levels were measured 5 to 3 d before the initial dexamethasone dosing (baseline) and on d 8 (after, i.e. 4 d after the final administration of dexamethasone). IMCL levels are well correlated with visceral (Visc) fat contents (right panel). Data are presented as mean ± SEM. * P < 0.05.

+1.54 ± 0.48 g vs. Δbody weight_vehicle +0.08 ± 0.52 g, P < 0.05; Fig. 2). Fasting levels of plasma glucose, insulin, and adiponectin as well as the HOME index remained unchanged on the dexamethasone treatment (Table 1). However, a significant increase in the glucose excursion values was observed in dexamethasone-treated animals in response to the oral glucose challenge, indicating an impairment of whole-body glucose tolerance in these animals (Fig. 3).

Adipose tissue and muscle lipids

When measured relative to body weight, visceral fat contents were approximately 50% greater in dexamethasone-treated mice (Fig. 4). In contrast, sc fat contents were similar in both treated and untreated groups. Under baseline conditions (after 2 months on the high fat diet), IMCL were comparable for both groups studied (IMCL/tCr_dexa: 2.06 ± 0.33 vs. IMCL/tCr_Saline: 1.92 ± 0.11, P > 0.05). Dexamethasone treatment resulted in 310% greater IMCL levels, compared with controls, this difference being partly due to a significant decrease in the vehicle group (Fig. 5). Interestingly on d 5, IMCL/tCr values appeared to be strongly related to the degree of visceral adiposity (r = 0.76, P < 0.01).

Histological analysis showed that the morphometry and integrity of plasma, nuclear, and mitochondrial membranes were similar in both muscle types studied (i.e. soleus and TA muscles), compared with the control animals, with no lesions noted. As derived from both the number and size of lipid droplets (Fig. 6), the lipid surface per fiber area (square nanometers) was approximately 2.4-fold higher in the TA of mice treated with dexamethasone, compared with saline mice (dexa: 0.0158 ± 0.0037 vs. vehicle: 0.0066 ± 0.0012 nm²/ nm² field area, P < 0.05). There was no significant difference between the amounts of lipids in the soleus muscle, compared with controls (dexa: 0.0068 ± 0.0014 vs. vehicle: 0.0094 ± 0.0019 nm²/ nm² field area, P = NS). Lipid droplets often accumulated within the mitochondrial network; however, no mitochondrial lesions were observed.

Metabolite profile analysis (perchloric acid extracts)

The concentrations of low-molecular-weight muscle metabolites, including the amino acids glutamine and arginine, ATP, and creatine were determined using NMR spectroscopy. No significant differences were observed between the dexamethasone and saline-dosed groups.

Lipid profile analysis (chloroform extracts)

Figure 7 shows the PLS-DA score plot generated from the ¹H-NMR spectra of the chloroform extracts of TA muscle from dexamethasone- (△) and saline (●)-dosed mice. Each symbol represents the ¹H-NMR spectrum of an individual spectrum. The separation of samples from dexamethasone- and saline-dosed mice indicates that dexamethasone treatment induced significant alterations in the composition of muscle FAs.
ration of the dexamethasone and saline groups can be seen in Figs. 8 and 9. Lipid resonances, which were altered by dexamethasone treatment, were integrated relative to the combined intensities of the FA \( \omega \)-methyl resonances (tCH\(_3\)), including the \( \omega \)-methyl resonances from \( \omega \)-3 FAs (\(^1\)H\(_6\) 0.98 ppm), as well as the main FA \( \omega \)-methyl resonance centered at \(^1\)H\(_6\) 0.89 ppm. The main FA \( \omega \)-methyl resonance is composed of resonances from \( \omega \)-6, \( \omega \)-9 and all other classes (except \( \omega \)-3) of FAs. It was assumed that each FA molecule contained a single \( \omega \)-methyl moiety and that the tCH\(_3\) intensity would reflect the total number of FA molecules.

Figure 10 describes the effects of dexamethasone on the saturated and unsaturated FA distribution in the TA muscle. Dexamethasone treatment reduced docosahexaenoic acid (DHA; 22:6) by approximately 43% and the total \( \omega \)-3 FA content by approximately 45% (\( P < 0.02 \)) in dexamethasone-treated rats, indicating an overall decrease of PUFAs in the lipid composition. Interestingly, the LA(\( \Delta \)-CH\(_2\)-\( \Delta \)) to tCH\(_3\) ratio was not significantly altered by dexamethasone treatment. The contribution of PUFAs to the pool of unsaturated FAs was reduced by approximately 17%, indicating an increase in the amount of monounsaturated fatty acids, presumably dietary oleic acid. Finally, dexamethasone treatment did not have a significant effect on the TAG to tCH\(_3\) ratio or the relative amounts of 1,2-diacylglycerides and 1,3-diacylglycerides in the lipid pool.

Discussion

Glucocorticoids exert orexigenic (38) and antithermogenic (39, 40) effects as well as stimulate the accumulation of both body fat and visceral fat (41, 42), which are concordant with the well-established effect of stress-related weight gain (43).

![Chemical Shift (ppm)](https://example.com/chemical-shift.png)

**Fig. 8.** Spectral expansion (\(^1\)H\(_6\) 5.6–2.3 ppm) of the chloroform extracts of the TA muscle from dexamethasone- (A) and saline (B)-dosed mice. Spectra are scaled to the FA \( \omega \)CH resonance. Note the reduction in DHA and longer-chain PUFAs in the muscle extract from the dexamethasone-treated mouse. MUFA, Monounsaturated fatty acid; DAG\(_{\omega,\omega}\), 1,2-diacylglyceride; DAG\(_{\omega,\omega}\), 1,2-diacylglyceride; \( \omega \)CH\(_3\), fatty acid \( \omega \)CH\(_2\) moiety.

**Fig. 9.** Spectral expansion (\(^1\)H\(_6\) 1.0–0.5 ppm) of the chloroform extracts the TA muscle from dexamethasone- (A) and saline (B)-dosed mice. The FA \( \omega \)CH\(_3\) resonance of \( \omega \)-3 FAs is significantly reduced in dexamethasone-treated animals. \( \omega \)-3 FA, \( \omega \)CH\(_3\) moiety from \( \omega \)-3 fatty acids; \( \omega \)CH\(_3\),\( \omega \)CH\(_3\) moiety from \( \omega \)-6, \( \omega \)-9, and saturated FAs; Cholest, C18 methyl from cholesterol.
Dexamethasone stimulates the release of leptin by adipocytes (47), which likely contributed to the drop in adiposity as observed in the aforementioned rat model. However, sensitivity to the weight-reducing action of leptin is significantly impaired by diet-induced obesity (48). This in turn would explain why already obese mice under acute dexamethasone treatment would continue to accumulate ectopic fat.

In the dexamethasone-aggravated DIO mouse model, IMCL was increased 2- to 3-fold in response to dexamethasone treatment. Saline-treated DIO mice showed an approximately 30% reduction in IMCL levels over the course of the study. The reason for the decrease in IMCL in saline-treated animals is unclear. In non-insulin-resistant rats, lipoprotein lipase activity has been reported to decrease in fast-twitch muscles as the animals mature (49). This would limit IMCL accumulation and combined with normal fatty acid oxidation may be partially responsible for an age-related decrease in IMCL. The decline in IMCL in the saline-treated mice can be contrasted with the DIO rat model (19) in which IMCL was found to increase over a similar period of time. The physiological basis for such divergence may have to do with the metabolic rate, which is known to be proportional to body mass (50). Whereas it can easily exceed 300 cal/d/kg for a normal mouse (51), the average energy expenditure for a rat is only 60 cal/d/kg (52). Given that a good relationship was found between IMCL contents and visceral adiposity in the DIO mouse model, it is conceivable that dexamethasone exacerbated lipid exchanges between fat adipose tissue and skeletal muscles far more in mice than rats, although a causal relationship between the two fat depots (i.e., visceral fat and IMCL) still remains to be determined.

It is noteworthy that IMCL levels in the soleus, in contrast to the TA muscle, were not significantly affected by dexamethasone treatment. Metabolic rates were not measured in the present study. Nonetheless, it is likely that the balance between fatty acid uptake and use is not significantly altered by dexamethasone, given that in oxidative muscles dexamethasone has been shown to increase both FA absorption and oxidation (53, 54). It remains for this to be tested in future studies.

The FA profile of the dexamethasone-treated group differed significantly from that of the saline-dosed group. There was a marked reduction in the percentage of ω-3 fatty acids and long-chain PUFAs, including the beneficial FA, DHA (22:6), of dexamethasone-treated animals. The decrease in muscle TA ω-3 and PUFA levels can be attributed to the increased accumulation of dietary FAs because the content of these FAs in lard, i.e., the main source of fat in the diet provided, is relatively low (55).

Despite the reduction of PUFAs in the dexamethasone-dosed animals, the percentage of LA and the LA to unsaturated FA ratio in the TA muscle were not decreased by dexamethasone treatment as would be expected if the effect of dexamethasone was simply to increase muscle accumulation of dietary lipids. If the TA muscle lipid profile reflected only fat accumulation, the percentage of LA would be expected to significantly decrease because LA constitutes approximately 16–17% of the fatty acid composition of dietary fat, whereas the monounsaturated FA, oleic acid, and the saturated fatty acids, palmitic acid, and stearic acid comprise approximately 43% and 32%, respectively. Because the meth-
ods used in the present study cannot rule out the possibility that the uptake and storage of LA in the TA muscle are not stimulated in the dexamethasone-treated group, our results would be consistent with the increased conversion of dietary oleic acid into LA through the up-regulation of stearoyl-CoA desaturase 2 (SCD2). The in vitro up-regulation of SCD2 activity by dexamethasone in Sertoli cells has been reported (56); however, our observation may be among the first, to the author’s knowledge, suggesting that dexamethasone increases SCD2 activity in vivo.

No significant differences in the TAG to FA or diacylglyceride (DAG) to FA ratios were found between the dexamethasone and saline-dosed groups, indicating that the percent contribution of DAG and fatty acyl CoA to the TA muscle lipid pool were not affected. These metabolites are thought to interfere with the insulin signaling cascade through chronic activation of protein kinase C, resulting ultimately in the impairment of GLUT4 translocation (57, 58). Although dexamethasone did not increase DAGs and fatty acyl CoA levels in terms of lipid composition, the absolute amounts of DAGs were certainly increased in the dexamethasone-treated animals because both in vivo and ex vivo IMCL measurements showed a 2- to 3-fold increase in muscle lipids. Given this fact, the effect of an elevation of DAG and FA CoA in absolute terms in this model warrants further consideration.

In the present study, dexamethasone had a minimal effect on the low-molecular-weight metabolite profile of the TA muscle. This is in contrast to our previous finding with the rat in which dexamethasone treatment resulted in a significant decrease in muscle concentrations of glutamine and arginine. The glutamine pool in particular plays a significant role in maintaining muscle mass. The depletion of muscle glutamine has been implicated in the muscle wasting associated with glucocorticoid treatment (59). Glucocorticoids alter muscle glutamine pools by stimulating both glutamine synthesis, via the up-regulation of SCD2 (59). Glucocorticoids alter muscle glutamine pools by thought to interfere with the insulin signaling cascade acting as a selective glycogen synthase kinase-3 inhibitor. Diabetologia 48:2119–2130

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