Increased Intramyocellular Lipid Concentration Identifies Impaired Glucose Metabolism in Women With Previous Gestational Diabetes

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Women with previous gestational diabetes (pGDM) are frequently insulin-resistant, which could relate to intramyocellular lipid content (IMCL). IMCL were measured with $^1$H nuclear magnetic resonance spectroscopy in soleus (IMCL-S) and tibialis-anterior muscles (IMCL-T) of 39 pGDM ($32 \pm 2$ years, waist-to-hip ratio $0.81 \pm 0.01$) and 22 women with normal glucose tolerance (NGT; $31 \pm 1$ years, $0.76 \pm 0.02$) at 4–6 months after delivery. Body fat mass (BFM) was assessed from bioimpedance analysis, insulin sensitivity index ($S_I$), and glucose effectiveness ($S_G$) from insulin-modified oral glucose tolerance tests. pGDM exhibited 45% increased BFM, 35% reduced $S_I$, and $S_G$ ($P < 0.05$), and 40% ($P < 0.05$) and 55% ($P < 0.005$) higher IMCL-S and IMCL-T, respectively. IMCL related to body fat (BFM $P < 0.005$, leptin $P < 0.03$), but only IMCL-T correlated ($P < 0.03$) with $S_I$ and glucose tolerance index independent of BMI. Insulin-resistant pGDM ($n = 17$) had higher IMCL-S ($+66\%$) and IMCL-T ($+86\%$) than NGT and insulin-sensitive pGDM ($+28\%$). IMCL were also higher ($P < 0.005$, $P = 0.05$) in insulin-sensitive pGDM requiring insulin treatment during pregnancy and inversely related to the gestational week of GDM diagnosis. Thus, IMCL-T reflects insulin sensitivity, whereas IMCL-S relates to obesity. IMCL could serve as an additional parameter of increased diabetes risk because it identifies insulin-resistant pGDM and those who were diagnosed earlier and/or required insulin during pregnancy. *Diabetes* 52:244–251, 2003

Gestational diabetes mellitus (GDM) is a frequent metabolic complication during pregnancy that does not completely normalize after delivery (1–3). Women with a history of previous gestational diabetes (pGDM) are often insulin-resistant and exhibit markedly increased risk for the later development of type 2 diabetes (4,5). The most prominent parameters that predict type 2 diabetes in later life are the need for insulin in addition to diet therapy to achieve normoglycemia, early diagnosis of GDM during pregnancy, and maternal BMI and plasma glucose during the oral glucose tolerance test (OGTT) at diagnosis as well as at the first postpartum assessment (4,6,7).

Skeletal muscle insulin resistance is a key feature of the metabolic syndrome and predisposes to type 2 diabetes and premature cardiovascular complications (8). Although lifestyle (9), obesity, and increased lipid supply play an important role in this disease (8,10), the hierarchy of events is still unclear. It was postulated that muscle fat content could contribute to insulin resistance and glucose intolerance (11–19), but only the advent of $^1$H nuclear magnetic resonance spectroscopy (NMRS) made it possible to quantify and distinguish between extramyocellular and intramyocellular lipid contents (IMCL) (11,14,15,17,18,20–22).

We tested the hypotheses that intracellular fat content in different muscles diverges relates to insulin sensitivity and correlates with established risk markers for type 2 diabetes in pGDM, such as gestational week at diagnosis, insulin treatment during pregnancy, glucose levels during OGTT at diagnosis and postpartum, and the degree of obesity. Thus, we applied $^1$H NMRS to measure rapidly and noninvasively IMCL in soleus (IMCL-S) and tibialis anterior muscles (IMCL-T) in pGDM. IMCL were correlated with parameters of glucose tolerance, insulin sensitivity, cardiovascular risk, body fat content, and distribution. Furthermore, the study extends to potential links between IMCL and the leptin system, which participates in the regulation of body weight (BW) and energy metabolism (19,23,25,26).

**RESEARCH DESIGN AND METHODS**

All women ingested an isocaloric diet containing 200 g of carbohydrates/day and refrained from exercise for at least 3 days before the studies. Metabolic

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AIRg 3–10, acute insulin response 3–10 min after glucose ingestion; BFM, body fat mass; BW, body weight; FFM, fat-free mass; GDM, gestational diabetes mellitus; IMCL, intramyocellular lipid content; IMCL-S, IMCL of soleus muscle; IMCL-T, IMCL of tibialis anterior; NGT, normal glucose tolerance; NMRS, nuclear magnetic resonance spectroscopy; OGIS, insulin sensitivity parameter; OGTT, oral glucose tolerance test; pGDM, previous gestational diabetes mellitus; $S_I$, insulin sensitivity index.

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tests were performed on different days during the first phase (days 5–8) of the menstrual cycle after 10- to 12-h overnight fasting. 

**Study participants.** Cross-sectional analysis was performed in 39 pGDM women at 4–6 months after delivery. They were recruited from our division's outpatient service, where they had been seen previously during pregnancy. GDM had been diagnosed according to the criteria of the 4th Workshop Conference of Gestational Diabetes (27). During pregnancy, 26 women were treated with diet plus insulin, because blood glucose exceeded 95 mg/dl at fasting and/or 130 mg/dl at 60 min postprandially. A total of 22 age-matched women without any risk for diabetes and with normal glucose tolerance during pregnancy served as a control group (NGT). All subjects gave written informed consent for participation in the study, which was approved by the local ethics committee.

Patients with previous ketoacidosis and/or β-cell antibodies (GAD, ICA, IA2) were excluded. The relationship among IMCL, insulin sensitivity, and metabolic parameters was analyzed both in the total pGDM population and for the insulin-sensitive (pGDM-S) and insulin-resistant subgroups (pGDM-R), which were separated by a cutoff value of 2.8 \(10^{-4}\) min \(^{-1}\) (µU/ml) for the insulin sensitivity index (SI). This value was derived from analyzing SI of the NGT of the present study plus another 26 matched female control subjects from other studies. The lower 2.5% quantile of the distribution gave an SI of 2.79 \(10^{-4}\) min \(^{-1}\) (µU/ml), which was defined as the cutoff point between normal and impaired (lower) SI.

All pGDM had higher waist circumference, diastolic blood pressure, HbA\(_{1c}\), and basal metabolic rate corrected for BW than NGT (Table 1). After correction for BMI, differences in waist-to-hip-ratio disappeared, whereas waist circumference remained different (\(P < 0.01\)).

**Clinical characteristics** were not different between NGT and pGDM-S except for HbA\(_{1c}\) (Table 1). In contrast, pGDM-R had higher BMI and systolic blood pressure but lower HDL cholesterol. Basal metabolic rate adjusted for BW was lowest in pGDM-R (~16% versus NGT and pGDM-S) but not different between pGDM-S and NGT. After correction for lean body mass, i.e., fat-free mass (FFM), the basal metabolic rate was not different between pGDM (30.5 ± 0.33 kJ/kg FFM) and NGT (30.57 ± 0.39 kJ/kg FFM) but lower in pGDM-R (29.35 ± 0.50 kJ/kg FFM) than pGDM-S (31.58 ± 0.42 kJ/kg FFM, \(P < 0.003\)) and NGT (\(P < 0.05\)).

**Frequently sampled intravenous glucose tolerance test.** Glucose (time 0–0.5 min: 300 mg/kg BW) and then normal insulin (time 20–25 min: 0.03 IU/kg, Humulin R; EI Lilly, Indianapolis, IN) were infused intravenously, and venous blood samples were taken in timed intervals (28). Analysis of glucose and insulin concentrations provided indexes for glucose tolerance (K\(_{gl}\)), insulin sensitivity (SI), and glucose effectiveness (S\(_{e}\)), which describe glucose disposal and the insulin effect on glucose disappearance (28). Insulin secretion was assessed from incremental short-term insulin response (ΔAIR\(_{15}\)) calculated by averaging insulin concentration above basal from times 3–10 min. The disposition index was calculated as S\(_{e}\) times ΔAIR\(_{15}\) and gives a measure of the combined effects of insulin secretion and sensitivity on glucose disposal (29).

**OGTT.** Participants ingested 75 g of glucose solution, and venous blood samples were collected for glucose, insulin, and C-peptide measurements at timed intervals (30). Modeling analysis yielded fasting prehepatic insulin secretion rate and the total amount of insulin per unit volume released during the OGTT in response to increments in glucose concentration (30). Insulin sensitivity from OGTT (OGIS) was derived as glucose clearance (ml \(\cdot \min^{-1}\) \(\cdot\) m\(^2\)) (31).

**Localised 1H NMR.** IMCL was measured with localised 1\(^{H}\) NMRs (17,20,21) on a 3.0-T/80-cm NMR spectrometer (Medspec; Bruker, Ettlingen, FRG) equipped with a whole-body gradient coil (40 mT/m; Fig. 1). A standard birdcage 1\(^H\) coil (inner diameter 25 cm) was used in the transmission/reception mode. The STEAM sequence (echo time 20 ms; mixing time 30 ms; relaxation time 6 s; number of scans 32) was complemented by CHESS water suppression and applied on the 1.73-cm\(^3\) volume of interest, which was placed in the soleus or tibialis anterior muscles of the subject’s right leg. Spectra were line-broadened and -fitted using the MacNUTS-PFC software (Acorn NMR, Livermore, CA). IMCL was quantified from processed spectra after T2-relaxation correction as a ratio of the intensity of (CH\(_2\))\(_3\) (1.25 ppm) group resonance to the intensity of the water resonance from non-water-suppressed spectra of the same volume of interest (Fig. 1). The T2 relaxation times were of 82 ± 3 ms for IMCL-S and 30 ± 2 ms for water in soleus muscle and 90 ± 8 ms for water in tibialis muscle.

**TABLE 1**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>GDM</th>
<th>GDM-R</th>
<th>GDM-S</th>
<th>NGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.1 ± 0.81</td>
<td>31.0 ± 1.4</td>
<td>31.2 ± 1.0</td>
<td>30.6 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>26.4 ± 1.1</td>
<td>29.8 ± 1.8</td>
<td>24.9 ± 0.8*</td>
<td>24.3 ± 0.9†</td>
</tr>
<tr>
<td>WHR</td>
<td>0.81 ± 0.01</td>
<td>0.81 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>89.1 ± 2.3§</td>
<td>96.1 ± 2.5</td>
<td>84.5 ± 2.2*</td>
<td>75.1 ± 2.3†‡</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>118.2 ± 22.0</td>
<td>136.3 ± 50.9</td>
<td>105.9 ± 14.3</td>
<td>75.2 ± 6.3</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>210.9 ± 7.6</td>
<td>200.3 ± 14.1</td>
<td>218.0 ± 8.5</td>
<td>198.0 ± 12.4</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>61.4 ± 2.7</td>
<td>54.8 ± 4.7</td>
<td>65.8 ± 3.9§</td>
<td>62.1 ± 2.9†</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>126.2 ± 6.5</td>
<td>118.9 ± 10.9</td>
<td>131.2 ± 7.9</td>
<td>120.8 ± 11.0</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>116.7 ± 2.2</td>
<td>123.7 ± 3.9</td>
<td>112.3 ± 2.3*</td>
<td>111.5 ± 2.6†</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80.0 ± 1.5§</td>
<td>83.0 ± 2.5</td>
<td>78.1 ± 1.9</td>
<td>74.1 ± 1.9†</td>
</tr>
<tr>
<td>HbA(_{1c}) (%)</td>
<td>5.40 ± 0.07§</td>
<td>5.36 ± 0.14</td>
<td>5.43 ± 0.08</td>
<td>5.1 ± 0.03†</td>
</tr>
<tr>
<td>Basal metabolic rate (kJ/kg BW)</td>
<td>20.10 ± 0.55§</td>
<td>18.35 ± 0.69</td>
<td>21.75 ± 0.65*</td>
<td>22.03 ± 0.51†</td>
</tr>
<tr>
<td>Basal metabolic rate (kJ/kg FFM)</td>
<td>30.50 ± 0.33</td>
<td>29.35 ± 0.50</td>
<td>31.58 ± 0.42*</td>
<td>30.57 ± 0.39†</td>
</tr>
</tbody>
</table>

* \(P < 0.05\) GDM-R versus GDM-S; † \(P < 0.05\) GDM-R versus NGT; ‡ \(P < 0.05\) GDM-S versus NGT; § \(P < 0.05\) GDM versus NGT.

**FIG. 1.** Left: Magnetic resonance cross-sectional image of human calf muscle. The rectangles indicate the positioning of the volumes of interest in soleus and tibialis anterior muscles. Right: 1\(^{H}\) NMR spectra acquired from the volumes of interest in both muscles.
**TABLE 2**

Metabolic parameters of the total group of women with pGDM (n = 39), the insulin-resistant subgroup (GDM-R), and the insulin-sensitive subgroup (GDM-S) compared with women with NGT 4–6 months after delivery.

<table>
<thead>
<tr>
<th></th>
<th>GDM</th>
<th>GDM-R</th>
<th>GDM-S</th>
<th>NGT</th>
</tr>
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<tbody>
<tr>
<td><strong>FSIGT</strong></td>
<td></td>
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<tr>
<td>Insulin sensitivity [10^{-4} min^{-1} (mU/ml)^{-1}]</td>
<td>4.00 ± 0.35§†</td>
<td>1.92 ± 0.10</td>
<td>5.39 ± 0.36‡</td>
<td>6.1 ± 0.5†</td>
</tr>
<tr>
<td>Glucose effectiveness (min^{-1})</td>
<td>0.022 ± 0.007§</td>
<td>0.021 ± 0.001</td>
<td>0.023 ± 0.006</td>
<td>0.027 ± 0.001†</td>
</tr>
<tr>
<td>Disposition index (10^{-2} min^{-1})</td>
<td>0.112 ± 0.011§</td>
<td>0.094 ± 0.020</td>
<td>0.124 ± 0.015</td>
<td>0.20 ± 0.03†</td>
</tr>
<tr>
<td>Glucose tolerance index (% min^{-1})_{10–20min}</td>
<td>1.85 ± 0.13</td>
<td>1.90 ± 0.25</td>
<td>1.79 ± 0.15</td>
<td>2.54 ± 0.29‡</td>
</tr>
<tr>
<td>A1Rg (pmol l^{-1} d^{-1})_{3–10min}</td>
<td>38.1 ± 4.7</td>
<td>35.3 ± 9.6</td>
<td>27.4 ± 3.4*</td>
<td>35.3 ± 4.5</td>
</tr>
<tr>
<td><strong>OGTT</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fasting glucose (mg/dl)</td>
<td>91.1 ± 1.4§</td>
<td>93.1 ± 1.9</td>
<td>90.0 ± 1.7</td>
<td>77.1 ± 1.2‡</td>
</tr>
<tr>
<td>1 h plasma glucose (mg/dl)</td>
<td>151.3 ± 7.3§</td>
<td>159.1 ± 10.1</td>
<td>145.1 ± 10.4</td>
<td>102 ± 4.4‡‡</td>
</tr>
<tr>
<td>2 h plasma glucose (mg/dl)</td>
<td>119.1 ± 5.8§</td>
<td>121.5 ± 9.0</td>
<td>117.3 ± 7.9</td>
<td>90.5 ± 3.0‡‡</td>
</tr>
<tr>
<td>OGIS (ml · min^{-1} · m^{-2})</td>
<td>432.5 ± 11.5§</td>
<td>385.4 ± 16.5</td>
<td>454.8 ± 11.4*</td>
<td>502.4 ± 12.6‡‡</td>
</tr>
<tr>
<td>Basal secretion rate (pmol · l^{-1} · min^{-1})</td>
<td>22.8 ± 2.4§</td>
<td>27.6 ± 4.9</td>
<td>18.8 ± 1.6</td>
<td>14.5 ± 1.1*</td>
</tr>
<tr>
<td>Dynamic insulin secretion (nmol/l)</td>
<td>51.7 ± 10.3</td>
<td>73.4 ± 20.0</td>
<td>31.83 ± 2.51*</td>
<td>33.6 ± 2.6*‡</td>
</tr>
</tbody>
</table>

*P < 0.05 GDM-R versus GDM-S; †P < 0.05 GDM-R versus NGT; ‡P < 0.05 GDM-S versus NGT; §P < 0.05 GDM versus NGT.

6 ms for IMCL-T and 27 ± 1 ms for water in tibialis anterior muscle. The coefficients of variation for 1H NMRs of IMCL as assessed from three measurements of the lipid content in each muscle in six healthy young subjects, were 0.3% for soleus muscle and 1.3% for tibialis anterior muscle, respectively.

**Body fat mass and basal metabolic rate.** Body fat mass (BFM) was assessed from bioimpedance measurements (Akern-RJL Systems, Florence, Italy) as was the basal metabolic rate. Measurement of resting energy expenditure with bioimpedance was validated in 10 subjects against indirect calorimetry yielding comparable results (r = 0.78, P < 0.01). Resting energy expenditure is expressed per kg BW as well as per kg FFM.

**Metabolites and hormones.** Plasma glucose was measured using an automated glucose analyzer (Beckman, Fullerton, CA). HbA1c (upper limit of expenditure is expressed per kg BW as well as per kg FFM.

**RESULTS**

**Glucose metabolism.** All pGDM featured lower S1 (−35%), glucose effectiveness (S2, −25%), and disposition index, a marker of overall glucose homeostasis (−47%), than NGT (Table 2). In the subgroup, pGDM-R, S1 was even 70 and 65% lower than in NGT and pGDM-S, respectively (Table 2). The disposition index was lower in pGDM-R than in NGT but not different between pGDM subgroups. The glucose tolerance index (Kc) was >30% lower in all pGDM, whereas ΔAIRg was 22% lower only in pGDM-S, reflecting reduced insulin release in response to a given glucose load. In contrast, pGDM-R featured 50% higher ΔAIRg than in NGT, indicating compensation by the β-cells for their insulin resistance.

Although fasting and postprandial plasma glucose concentrations during OGTT were higher in pGDM, no woman was diabetic according to American Diabetes Association and World Health Organization criteria (Table 2). The insulin sensitivity index (OGIS) was reduced by 10 and 25% in pGDM-S and pGDM-R, respectively. The pGDM-R compensated their marked insulin resistance by doubling basal and dynamic insulin secretion, which in contrast were not different between pGDM-S and NGT. Correction for BMI or BFM as a result of the higher degree of obesity in pGDM-R did not affect the differences in insulin sensitivity (P < 0.001) and glucose effectiveness (P < 0.05) between groups. Fasting plasma FFA were higher in both the total group of pGDM (0.63 ± 0.04 μmol/l, P < 0.005) and the subgroups (P < 0.01) pGDM-R (0.61 ± 0.09 mmol/l) and pGDM-S (0.60 ± 0.05 mmol/l) than in NGT (0.38 ± 0.03 mmol/l) but did not differ (P = 0.07) between subgroups.

**IMCL.** Mean IMCL-S and IMCL-T were 31 and 61% higher in the total group of pGDM than in NGT (Table 3). In the subgroup pGDM-R, both IMCL were also higher (IMCL-S 66%; IMCL-T 80%) than in NGT and one third higher in pGDM-R than in pGDM-S. Also, BFM was 78 and 50% higher in GDM-R than in GDM-S and GDM-S, respectively (Table 3). After correction for either BMI or BFM, the differences in IMCL-S disappeared (P = 0.10 for BMI, P = 0.61 for BFM), whereas IMCL-T remained increased in pGDM-R (P = 0.01 for BMI, P = 0.04 for BFM), indicating that obesity strongly influences only IMCL-S.

Nearly all pGDM-R (n = 15 of 17; 88%) but only 50% of GDM-S (n = 11 of 22) had required insulin therapy during pregnancy. The pGDM-S treated previously with insulin presented with higher IMCL-S (P < 0.005) and IMCL-T (P = 0.05) than insulin-naïve patients who were treated with diet despite no difference in metabolic or anthropometric parameters (Fig. 2).

**Leptin system.** Plasma total leptin was highest in pGDM-R (Table 3). The fraction of bound leptin was not different between pGDM-R and NGT but was 50% lower in
pGDM-S. However, when adjusted for BFM (Table 3) and corrected for BMI, bound leptin became markedly \( (P < 0.001) \) different, being lower in both GDM subgroups than in NGT. Plasma concentrations of soluble plasma leptin receptor were decreased in pGDM independent of BMI, whereas total leptin was no more different between groups after correction for BMI.

**Correlation analysis.** In all women, IMCL correlated positively with BMI and BFM and negatively with the basal metabolic rate after adjustment for BW (Fig. 3). However, when expressed per FFM, basal metabolic rate did not relate to IMCL-S or IMCL-T \( (P = 0.09) \) but still to IMCL-T in pGDM-R \( (r = -0.55, P < 0.04) \). IMCL was also associated with 2-h plasma glucose during OGTT (Fig. 4), waist circumference \( (r = 0.4, P < 0.001) \), plasma total leptin \( (r = 0.32, P < 0.03) \), and plasma FFA (IMCL-S: \( r = 0.31, P < 0.03 \); IMCL-T: \( r = 0.55, P < 0.001 \)). In all women, IMCL also inversely related to the gestational week during which GDM was diagnosed (IMCL-S \( r = -0.30, P < 0.05 \); IMCL-T \( r = -0.44, P < 0.02 \)), but only IMCL-T also negatively related to systolic blood pressure and negatively correlated with SI, Sg, and KG \( (r = -0.31, P < 0.03 \); Fig. 4). In pGDM-R, IMCL-T negatively related to the disposition index derived from both intravenous \( (r = 0.02, P < 0.02) \) and oral glucose challenge \( (r = -0.67, P < 0.014 \); Spearman, ANOVA). Multiple regression analysis including SI, BMI, and 2-h plasma glucose as explanatory variables showed that for IMCL-S only BMI and for IMCL-T only the 2-h plasma glucose had an independent influence. With only BMI and SI in the model, Sg remained as the only significant independent parameter to explain IMCL-T. When BFM entered the multiple regression analysis instead of BMI, again only BFM influenced IMCL-S \( (P < 0.02) \), and 2-h plasma glucose \( (P < 0.04) \) and BFM \( (P < 0.05) \) influenced IMCL-T. With only BMI and Sg in the model, BFM still significantly affected IMCL-S \( (P < 0.01) \), whereas no parameter independently affected IMCL-T.

Linear regression analysis for plasma leptin revealed its correlation to BMI \( (r = 0.70, P < 0.0001) \), waist-to-hip ratio \( (r = 0.40, P < 0.003) \), 2-h glucose during OGTT \( (r = 0.45, P < 0.002) \), systolic and diastolic blood pressures \( (r = 0.38, P < 0.009) \), triglycerides \( (r = 0.30, P < 0.05) \), HDL cholesterol \( (r = -0.31, P < 0.03) \), insulin sensitivity \( (r = -0.30 P < 0.04) \), and insulin secretion \( (r = 0.38, P < 0.009) \). In contrast, bound leptin correlated inversely only with 2-h glucose \( (r = -0.30, P < 0.03) \) and positively with the soluble leptin receptor \( (r = 0.53, P < 0.005) \) as well as parameters of glucose effectiveness \( (r = 0.33, P < 0.01) \) and insulin sensitivity \( (r = 0.43, P < 0.001) \). Soluble leptin receptor concentrations were inversely related to IMCL-T \( (-0.43, P < 0.05) \) and positively to HDL cholesterol \( (r = 0.57, P < 0.02) \).

**TABLE 4**

<table>
<thead>
<tr>
<th>GDM-R</th>
<th>Spearman</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGTT disposition index (mmol/m³)</td>
<td>-0.67</td>
<td>0.014</td>
</tr>
<tr>
<td>FSI GT disposition index</td>
<td>-0.62</td>
<td>0.028</td>
</tr>
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</table>

**FIG. 2.** Box plots for IMCL-S and IMCL-T in insulin-sensitive women who had pGDM-S and were treated by diet \( (n = 11) \) or insulin \( (n = 11) \) during pregnancy. The median is represented by the horizontal line inside the box; the top and bottom of the box represent the third quartile \( (75th \text{ percentile}) \) and the first quartile \( (25th \text{ percentile}) \), respectively. Whiskers are drawn from the edge of the box to the farthest observation within 1.5 times the interquartile range of the edge of the box. Observations beyond the whiskers are individually identified \( (*) \).
DISCUSSION

This study confirms that the disturbances in glucose metabolism persist in women with pGDM. Of the present population, 44% were severely insulin-resistant (pGDM-R) with excessive insulin secretion and higher degree of abdominal fat as indicated by their higher waist circumference and waist-to-hip ratio than the other groups. However, 56% were insulin-sensitive (pGDM-S) but showed distinct abnormalities of early and dynamic insulin secretion during intravenous and oral glucose challenge. Both metabolic abnormalities resulted in similarly higher plasma glucose in both pGDM subgroups than in NGT.

Although parameters of long-term glucose control such as plasma glucose during OGTT and HbA1c were comparable at 4–6 months after delivery, almost all pGDM-R and half of the lean pGDM-S had required insulin therapy during pregnancy. Glucose control was similar throughout pregnancy between pGDM-S and pGDM-R as reflected by HbA1c and self-recorded daily pre- and postprandial glucose profiles. As the requirement of insulin therapy indicates a more severe derangement of glucose metabolism and is considered an important risk factor for the progression to overt diabetes, it seems that pGDM-R are at higher risk of type 2 diabetes than pGDM-S. It is of note that these women with characteristic features of the metabolic syndrome also presented with highest IMCL. Moreover, even the 50% of pGDM-S on previous insulin therapy had higher IMCL than the insulin-naive pGDM-S whose IMCL were almost identical to those of NGT. As increased IMCL was the only parameter that differed in these pGDM-S subgroups, it might be an important marker to identify even women who are lean and otherwise insulin-sensitive but at increased risk for type 2 diabetes. Furthermore, in all pGDM, independent of their insulin sensitivity, the higher IMCL was related to earlier diagnosis of GDM, another widely known risk factor for deterioration of glucose tolerance at follow-up (6).

IMCL was higher in GDM-R than in the other groups and correlated with body fat, insulin sensitivity, and cardiovascular risk parameters in all women. Thus, increased IMCL may be added to the characteristics of the metabolic syndrome. Previous studies reported divergent results on the relationship between IMCL and anthropometric parameters (34,35), which possibly results from differences between individual skeletal muscle groups.

We found that IMCL-S more strongly relate to measures of obesity, whereas IMCL-T are more tightly associated with insulin resistance per se. This is in line with one report (11) but partly in contrast to another study report-
ing a better correlation of insulin resistance with IMCL-S and increased IMCL-T only in women (20). Although the correlation of IMCL-S with the degree of obesity might suggest that increased intracellular muscle fat simply reflects whole-body adiposity, the correlation of IMCL-T with parameters of the insulin resistance syndrome was found to be independent of BMI. In untrained humans, skeletal muscle represents a mixed fiber type (36) containing fiber types of different insulin sensitivity (37). Soleus muscle is prevalently composed of slow-twitch oxidative type I fibers, whereas tibialis anterior muscle contains more fast-twitch glycolytic type IIb fibers. Type I fibers generally have a higher lipid content yet also higher oxidative enzyme capacity and greater insulin sensitivity than type IIb fibers (38). In obesity and type 2 diabetes, the proportion of type IIb fibers with reduced oxidative enzyme activity may increase (39,40) so that a dynamic interaction between fiber type and metabolic capacity with more lipid stored in relation to oxidative capacity can be postulated for metabolic disorders. Furthermore, reduction of type I fibers with decreased expression of insulin sensitive glucose transporters (GLUT4) was detected in type 2 diabetes (41).

At present, a cause–effect relationship for IMCL and insulin sensitivity is not clear and the mechanisms of interaction are not yet fully understood. Plasma FFA resulting from dietary fat supply and/or increased lipolysis in fat tissue may directly induce insulin resistance or could be channeled preferentially into triglycerides (8, 10, 19, 42, 43). Increased FFA uptake or lipolysis of IMCL would increase cytosolic long-chain acyl-CoA (LCA-CoA), which correlate well with insulin resistance (43) and can inhibit insulin action via decreased insulin-receptor substrate-1 phosphorylation (42,43). In line with this hypothesis, plasma FFA elevation induces insulin resistance and gives rise to IMCL under high (21,44) but not fasting insulin conditions (33). Likewise, improvement of insulin resistance by the thiazolidinedione pioglitazone reduces muscle LCA-CoA and lipid accumulation in high-fat–fed rats (45) so that the beneficial effect of troglitazone to improve insulin action and reduce progression to type 2 diabetes in high-risk Latino women with pGDM (46) could at least partly result from altered muscle lipid supply.

Alternatively, the correlation between IMCL-T and 2-h plasma glucose during OGTT hints at an interaction between postprandial hyperglycemia and IMCL. Kelley et al. (47) proposed the concept that provision of glucose inhibits lipid oxidation, which could contribute to pathogenesis of lipid accumulation in obesity. In contrast to the present study, Phillips et al. (15) detected no correlation between 2-h plasma glucose and IMCL as measured from muscle biopsies of women at a mean age of 52 years. Cross-sectional studies in other populations (11,12,20,22) found no significant correlation for fasting plasma glucose and IMCL. We have previously reported that short-term hyperglycemia for 2 h in the presence of fasting (peripheral)
insulin decreases glucose disposal without changes in IMCL-S (33). Nevertheless, it cannot be excluded that chronic postprandial increases in plasma glucose may contribute to IMCL accumulation in pGDM.

The close association between IMCL and elevated plasma total leptin concentrations correlating with insulin secretion, insulin resistance, and BFM in pGDM could point at increased weight retention postpartum (48,49) and risk for later type 2 diabetes (50,51). In the present study, increased total leptin was mostly explained by increased free leptin, because bound leptin and soluble leptin receptor were even lower in pGDM. Of note, the bound form was positively associated with glucose disposal and tolerance, whereas the soluble leptin receptor was inversely related to IMCL-T. The soluble leptin receptor represents the major leptin binding protein in human blood and may therefore determine the circulating amount of total leptin (26,52). It was postulated that the bound form is involved in the regulation of energy expenditure, whereas the free form simply reflects the degree of adiposity (25,32). The present study supports this hypothesis, because the degree of adiposity explains variations in plasma total leptin, whereas the protein-bound form and the soluble leptin receptor were similarly reduced in all pGDM after correction for BF.

In conclusion, IMCL-T as measured with 1H NMR spectroscopy reflects insulin sensitivity and glucose homeostasis, whereas IMCL-S predominantly relates to the degree of obesity in women with pGDM. Increased IMCL particularly identifies those women who are markedly insulin-resistant and/or require insulin during pregnancy and who receive a diagnosis earlier in the course of pregnancy. Thus, higher IMCL relate to classical risk factors for type 2 diabetes in this cohort of young women and could be added to features of the metabolic syndrome and serve as an additional marker of risk for later type 2 diabetes in women with pGDM.

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