Body and Liver Fat Mass Rather Than Muscle Mitochondrial Function Determines Glucose Metabolism in Women with a History of Gestational Diabetes

Thomas Prikoszovich, MD\textsuperscript{1}, Christine Winzer, MD\textsuperscript{1}, Albrecht Ingo Schmid, PhD\textsuperscript{2,3}, Julia Szendroedi, MD, PhD\textsuperscript{2,4}, Marek Chmelik, PhD\textsuperscript{2,3}, Giovanni Pacini, DSc\textsuperscript{5}, Martin Krššák, PhD\textsuperscript{1,3,6}, Ewald Moser, PhD\textsuperscript{3}, Thoru Funahashi, MD, PhD\textsuperscript{7}, Werner Waldhäusl, MD\textsuperscript{1}, Alexandra Kautzky-Willer, MD\textsuperscript{1}, Michael Roden, MD\textsuperscript{1,2,4}

\textsuperscript{1}Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria, \textsuperscript{2}Karl-Landsteiner Institute of Endocrinology and Metabolism, Vienna, Austria, \textsuperscript{3}High-Field Magnetic Resonance Centre of Excellence, Medical University of Vienna, Vienna, Austria, \textsuperscript{4}Institute for Clinical Diabetology, German Diabetes Center (Leibniz Center for Diabetes Research) and Department of Metabolic Diseases, Heinrich-Heine University Düsseldorf, Germany, \textsuperscript{5}Metabolic Unit, Institute of Biomedical Engineering, National Research Council, Padova, Italy, \textsuperscript{6}Department of Radiology, Medical University of Vienna, Austria, \textsuperscript{7}Department of Metabolic Medicine, University Graduate School of Medicine, Osaka, Japan

Short title: ATP Synthesis in Gestational Diabetes

Corresponding Author:
Michael Roden, MD
Email: michael.roden@ddz.uni-duesseldorf.de

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**Objective:** Ectopic lipid storage in muscle (IMCL) and liver (HCL) coexists with impaired myocellular flux through ATP synthase (fATPase) in certain cohorts with increased risk of type 2 diabetes. As women with a history of gestational diabetes (pGDM) have elevated ectopic lipids and diabetes risk, we tested whether deteriorated energy metabolism contributes to these abnormalities.

**Research Design and Methods:** 23 glucose tolerant nonobese pGDM and 8 women with normal glucose metabolism during pregnancy with similar age, body mass and physical activity underwent oral (OGIS) and intravenous glucose tolerance tests at 4-5 years after delivery. OGIS values less than 463 ml.min$^{-1}$.m$^{-2}$ were considered to indicate insulin resistance. pGDM were further stratified into insulin resistant and sensitive groups (pGDM-IR, pGDM-IS). IMCL, HCL and fATPase were measured with $^1$H/$^{31}$P magnetic resonance spectroscopy.

**Results:** pGDM had 36% higher fat mass and 12% lower insulin-sensitivity. Log-transformed fATPase was lower in pGDM (10.6±3.8 vs. 12.1±1.4 µmol.ml muscle$^{-1}$.min$^{-1}$, p<0.03) and related to plasma adiponectin after adjustment for body fat (r=0.44, p<0.04). IMCL was 61% and 69% higher in pGDM-IR (p<0.05 vs. pGDM-IS) and in insulin resistant (p<0.003 vs. insulin sensitive) women. HCL was doubled (p<0.05) in pGDM and insulin resistant women, correlated positively with body fat mass (r=0.50, p<0.01) and inversely with insulin sensitivity (r=-0.46, p<0.05).

**Conclusions:** Glucose tolerant pGDM show increased liver fat but only slightly lower muscular insulin sensitivity and ATP synthesis. This suggests that alteration of hepatic lipid storage represents an early and predominant abnormality in this cohort.

Insulin resistance tightly relates to abdominal obesity and ectopic fat deposition in skeletal muscle (intramyocellular lipids, IMCL) and liver (hepatocellular lipids, HCL) (1). Obesity-associated exposure of both tissues to elevated free fatty acids (FFA) (1) and/or impaired secretion of the adipocytokine, adiponectin, which regulates lipid metabolism and insulin action (2), contribute to insulin resistance. Recently, impaired myocellular mitochondrial fitness (1) has also been linked to insulin resistance.

In skeletal muscle, elevation of IMCL associates with diminished maximal oxidative capacity (3). Insulin resistant humans with type 2 diabetes and their first-degree relatives can present with impaired flux through myocellular ATP synthase (fATPase), the final step of mitochondrial oxidative phosphorylation (4-6). Women with a history of gestational diabetes (pGDM) are also frequently insulin resistant and more obese than women after normoglycemic pregnancy. Their markedly higher diabetes risk renders them as a suitable model of early metabolic alterations preceding type 2 diabetes (7). The strongest predictors for an early onset of diabetes include insulin requirement and early diagnosis of GDM during pregnancy and maternal body mass index (BMI) (7).

Increased IMCL identifies women who are more insulin resistant, earlier diagnosed and prone to require insulin rather than diet only during pregnancy (8). Likewise, elevated HCL correlate tightly with insulin resistance in pGDM (9) who also exhibit lower plasma
adiponectin, another predictor of glycemic deterioration (10). However, the role of myocellular energy metabolism for insulin resistance and accumulation of IMCL and HCL in this cohort is unknown. Here, we tested the primary hypothesis that pGDM exhibit lower myocellular fATPase than women with normal glucose tolerance during pregnancy. We further hypothesized that fATPase correlates inversely with IMCL and insulin resistance and that insulin resistant pGDM have higher HCL and/or lower plasma adiponectin.

RESEARCH DESIGN AND METHODS
Volunteers. Twenty-three pGDM and eight women without any risk factors for type 2 diabetes serving as controls (CON) were recruited from the outpatient service of the Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna. GDM was diagnosed according to the criteria of the 4th Workshop Conference of Gestational Diabetes. The women had neither any disease nor were taking medications. During their pregnancies they were continuously seen in the outpatient service so that all data on diagnosis and treatment have been recorded and validated. 15 pGDM had been on insulin-therapy (IT) and 8 on diet-treatment only (DT). They were instructed to ingest an isocaloric diet (carbohydrate/protein/fat: 60/20/20%) and refrain from any physical exercise during the three days preceding the examinations. Metabolic tests were performed on different days during the first phase (days 5-8) of the menstrual cycle after 10-12-hours overnight fasting. All participants gave written informed consent to the protocol which had been approved by the institutional ethics board of the Medical University of Vienna.

Oral glucose tolerance test (OGTT). A solution containing 75 grams of glucose was ingested within 2 minutes and venous blood samples were collected for measurements of glucose and hormones. During fasting, insulin sensitivity was assessed with QUICKI=1/[log(fasting-glucose)+log(fasting-insulin)] which mostly indicates hepatic insulin sensitivity (11). Under dynamic conditions, the Oral Glucose Insulin Sensitivity (OGIS) index (12) was used, which quantifies glucose clearance per unit change of insulin and has been validated against the glucose clamp (12-13) and in terms of reproducibility and variability (14). The OGIS correlates more closely with total glucose disposal than with hepatic insulin sensitivity in a study directly comparing the glucose clamp with the OGTT (11). Thus, we used OGIS as a measure of whole body insulin sensitivity during glucose ingestion. Insulin delivered into peripheral circulation was assessed with the total area under the insulin concentration curve ($\text{AUC}_{\text{INS}}$), calculated with the trapezoidal rule. An integrated index of beta-cell function (adaptation index), as its ability to compensate insulin resistance by increasing insulin release, was calculated as $\text{OGIS} \times \Delta \text{AUC}_{\text{CP}}$, where $\Delta \text{AUC}_{\text{CP}}$ is the suprabasal area under the C-peptide concentration curve. In addition, the frequent sampling intravenous glucose tolerance test was performed and analyzed for assessing $S_1$ as a measure of insulin sensitivity and DI, the disposition index=$S_1$ times $\Delta \text{AIR}_{\text{GLUC}}$ as a measure of combined effects of insulin secretion and sensitivity on glucose disposal as described before (15).

In vivo magnetic resonance spectroscopy (MRS). Measurements were performed in participants lying supine inside a 3-T whole body spectrometer (Bruker Biospin, Ettlingen, Germany). $^{31}$P MRS. A 10-cm circular double-resonant surface coil was positioned over the medial head of the right gastrocnemius muscle. Intramyocellular concentrations of inorganic phosphate, ATP as well as rate constant (kATP) and unidirectional fATPase, employing the
saturation transfer experiment, were measured as described (4; 16). \( ^1H \) MRS. A 10-cm circular double resonant surface coil was applied above the right soleus muscle to measure IMCL from localized \( ^1H \) spectra using the series stimulated echo acquisition mode (STEAM) sequence within a volume of interest (VOI) of 1.73 cm\(^3\) (5). HCL was quantified using STEAM within a VOI of 27 cm\(^3\) (5).

**Body fat mass (BFM) and resting energy expenditure (REE).** BFM was assessed from bioimpedance analysis (Akern-RJL Systems, Florence, Italy). Prediction errors of body composition equations estimating percent fat free mass (FFM) are based on empirically derived measurement errors associated with the reference method, hydrodensitometry. REE was assessed from indirect calorimetry as described before (8).

**Laboratory tests.** Plasma glucose was measured by the hexokinase method (Hitachi Ltd., Tokyo, Japan), HbA1c by high performance liquid chromatography (VARIANT Hemoglobin Testing System, Hercules, USA) and ultrasensitive C-reactive protein (usCRP) by particle-enhanced immunonephelometry (N High-Sensitivity-CRP Reagent, BN Systems; Dade Behring, Deerfield, IL). HDL- and LDL-cholesterol were quantified using standard laboratory procedures. Fasting plasma adiponectin was measured in duplicate using an enzyme-linked immunosorbent assay system (Otsuka Pharmaceutical Co. Ltd.) with human recombinant adiponectin as a standard.

**Calculations and statistics.** Data were compared between pGDM and CON. Relationships between fATPase, IMCL, HCL, insulin sensitivity, metabolic and inflammatory parameters were further analyzed in pGDM and CON, insulin sensitive (IS, n=15), insulin resistant (IR, n=16) subjects, insulin-sensitive (pGDM-IS, n=9) and insulin-resistant pGDM (pGDM-IR, n=14). The cut-off value (462.8 ml·min\(^{-1}\)·m\(^{-2}\)) for insulin resistance was derived from OGIS values in control women of this and other studies. The lowest quantile of the distribution gave the value defined as cut-off point between normal and impaired (lower) OGIS. The identical approach has been already applied in previous studies (8; 10). Further, all women and all pGDM were divided into three groups according to OGIS tertiles.

Statistical analyses were performed using SAS\textsuperscript{®} Software 9.1.3 (Cary, NC, USA). Data are presented as means±standard deviation (SD) in text and tables or Box-and-Whisker plots in figures. Data exhibiting skewed distribution were log-transformed before statistical analysis. Comparisons between groups were performed using analysis of variance (ANOVA) for metric parameters. For post hoc testing, the Tukey was applied and the Wilcoxon rank sum test for non-parametric parameters. Pearson correlations coefficients were computed for normally distributed data, Spearman correlation coefficients in case of non-normal distribution. Bivariate correlation analyses were performed by applying partial correlations adjusting for BFM. P values <0.05 were considered to indicate significant differences.

**RESULTS**

**Study population.** All women had normal glucose tolerance based on the 75-g OGTT and comparable habitual physical activities according to Baecke’s questionnaire (Table 1). pGDM had greater BFM and waist circumference than CON (Table 1). This also held true for pGDM-IR, but not for pGDM-IS. Adjustment for BFM abolished the difference of HDL-cholesterol between pGDM and CON.

**Insulin sensitivity and secretion.** pGDM had marginally higher post-load glycemia (Table 2) and lower OGIS than CON (Fig. 1B). Within pGDM, OGIS was 21% lower (p<0.007) in the insulin resistant than in the
insulin sensitive subgroup. After adjustment for BFM, the difference in OGIS between pGDM-IR and pGDM-IS remained (adjusted means: 408 ml.min⁻¹.m⁻² vs. 513 ml.min⁻¹.m⁻²; p<0.0001) and tended to be lower even in pGDM-IS compared to CON (adjusted means: 412 ml.min⁻¹.m⁻² vs. 514 ml.min⁻¹.m⁻²; p=0.06). The intergroup difference for post-load glycemia disappeared. Insulin delivery (AUCINS) was higher in pGDM-IR compared to pGDM-IS (Table 2). The adaptation index was not different between pGDM and CON reflecting normal glucose tolerance due to adequate beta-cell compensation for a minor degree of insulin resistance in pGDM (data not shown). Fasting c-peptide reflecting basal insulin secretion rates and AUCGLUC were higher in pGDM-IR than in pGDM-IS (p<0.05 and p=0.0007; Table 2), although these differences disappeared after adjustment for BFM.

**Myocellular energy metabolism.** pGDM had 12% lower fATPase than CON (Fig. 1A) only upon log-transformation of the data; (p<0.03), which had to be performed because of outliers. Adjusting for BFM diminished the difference (p=0.37). Interestingly, fATPase did not differ between the IR- and IS-subgroups of pGDM and the total population (p=0.30; Fig. 1C). Further, fATPase did not differ between lowest and highest OGIS tertiles, both in all women (p>0.2) and in pGDM alone (p>0.4). kATP was neither different between pGDM and CON (0.06±0.02 1/s vs. 0.07±0.01 1/s; p=0.1) nor between subgroups (pGDM-IR vs. pGDM-IS: 0.06±0.02 1/s vs. 0.06±0.02 1/s; p=0.6). fATPase was also comparable between pGDM with and without insulin therapy during their pregnancies.

**Ectopic lipids.** IMCL was not different between pGDM and CON (Fig. 2A) but 61% higher in pGDM-IR than in pGDM-IS (p<0.05). Among all women, IR had 69% higher IMCL than IS (p<0.007; Fig. 2C). pGDM, who had been on insulin during their pregnancies, had higher IMCL than those on diet (IT vs. DT: 0.86±0.29% vs. 0.51±0.25%; p=0.01).

pGDM had 2.5fold higher HCL (p<0.05) than CON (Fig. 2B). Likewise, HCL were twice as high in IR than in IS (p<0.05; Fig. 2D), but not different among pGDM-subgroups. Adjusting for BFM diminished these differences. HCL were not different between pGDM with or without insulin therapy during pregnancy (DT vs. IT: HCL 4.8±5.0% vs. 3.1±2.4%)

**Correlation analyses for fATPase and HCL.** Partial correlation analysis, controlled for BFM, revealed a relationship between fATPase and plasma adiponectin across all women (p<0.04, r=0.44). fATPase did not relate to measures of insulin sensitivity (OGIS: r²=0.002, S_I: r²=0.07) or glucose tolerance (AUCGLUC: r²=0.005) in any subgroup. However, the disposition index related to HCL (r=0.59, p=0.002) and fATPase (r=0.37, p=0.04) in all women. Fasting plasma FFA did not relate to fATPase, whereas 2-hour post-load plasma FFA related to HCL (r=0.59, p=0.01) and insulin sensitivity as assessed from OGIS (r=0.44, p=0.04) or S_I (r=-0.42, p=0.04) in pGDM.

HCL related to insulin sensitivity (r=-0.47, p<0.02) across all women and to IMCL (r=-0.59, p=0.05) and HbA1c across IR (r=-0.64, p<0.03). After adjustment for BFM, HCL still related to HbA1c (r=0.57, p<0.02) in all women. Within pGDM, the correlation of HCL with hip circumference (r=0.59, p=0.04) and HbA1c (r=0.62, p=0.03) remained after adjustment for BFM.

**CONCLUSIONS**
We found that myocellular fATPase is lower in pGDM than in controls, neither relates to insulin sensitivity nor to IMCL, but correlates positively with plasma adiponectin across all women suggesting a yet unknown relationship between energy metabolism and adipocyte
function. HCL were greater in pGDM and HCL and postload FFA negatively related to insulin sensitivity.

Our pGDM featured slightly greater fat mass, post-load glycemia and insulin resistance than CON. Except for HDL-cholesterol, they did not differ from controls in other variables including physical activity and REE. This held true even for pGDM-IR suggesting that this cohort is at lower risk for type 2 diabetes than other mostly obese and glucose intolerant pGDM but already exhibits distinct metabolic features.

We found moderately lower myocellular fATPase in pGDM compared with CON which was significant only upon log-transformation. Our cohort was carefully matched to CON, except for insulin sensitivity, and probably bears a low risk for rapid progression to diabetes. The observed small differences could also be due to this specific cohort maintaining normal glucose tolerance for up to 4 years post partum. The difference in fATPase between pGDM and CON, however, vanished upon correction for BFM and was not found when comparing pGDM-IR with pGDM-IS. Although one might speculate that the reduction of fATPase resulted from greater body fat content as reported for obese insulin resistant as well as overweight type 2 diabetes cohorts (17), our data do not allow to draw such conclusion. Basal fATPase, the final step of mitochondrial oxidative phosphorylation, is impaired only in some cohorts presenting with severe insulin resistance and elevated IMCL such as elderly and lean relatives of patients with type 2 diabetes (4). The overall higher insulin sensitivity compared with other populations at risk of or having overt type 2 diabetes (5-6; 18) suggests that muscle metabolism was almost normal in our pGDM.

The finding that IMCL were not different between pGDM and CON but ~69% higher in IR than IS, underlines the contention that IMCL generally correlate with insulin resistance (8; 19) and can be elevated in patients with type 2 diabetes and their insulin resistant first-degree relatives (4). Further, the pGDM of our previous study exhibited higher IMCL along with greater insulin resistance and BFM (8). Of note, baseline fATPase is not necessarily different in patients with overt type 2 diabetes (5-6) indicating that mitochondrial dysfunction is not a uniform feature of type 2 diabetes (5). The increase in IMCL not only relates to diminished mitochondrial capacity for oxidative phosphorylation but also to lipid availability. Plasma FFA elevation decreases insulin-stimulated fATPase and insulin sensitivity prior to changes in IMCL (16). Of note, post-challenge plasma FFA were not different between pGDM and CON and only slightly higher in IR-pGDM than in IS-pGDM. The increase in insulin secretion further argues against mitochondrial dysfunction at the level of the beta-cell which has been observed in GDM and some pGDM (20).

Despite the only discrete alterations in muscular metabolism, liver fat content was more than twice in all pGDM and even trice in IR-pGDM compared with CON being still below the detection limits of clinical routine ultrasound. Higher HCL have been reported in obese pGDM (9) and type 2 diabetes, in whom HCL tightly correlates with whole body and hepatic insulin resistance (5). Steatosis even predicts the development of insulin resistance, type 2 diabetes and cardiovascular endpoints (1; 21). Of note, similar to skeletal muscle, impaired mitochondrial function has been recently detected in liver of patients with type 2 diabetes (1; 22). It has been proposed that primary muscular insulin resistance promotes shifting of ingested carbohydrates away from skeletal muscle to hepatic de-novo lipogenesis, thereby raising HCL independently of obesity (18). But alternatively, steatosis and/or impaired hepatic mitochondrial function could lead to
muscular insulin resistance (1; 22). We cannot address this issue directly, because liver biopsies are not permitted in healthy humans and non-invasive assessment with in vivo $^{31}$P MRS was not available.

In pGDM, the 2-hour post glucose load plasma FFA further correlated positively with HCL and negatively with whole-body insulin sensitivity. Even after correction for BFM, pGDM and their IR-subgroup also exhibited positive relationships of HCL with hip-circumference and HbA1c. Of note, despite the greater BFM none of the anthropometric parameters reached the cut-off values of the metabolic syndrome. From this it appears that impaired insulin action in these pGDM is primarily located at the level of the liver and already present in the glucose tolerant state.

Steatosis may not only results from abnormal fat metabolism but also from disturbed cytokine release. Of note, pGDM exhibit higher HCL and lower plasma adiponectin which is a sensitive predictor for future deterioration of glucose metabolism (10). Adiponectin has been linked to improved muscular and hepatic insulin sensitivity, which might result from anti-inflammatory and anti-atherogenic activity, decreased triglyceride synthesis and stimulated $\beta$-oxidation (2). Adiponectin may also increase muscular mitochondrial number and function, and exert antidiabetic effects (23). Supporting this contention, this paper showed that plasma adiponectin relates positively to fATPase in all women after adjustment for BFM. Moreover, prevention of the development of type 2 diabetes in pGDM by thiazolidinediones (24-25) could be mediated by increased plasma adiponectin with subsequent reduction of HCL and hepatic as well as peripheral insulin resistance. In addition, primary abnormal hepatic mitochondrial function could lead to steatosis, as patients with type 2 diabetes have reduced hepatocellular ATP levels which correlate with hepatic insulin sensitivity and HCl (22). The limitations of the study are the small sample size and the specific selection of pGDM which restricts the extrapolation of the results to all pGDM. On the other hand, the selected group underwent intensive phenotyping including tissue-specific metabolic assessment.

In summary, glucose tolerant nonobese pGDM do not show major alterations of muscle glucose and energy metabolism, but already exhibit a subclinical rise of liver fat content suggesting early abnormalities or adaptations of hepatic metabolism.

Author contribution. T.P. researched data, wrote manuscript, contributed to discussion. C.W. researched data, wrote manuscript, contributed to discussion. A.S. researched data. J.S. contributed to discussion and reviewed manuscript. M.C. researched data, contributed to discussion. G.P. performed insulin sensitivity calculations and contributed to discussion. M.K. researched data, contributed to discussion. E.M. contributed to discussion. T.F. researched data. W.W. contributed to discussion. A.K.-W. contributed to discussion. M.R. planned study, wrote manuscript, contributed to discussion and reviewed/edited manuscript.

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REFERENCES
Table 1. Clinical characteristics and metabolic parameters (means±SD) of women with a history of gestational diabetes (pGDM) and their insulin resistant (pGDM-IR) and insulin sensitive (pGDM-IS) subgroups compared with controls (CON). IR was defined by means of OGIS < 462.8 ml.min⁻¹.m⁻².

<table>
<thead>
<tr>
<th></th>
<th>pGDM</th>
<th>pGDM-IR</th>
<th>pGDM-IS</th>
<th>CON</th>
<th>p value</th>
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<tbody>
<tr>
<td>N</td>
<td>23</td>
<td>14</td>
<td>9</td>
<td>8</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>37±5</td>
<td>37±5.9</td>
<td>39±3</td>
<td>35±4</td>
<td>*n.s.</td>
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<tr>
<td>Time after delivery (m)</td>
<td>57±11</td>
<td>56±14</td>
<td>59±6</td>
<td>45±15</td>
<td>*0.02</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>25.5±3.6</td>
<td>26.5±3.0</td>
<td>24.2±4.1</td>
<td>25.0±2.9</td>
<td>*n.s.</td>
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<tr>
<td>Body fat mass (kg)</td>
<td>24.5±6</td>
<td>26.1±5.1</td>
<td>22.2±6.8</td>
<td>18.0±3.3</td>
<td>*&lt;0.03</td>
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<td>Waist circumference (cm)</td>
<td>85.5±9.4</td>
<td>87.9±7.3</td>
<td>82.2±11.2</td>
<td>76.0±8.2</td>
<td>*0.03</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>85.2±38.6</td>
<td>94.1±42.4</td>
<td>71.3±28.7</td>
<td>97.0±37.5</td>
<td>*n.s.</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>55.6±12.5</td>
<td>52.8±13.3</td>
<td>59.9±10.4</td>
<td>64.5±10.1</td>
<td>*&lt;0.05</td>
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<td>HbA1c (%)</td>
<td>5.4±0.4</td>
<td>5.3±0.5</td>
<td>5.4±0.3</td>
<td>5.2±0.2</td>
<td>*n.s.</td>
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<td>GGT (U/l)</td>
<td>18±7</td>
<td>20±7</td>
<td>15±6</td>
<td>23±24</td>
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<td>Adiponectin (µg/ml)</td>
<td>7.9±2.5</td>
<td>7.3±2.2</td>
<td>8.9±2.7</td>
<td>9.1±2.3</td>
<td>*n.s.</td>
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<tr>
<td>usCRP (mg/dl)</td>
<td>0.19±1.67</td>
<td>0.20±0.17</td>
<td>0.17±0.18</td>
<td>0.25±0.24</td>
<td>*n.s.</td>
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<td>Physical activity score</td>
<td>2.68±0.51</td>
<td>2.58±0.5</td>
<td>2.85±0.53</td>
<td>2.75±0.12</td>
<td>*n.s.</td>
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<tr>
<td>Resting energy expenditure (kcal/24h)</td>
<td>1541±255</td>
<td>1587±250</td>
<td>1449±270</td>
<td>1440±215</td>
<td>*n.s.</td>
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</table>

*pGDM vs. CON, †pGDM-IS vs. pGDM-IR, ‡pGDM-IR vs. CON, §pGDM-IS vs. CON
Table 2. Glucose metabolism, insulin sensitivity and secretion (all expressed as means±SD) in women with a history of gestational diabetes (pGDM) and their insulin resistant (pGDM-IR) and insulin sensitive (pGDM-IS) subgroups compared with controls (CON). IR was defined by means of OGIS < 462.8 ml.min^{-1}.m^{-2}.

<table>
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<th>CON</th>
<th>p-value</th>
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<tbody>
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<td>23</td>
<td>14</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>90±9</td>
<td>92.3±11</td>
<td>86.3±3</td>
<td>84±9</td>
<td>* n.s.</td>
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<tr>
<td>Fasting insulin (µU/ml)</td>
<td>9.8±5.7</td>
<td>11.6±6.5</td>
<td>7.2±2.5</td>
<td>7.6±1.6</td>
<td>* n.s.</td>
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<tr>
<td>Fasting C-peptide (ng/ml)</td>
<td>1.89±0.98</td>
<td>2.26±1.12</td>
<td>1.37±0.42</td>
<td>2.00±0.43</td>
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<tr>
<td>QUICKI</td>
<td>0.348±0.027</td>
<td>0.337±0.024</td>
<td>0.364±0.024</td>
<td>0.357±0.018</td>
<td>* n.s.</td>
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<tr>
<td>1-h glucose (mg/dl)OGTT</td>
<td>144±42</td>
<td>155±44</td>
<td>126±32</td>
<td>106±47</td>
<td>* n.s.</td>
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<tr>
<td>2-h glucose (mg/dl)OGTT</td>
<td>109±32</td>
<td>117±39</td>
<td>98±12</td>
<td>85±22</td>
<td>* 0.05</td>
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<td>Fasting FFA (µmol/l)OGTT</td>
<td>551±206</td>
<td>522±150</td>
<td>597±277</td>
<td>659±256</td>
<td>* n.s.</td>
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<tr>
<td>2-h FFA (mmol/l)OGTT</td>
<td>33±18</td>
<td>39±19</td>
<td>23±12</td>
<td>42±25</td>
<td>* n.s.</td>
</tr>
<tr>
<td>AUCGLUC (mol/l min)</td>
<td>1.21±0.13</td>
<td>1.30±0.08</td>
<td>1.10±0.07</td>
<td>1.07±0.16</td>
<td>* n.s.</td>
</tr>
<tr>
<td>AUCINS (nmol/l min)</td>
<td>61.75±41.0</td>
<td>74.76±47.8</td>
<td>41.52±12.4</td>
<td>50.39±41.6</td>
<td>* n.s.</td>
</tr>
</tbody>
</table>

*pGDM vs. CON, † pGDM-IR vs. pGDM-IS, ‡ pGDM-IR vs. CON, § pGDM-IS vs. CON

Fasting FFA: basal free fatty acid; 2h-FFA: free fatty acid at 120 minutes; QUICKI: quantitative insulin-sensitivity check index; AUCGLUC: area under the glucose concentration curve; AUCINS: area under the insulin concentration curve.
FIGURE LEGENDS

Figure 1. Myocellular flux through ATP synthase and insulin-sensitivity (OGIS).
(A, C) Myocellular flux through ATP synthase (fATPase) and (B, D) insulin-sensitivity as assessed from the oral glucose tolerance test (OGIS) in women with a history of gestational diabetes (pGDM) compared with controls (CON) (A and B) and in insulin-resistant (IR) and insulin-sensitive (IS) subgroups of the total cohort according to their insulin-sensitivity (OGIS) (C and D). Data are presented as Box-and-Whisker plots. Boxes delineate lower and upper quartile, Whiskers represent minima and maxima, medians are indicated by solid line within boxes, small circles/asterisks represent experimental outliers.

fATPase: pGDM vs. CON (10.6±3.8 vs. 12.1±1.4 µmol.mL muscle⁻¹.min⁻¹; p<0.12, ** after log transformation: p<0.03),
OGIS (glucose clearance): pGDM vs. CON (447±67 vs. 508±87 mL.min⁻¹.m⁻²; p<0.05),
fATPase: IR vs. IS (10.7±3.7 vs. 11.4±3.0 µmol.mL muscle⁻¹.min⁻¹; p=0.6 ns),
OGIS (glucose clearance): IR vs. IS (402±38 vs. 528±46 mL.min⁻¹.m⁻²; p<0.0001)

Figure 2. Intramyocellular and hepatocellular lipids.
Ectopic lipids in skeletal muscle (IMCL; A and C) and in liver (HCL; B and D) of women with a history of gestational diabetes (pGDM) compared with controls (CON) (A and B) and in insulin-resistant (IR) and insulin-sensitive (IS) subgroups of the total cohort according to their insulin-sensitivity (OGIS) (C and D). Data presented as Box-and-Whisker plots. Boxes delineate lower and upper quartile, Whiskers represent minima and maxima respectively, medians are indicated by solid line within boxes, small circles/asterisks represent experimental outliers.

IMCL: pGDM vs. CON (0.73±0.32 vs. 0.69±0.5 %H₂O; p=0.08 ns)
HCL: pGDM vs. CON (3.7±3.5 vs. 1.5±0.9 %signal; p<0.05)
IMCL: IR vs. IS (0.90±0.3 vs. 0.54±0.32 %H₂O; p=<0.003)
HCL: IR vs. IS (4.0±3.3 vs. 2.0±1.8 %signal; p<0.05)
Figure 1

A) fATPase

B) OGIS

C) fATPase

D) OGIS

ATP Synthesis in Gestational Diabetes
Figure 2

A

IMCL

% of H2O intensity

pGDM CON

B

HCL

% signal

pGDM CON

C

IMCL

% of H2O intensity

IR IS

D

HCL

% signal

IR IS