

Body and Liver Fat Mass Rather Than Muscle Mitochondrial Function Determine Glucose Metabolism in Women With a History of Gestational Diabetes Mellitus

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

RESEARCH DESIGN AND METHODS—A total of 23 glucose-tolerant nonobese pGDM and eight women with normal glucose metabolism during pregnancy with similar age, body mass, and physical activity underwent oral glucose tolerance tests (OGTT) and intravenous glucose tolerance tests at 4–5 years after delivery. OGTT values $<463 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ were considered to indicate insulin resistance. pGDM were further stratified into insulin-resistant (pGDM-IR) and insulin-sensitive (pGDM-IS) groups. IMCL, HCL, and fATPase were measured with $^1\text{H}/^{31}\text{P}$ magnetic resonance spectroscopy.

RESULTS—pGDM had 36% higher fat mass and 12% lower insulin sensitivity. Log-transformed fATPase was lower in pGDM ($10.6 \pm 3.8 \mu\text{mol} \cdot \text{mL muscle}^{-1} \cdot \text{min}^{-1}$ vs. $12.1 \pm 1.4 \mu\text{mol} \cdot \text{mL muscle}^{-1} \cdot \text{min}^{-1}$, $P < 0.03$) and related to plasma adiponectin after adjustment for body fat ($r = 0.44$, $P < 0.04$). IMCL were 61% and 69% higher in pGDM-IR ($P < 0.05$ vs. pGDM-IS) and insulin resistant women ($P < 0.003$ vs. insulin sensitive), respectively. HCL were doubled ($P < 0.05$) in pGDM and insulin resistant women, and 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.

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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 (FFAs) (1) or impaired secretion of the adipocytokine adiponectin, which regulates lipid metabolism and insulin action (2), contributes to insulin resistance. Impaired myocellular mitochondrial fitness (1) has also been linked to insulin resistance.

In skeletal muscle, elevation of IMCL is associated 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 mellitus (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 BMI (7).

Increased IMCL identifies women who are more insulin resistant, diagnosed earlier, 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.

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 Fourth Workshop Conference of Gestational Diabetes. The women had no disease and were not taking medications. During their pregnancies, the women were continuously seen in the outpatient service so that all data on diagnosis and treatment were recorded and validated. Fifteen pGDM had been on insulin therapy (IT), and eight pGDM had been on diet treatment (DT) only. They were instructed to ingest an isocaloric diet (carbohydrate/protein/fat: 60/20/20%) and refrain from any physical exercise during the 3 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-h 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

A solution containing 75 g of glucose was ingested within 2 min, and venous blood samples were collected for measurements of glucose and hormones. During fasting, insulin sensitivity was assessed with the quantitative insulin-sensitivity check index (QUICKI) = $1/[\log(\text{fasting-glucose}) + \log(\text{fasting-insulin})]$, 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 oral glucose tolerance test (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 (AUC_{INS}), calculated with the trapezoidal rule. An integrated index of β -cell function (adaptation index), as its ability to compensate insulin resistance by increasing insulin release, was calculated as $OGIS \times \Delta AUC_{CP}$, where ΔAUC_{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_I as a measure of insulin sensitivity and disposition index, $DI = S_I \times \Delta AIR_{GLUC}$ as a measure of combined effects of insulin secretion and sensitivity on glucose disposal as described previously (15).

In vivo magnetic resonance spectroscopy

Measurements were performed in participants lying supine inside a 3-T whole-body spectrometer (Bruker Biospin, Ettlingen, Germany). 1) ^{31}P MRS (magnetic resonance spectroscopy): A 10-cm circular double-resonant surface coil was positioned over the medial head of the right gastrocnemius muscle. Intramyocellular concentrations of inorganic phosphate and ATP, as well as rate constant (kATP) and unidirectional fATPase, using the saturation transfer experiment, were measured as described (4,16). 2) 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 sequence within a volume of interest of 1.73 cm^3 (5). HCL were quantified using the stimulated echo acquisition mode within a volume of interest of 27 cm^3 (5).

Body fat mass and resting energy expenditure

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

Laboratory tests

Plasma glucose was measured by the hexokinase method (Hitachi Ltd., Tokyo, Japan), HbA_{1c} (A1C) was measured by high-performance liquid chromatography

(VARIANT Hemoglobin Testing System, Hercules, CA), and ultrasensitive C-reactive protein was measured 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., Tokyo, Japan) with human recombinant adiponectin as a standard.

Calculations and statistics

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

Statistical analyses were performed using SAS Software 9.1.3 (SAS Institute, Inc., Cary, NC). Data are presented as means \pm 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 ANOVA for metric parameters. The Tukey test was applied for post hoc testing, and the Wilcoxon rank-sum test was applied for nonparametric parameters. Pearson correlation coefficients were computed for normally distributed data. Spearman correlation coefficients were computed in case of non-normal distribution. Bivariate correlation analyses were performed by applying partial correlations adjusting for BFM. $P < 0.05$ was considered statistically significant.

RESULTS

Study population

All women had normal glucose tolerance based on the 75-g OGTT and comparable habitual physical activities according to

Table 1—Clinical characteristics and metabolic parameters (means \pm SD) of women with pGDM and their insulin-resistant (pGDM-IR) and insulin-sensitive (pGDM-IS) subgroups compared with control subjects (CON)

	pGDM	pGDM-IR	pGDM-IS	CON	P			
N	23	14	9	8				
Age (years)	37 \pm 5	37 \pm 5.9	39 \pm 3	35 \pm 4	NS*	NS†	NS‡	0.06§
Time after delivery (m)	57 \pm 11	56 \pm 14	59 \pm 6	45 \pm 15	0.02*	NS†	NS‡	NS§
BMI (kg/m ²)	25.5 \pm 3.6	26.5 \pm 3.0	24.2 \pm 4.1	25.0 \pm 2.9	NS*	NS†	NS‡	NS§
BFM (kg)	24.5 \pm 6	26.1 \pm 5.1	22.2 \pm 6.8	18.0 \pm 3.3	<0.03*	NS†	<0.02‡	NS§
Waist circumference (cm)	85.5 \pm 9.4	87.9 \pm 7.3	82.2 \pm 11.2	76.0 \pm 8.2	0.03*	NS†	0.02‡	NS§
Triglycerides (mg/dL)	85.2 \pm 38.6	94.1 \pm 42.4	71.3 \pm 28.7	97.0 \pm 37.5	NS*	NS†	NS‡	NS§
HDL-cholesterol (mg/dL)	55.6 \pm 12.5	52.8 \pm 13.3	59.9 \pm 10.4	64.5 \pm 10.1	<0.05*	NS†	0.02‡	NS§
A1C (%)	5.4 \pm 0.4	5.3 \pm 0.5	5.4 \pm 0.3	5.2 \pm 0.2	NS*	NS†	NS‡	NS§
GGT (units/L)	18 \pm 7	20 \pm 7	15 \pm 6	23 \pm 24	NS*	0.03†	NS‡	NS§
Adiponectin (μ g/mL)	7.9 \pm 2.5	7.3 \pm 2.2	8.9 \pm 2.7	9.1 \pm 2.3	NS*	NS†	NS‡	NS§
us-CRP (mg/dL)	0.19 \pm 1.67	0.20 \pm 0.17	0.17 \pm 0.18	0.25 \pm 0.24	NS*	NS†	NS‡	NS§
Physical activity score	2.68 \pm 0.51	2.58 \pm 0.5	2.85 \pm 0.53	2.75 \pm 0.12	NS*	NS†	NS‡	NS§
Resting energy expenditure (kcal/24 h)	1541 \pm 255	1587 \pm 250	1449 \pm 270	1440 \pm 215	NS*	NS†	NS‡	NS§

IR was defined by means of OGIS $<462.8 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$. GGT, gamma glutamyl transferase; us-CRP, ultra-sensitive C-reactive protein. *pGDM vs. CON. †pGDM-IS vs. pGDM-IR. ‡pGDM-IR vs. CON. §pGDM-IS vs. CON.

Baekke'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 postload 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: $408 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ vs. $513 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$; $P < 0.0001$) and tended to

be lower even in pGDM-IS compared with CON (adjusted means: $412 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ vs. $514 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$; $P = 0.06$). The intergroup difference for postload glycemia disappeared. Insulin delivery (AUC_{INS}) was higher in pGDM-IR compared with pGDM-IS (Table 2). The adaptation index was not different between pGDM and CON, reflecting normal glucose tolerance due to adequate β -cell compensation for a minor degree of insulin resistance in pGDM (data not shown). Fasting C-peptide reflecting basal insulin secretion rates and AUC_{GLUC} 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 on 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 not different between pGDM and CON ($0.06 \pm 0.02 \text{ l/s}$ vs. $0.07 \pm 0.01 \text{ l/s}$; $P = 0.1$) or between subgroups (pGDM-IR vs. pGDM-IS: $0.06 \pm 0.02 \text{ l/s}$ vs. $0.06 \pm 0.02 \text{ l/s}$; $P = 0.6$). fATPase was also comparable

Table 2—Glucose metabolism, insulin sensitivity, and secretion (all expressed as means \pm SD) in women with pGDM and their insulin-resistant (pGDM-IR) and insulin-sensitive (pGDM-IS) subgroups compared with control subjects (CON)

	pGDM	pGDM-IR	pGDM-IS	CON	P			
N	23	14	9	8				
Fasting glucose (mg/dL)	90 \pm 9	92.3 \pm 11	86.3 \pm 3	84 \pm 9	NS*	NS†	NS‡	NS§
Fasting insulin (μ U/mL)	9.8 \pm 5.7	11.6 \pm 6.5	7.2 \pm 2.5	7.6 \pm 1.6	NS*	NS†		
Fasting C-peptide (ng/mL)	1.89 \pm 0.98	2.26 \pm 1.12	1.37 \pm 0.42	2.00 \pm 0.43	NS*	<0.05†		
QUICKI	0.348 \pm 0.027	0.337 \pm 0.024	0.364 \pm 0.024	0.357 \pm 0.018	NS*	<0.05†		
1-h glucose (mg/dL) _{OGTT}	144 \pm 42	155 \pm 44	126 \pm 32	106 \pm 47	NS*	NS†	0.04‡	NS§
2-h glucose (mg/dL) _{OGTT}	109 \pm 32	117 \pm 39	98 \pm 12	85 \pm 22	0.05*	NS†	NS‡	NS§
Fasting FFA (μ mol/L) _{OGTT}	551 \pm 206	522 \pm 150	597 \pm 277	659 \pm 256	NS*	NS†		
2-h FFA (mmol/L) _{OGTT}	33 \pm 18	39 \pm 19	23 \pm 12	42 \pm 25	NS*	<0.04†	NS‡	NS§
AUC_{GLUC} (mol/L min)	1.21 \pm 0.13	1.30 \pm 0.08	1.10 \pm 0.07	1.07 \pm 0.16	NS*	<0.05†		
AUC_{INS} (nmol/L min)	61.75 \pm 41.0	74.76 \pm 47.8	41.52 \pm 12.4	50.39 \pm 41.6	NS*	<0.05†		

IR was defined by means of OGIS $<462.8 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$. Fasting FFA, basal free fatty acid; 2-h FFA, free fatty acid at 120 min; QUICKI, quantitative insulin-sensitivity check index; AUC_{GLUC} , area under the glucose concentration curve; AUC_{INS} , area under the insulin concentration curve. *pGDM vs. CON. †pGDM-IR vs. pGDM-IS. ‡pGDM-IR vs. CON. §pGDM-IS vs. CON.

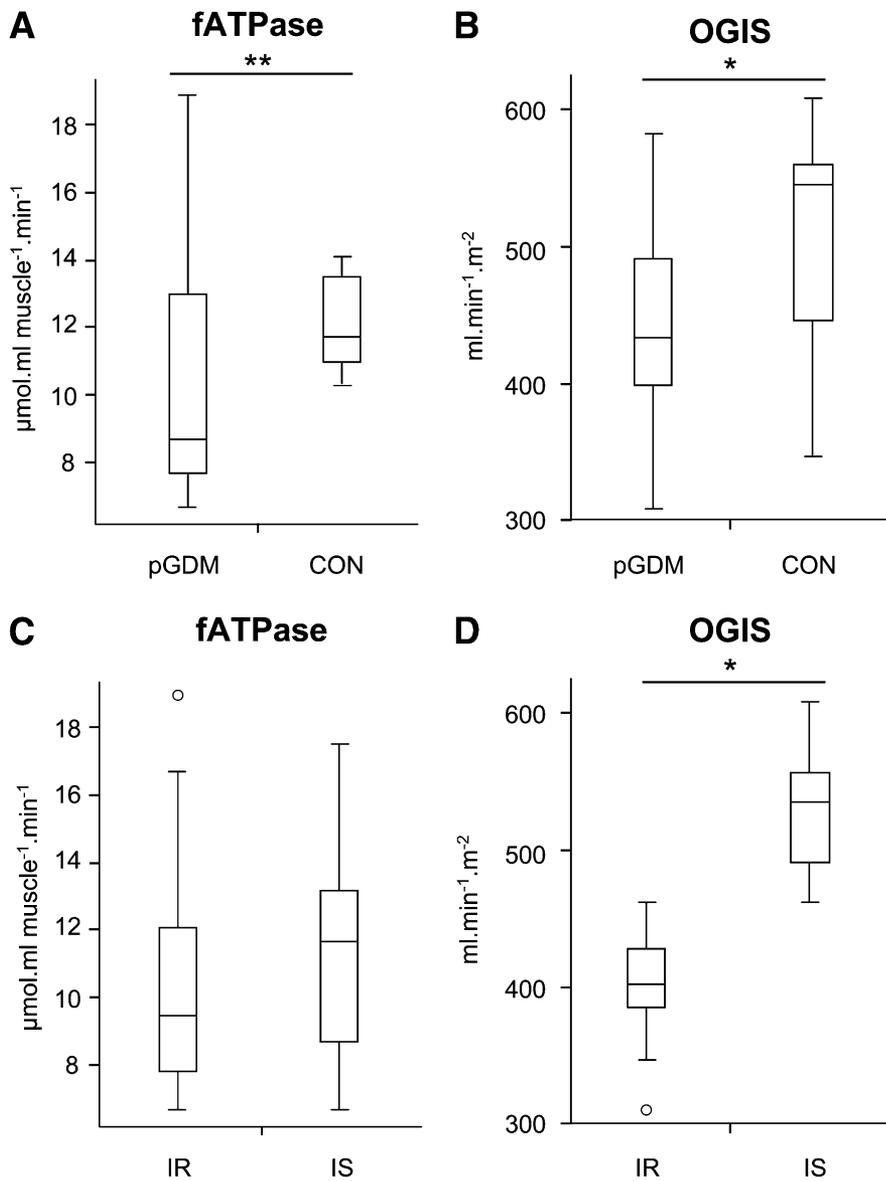


Figure 1—Myocellular fATPase (A and C) and insulin sensitivity (B and D) as assessed from the OGTT (OGIS) in pGDM compared with CON (A and B) and in IR and IS subgroups of the total cohort according to their insulin sensitivity (OGIS) (C and D). Data are presented as box-and-whisker plots. Boxes delineate lower and upper quartile, whiskers represent minima and maxima, medians are indicated by solid line within boxes, and small circles/asterisks represent experimental outliers. fATPase: pGDM vs. CON ($10.6 \pm 3.8 \mu\text{mol} \cdot \text{mL muscle}^{-1} \cdot \text{min}^{-1}$ vs. $12.1 \pm 1.4 \mu\text{mol} \cdot \text{mL muscle}^{-1} \cdot \text{min}^{-1}$; $P < 0.12$, **after log transformation: $P < 0.03$), OGIS (glucose clearance): pGDM vs. CON ($447 \pm 67 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ vs. $508 \pm 87 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$; $P < 0.05$), fATPase: IR vs. IS ($10.7 \pm 3.7 \mu\text{mol} \cdot \text{mL muscle}^{-1} \cdot \text{min}^{-1}$ vs. $11.4 \pm 3.0 \mu\text{mol} \cdot \text{mL muscle}^{-1} \cdot \text{min}^{-1}$; $P = 0.6 \text{ NS}$), OGIS (glucose clearance): IR vs. IS ($402 \pm 38 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ vs. $528 \pm 46 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$; $P < 0.0001$).

between pGDM with and without insulin therapy (IT) during their pregnancies.

Ectopic lipids

IMCL were 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 women had 69% higher IMCL than IS women ($P < 0.007$;

Fig. 2C). pGDM on IT during their pregnancies had higher IMCL than those on diet treatment (DT) (IT vs. DT: $0.86 \pm 0.29\%$ vs. $0.51 \pm 0.25\%$, $P = 0.01$).

pGDM had 2.5-fold higher HCL ($P < 0.05$) than CON (Fig. 2B). Likewise, HCL were twice as high in IR than in IS subjects ($P < 0.05$; Fig. 2D), but not different among pGDM subgroups. Adjusting for

BFM diminished these differences. HCL were not different between pGDM with or without IT during pregnancy (DT vs. IT: HCL $4.8 \pm 5.0\%$ signal vs. $3.1 \pm 2.4\%$ signal).

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: $r^2 = 0.002$, S_1 : $r^2 = 0.07$) or glucose tolerance (AUC_{GLUC} : $r^2 = 0.005$) 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-h postload 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_1 ($r = -0.42$, $P = 0.04$) 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 A1C across IR ($r = 0.64$, $P < 0.03$). After adjustment for BFM, HCL still related to A1C ($r = 0.57$, $P < 0.02$) in all women. Within pGDM, the correlation of HCL with hip circumference ($r = 0.59$, $P = 0.04$) and A1C ($r = 0.62$, $P = 0.03$) remained after adjustment for BFM.

CONCLUSIONS—We found that myocellular fATPase is lower in pGDM than in CON and does not relate to insulin sensitivity or 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 were negatively related to insulin sensitivity.

Our pGDM featured slightly greater fat mass, postload glycemia, and insulin resistance than CON. Except for HDL-cholesterol, they did not differ from CON in other variables, including physical activity and resting energy expenditure. 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 on log-transformation. Our cohort was carefully matched to CON, except for insulin sensitivity, and probably bears a low risk

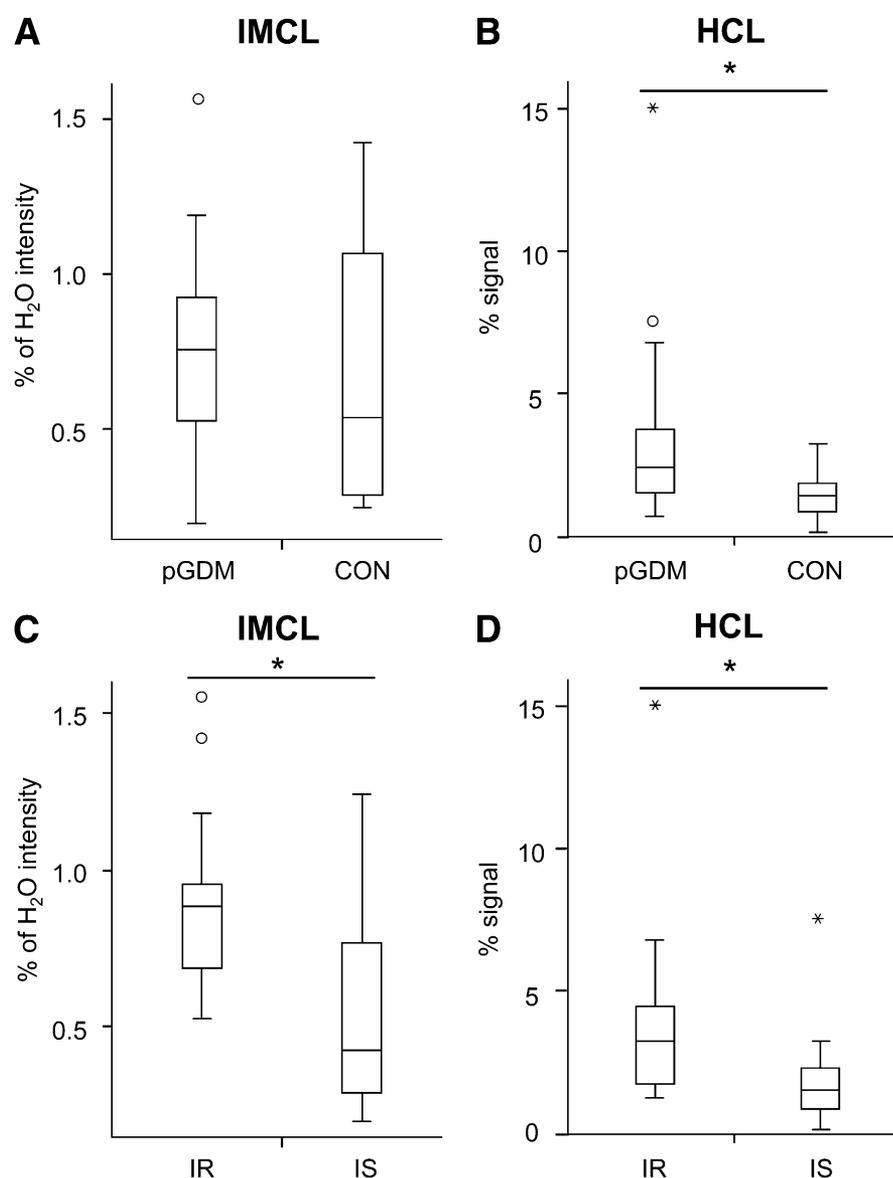


Figure 2—IMCL and HCL. Ectopic lipids in skeletal muscle (IMCL; A and C) and liver (HCL; B and D) of pGDM compared with CON (A and B) and in IR and IS subgroups of the total cohort according to their insulin sensitivity (OGIS) (C and D). Data presented as box-and-whisker plots. Boxes delineate lower and upper quartile, whiskers represent minima and maxima, respectively, medians are indicated by solid line within boxes, and small circles/asterisks represent experimental outliers. IMCL: pGDM vs. CON ($0.73 \pm 0.32\%$ H₂O vs. $0.69 \pm 0.5\%$ H₂O; $P = 0.08$ NS) HCL: pGDM vs. CON ($3.7 \pm 3.5\%$ vs. $1.5 \pm 0.9\%$ signal; $P < 0.05$) IMCL: IR vs. IS ($0.90 \pm 0.3\%$ H₂O vs. $0.54 \pm 0.32\%$ H₂O; $P < 0.003$) HCL: IR vs. IS ($4.0 \pm 3.3\%$ vs. $2.0 \pm 1.8\%$ signal; $P < 0.05$).

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 postpartum. The difference in fATPase between pGDM and CON, however, vanished on 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 and overweight type 2 diabetes cohorts (17), our data do not allow such a 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 for 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 relates to not only diminished mitochondrial capacity for oxidative phosphorylation but also lipid availability. Plasma FFA elevation decreases insulin-stimulated fATPase and insulin sensitivity before changes in IMCL (16). Of note, postchallenge plasma FFAs 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 β -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 double in all pGDM and even three times more in IR-pGDM compared with that in CON, which was still below the detection limits of clinical routine ultrasound. Higher HCL have been reported in obese pGDM (9) and type 2 diabetic patients, in whom HCL tightly correlate with whole-body and hepatic insulin resistance (5). Steatosis even predicts the development of insulin resistance, type 2 diabetes, and cardiovascular end points (1,21). Of note, similar to skeletal muscle, impaired mitochondrial function has been recently detected in livers 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 increasing HCL independently of obesity (18). 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 noninvasive assessment with in vivo ^{31}P MRS was not available.

In pGDM, the 2-h 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 A1C. Of note, despite the greater BFM, none of the anthropometric parameters reached the cutoff values of the metabolic syndrome. From this it seems 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 result not only 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 β -oxidation (2). Adiponectin may also increase muscular mitochondrial number and function, and exert antidiabetic effects (23). In support of this contention, the current study 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 and peripheral insulin resistance. In addition, primary abnormal hepatic mitochondrial function could lead to steatosis, because patients with type 2 diabetes have reduced hepatocellular ATP levels that 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 restrict 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 non-obese pGDM do not show major alterations of muscle glucose and energy metabolism, but already exhibit a subclinical increase of liver fat content suggesting early abnormalities or adaptations of hepatic metabolism.

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