Effects of high-fat overfeeding on mitochondrial function, glucose and fat metabolism, and adipokine levels in low-birth-weight subjects

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Brøns C, Jacobsen S, Hiscock N, White A, Nilsson E, Dunger D, Astrup A, Quistorff B, Vaag A. Effects of high-fat overfeeding on mitochondrial function, glucose and fat metabolism, and adipokine levels in low-birth-weight subjects. Am J Physiol Endocrinol Metab 302: E43–E51, 2012. First published September 13, 2011; doi:10.1152/ajpendo.00095.2011.—Low birth weight (LBW) is associated with an increased risk of insulin resistance and downregulation of oxidative phosphorylation (OXPHOS) genes when exposed to a metabolic challenge of high-fat overfeeding (HFO). To elaborate further on the differential effects of HFO in LBW subjects, we measured in vivo mitochondrial function, insulin secretion, hepatic glucose production, and plasma levels of key regulatory hormones before and after 5 days of HFO in 20 young LBW and 26 normal-birth-weight (NBW) men. The LBW subjects developed peripheral insulin resistance after HFO due to impaired endogenous glucose storage (9.42 ± 4.19 vs. 5.91 ± 4.42 mg·kg FFM−1·min−1, P = 0.01). Resting muscle phosphorcreatine and total ATP in muscle increased significantly after HFO in LBW subjects only, whereas additional measurements of mitochondrial function remained unaffected. Despite similar plasma FFA levels, LBW subjects displayed increased fat oxidation during insulin infusion compared with normal-birth-weight (NBW) subjects after HFO (0.37 ± 0.35 vs. 0.17 ± 0.33 mg·kg FFM−1·min−1, P = 0.02). In contrast to NBW subjects, the plasma lepentin levels of LBW subjects did not increase, and the plasma gastric inhibitory polypeptide (GIP) as well as pancreatic polypeptide (PP) levels increased less in LBW compared with NBW subjects during HFO. In conclusion, HFO unmasked dissociation between insulin resistance and mitochondrial dysfunction in LBW subjects, suggesting that insulin resistance may be a cause, rather than an effect, of impaired muscle OXPHOS gene expression and mitochondrial dysfunction. Reduced increments in response to HFO of fasting plasma leptin, PP, and GIP levels may contribute to insulin resistance, lower satiety, and impaired insulin secretion in LBW subjects.

insulin resistance; oxidative phosphorylation genes; peroxisome proliferator-activated receptor-γ coactivator-1α; incretin hormones

IMPAIRED MITOCHONDRIAL FUNCTION and expression of genes involved in oxidative phosphorylation (OXPHOS) in skeletal muscles has been proposed to play an important role in the pathogenesis of insulin resistance and type 2 diabetes (T2D) (19, 23, 29–31). However, recent investigations have reported discordance between mitochondrial function and muscle insulin sensitivity (1, 10, 38), and the extent to which muscle mitochondrial dysfunction represents a primary defect resulting in insulin resistance, or rather a secondary defect develop-
E44 EFFECTS OF HIGH-FAT OVERFEEDING IN LOW-BIRTH-WEIGHT SUBJECTS

MATERIALS AND METHODS

The data presented in this article are a part of a larger study investigating the effect of LBW and HFO on T2D pathophysiology (see study outline in the supplemental appendix). Therefore, the data on the effects of HFO in NBW subjects (5) and metabolic differences between LBW and NBW subjects prior to overfeeding (4) as well as the data on OXPHOS gene expressions (3) have been published previously but are summarized briefly here to provide the background of the current findings.

Subjects. Briefly, 46 healthy male volunteers 23–27 yr of age (24.4 ± 0.9 yr) were recruited from the Danish Medical Birth Register according to birth weight, as described previously (4). Twenty subjects had LBW (≤10th percentile, 2,717 ± 268 g) and 26 subjects were control subjects with NBW between the 50th and the 90th percentile (3,893 ± 207 g).

The study protocol was approved by the ethics committee for Copenhagen County, Denmark (KA 03129gm), and the procedures were performed according to the principles of the Declaration of Helsinki. After thorough written and oral information of the study details was given, all subjects signed an informed consent form prior to participation.

Experimental protocol. The study was designed as a randomised crossover study and identical to that reported previously in healthy control and LBW subjects (3, 5). All subjects were studied on two identical occasions following different diets and separated by a 6- to 8-wk washout period. The study activities were carried out over 3 days, and 5 days prior to each examination period all subjects were standardized with regard to alcohol consumption, physical activity, and food intake (see supplemental appendix). Furthermore, subjects were asked to remain weight stable between the two examination periods. The HFO diet was delivered to participants for 5 days, starting 3 days prior to the first examination day. Besides the standardization period, the control experiment was optimized even further by providing all foods for the 3 days before the first examination day (see supplemental appendix).

Diet. The dietary intervention was described in detail previously (5). In brief, the control diet reflected a balanced weight-maintaining diet containing 30% fat, and the overfeeding diet was high in fat (60%) and contained 50% more calories than each participant required. The energy requirement was multiplied by a factor of 1.5 corresponding to a low physical activity level during the examination days. The average amount of food was individualized according to the participant’s energy requirement determined by a World Health Organization equation for men under the age of 30 yr (8). The energy intake of each participant was assessed on day 1 by dual energy X-ray absorptiometry (DEXA; Lunar Radiation, Madison, WI).

Dual-energy X-ray absorptiometry scanning. Body composition was assessed on day 1 by dual energy X-ray absorptiometry (DEXA; Lunar Radiation, Madison, WI).

Phosphorus magnetic resonance spectroscopy. On day 2, we examined in vivo mitochondrial function and aerobic capacity with a noninvasive real-time 31P-MRS technique, using an Otsuka Electronics VivoSpec spectrometer interfaced with a 2.9 Tesla magnet (MagneX Scientific, Oxford, UK) with a 26-cm bore. 31Phosphorus magnetic resonance spectroscopy (31P-MRS) was recorded at rest and during recovery from exercise in two different muscle groups, namely the forearm flexor muscles with a high content of glycolytic type II fibers, and in the tibialis anterior of the leg with a high content of oxidative type I fibers (16, 32). Initially, maximal voluntary contrac-

tion (MVC) was determined by pulling the in-magnet ergometer two to three times using maximal force. The protocol involved 3 min of rest, 3 min of intermittent static exercise, and 6 min of recovery, where the intermittent isometric contractions were performed at 50% MVC, each lasting 7 s, interspersed by 3 s of rest. Data acquisition and calculations were performed as described in the supplemental appendix.

Hyperinsulinemic euglycemic clamp. The clamp examination was initiated at 7 AM on day 3 after an overnight fast and carried out as described previously in detail (4). A primed, continuous infusion of [3-3H]glucose (bolus 10.9 μCi, 0.109 μCi/min) was given at 0 h, and the examination was initiated by a 2-h basal period, followed by a 30-min intravenous glucose tolerance test (IVGTT). After the IVGTT, a primed, continuous insulin infusion was initiated and fixed at 80 mU·m−2·min−1 throughout the 180-min clamp. Variable infusion of glucose (180 g/l) enriched with [3-3H]glucose (110 μCi/500 ml) was used to maintain euglycemia of 5 mmol/l during insulin infusion. Indirect calorimetry with a computerized flowthrough can-opy gas analyzer system was used to measure oxygen consumption (VO2) and carbon dioxide production (VCO2) during steady state to determine substrate oxidation rates (Dateltrac; Datex, Helsinki, Finland) (39). Both steady-state periods (basal and insulin stimulated) were followed by excision of biopsies from the vastus lateralis muscle. Analytical procedures and calculations of glucose turnover and insulin secretion rates are described in the supplemental appendix.

Luminex xMAP system was used (controlled using the Bio-Plex Manager software version 5.0: Bio-Rad). Human adipokine panel A (code HADK1–61K-A; adiponectin, plasminogen activator inhibitor 1 (PAI-1), resistin) and human gut hormones [code HGT-68K; amylin, ghrelin, GIP, glucagon-like peptide-1 (GLP-1), insulin, leptin, and pancreatic polypeptide (PYY)] were used (Millipore). For each assay, supernatant samples, solutions, antibody-conjugated beads, and standards were prepared according to the manufacturers’ instructions. Ninety-six-well filter bottom plates were set up according to defined plate layouts and incubated. After an incubation period, plates were washed and analyzed using the Luminex system. For additional details, see the supplemental appendix.

Quantitative real-time PCR. The expression of OXPHOS genes shown previously to be associated with T2D (23, 29), namely PPARGC1A, NADH dehydrogenase-1 alpha subcomplex 6 (NDUFS6), ubiquinol-cytochrome c reductase subunit II (UQCRB), cytochrome c oxidase subunit VIIA polypeptide 1 (COX7A1) and ATP synthase, H+ transporting, mitochondrial F1 complex, and O subunit (ATP5O) in tissue from the m. vastus lateralis, was performed as described previously (3).

Statistics. Statistical analysis was performed with the SAS Statist-ical Analysis Package (version 9.1; SAS Institute, Cary, NC). To account for the correlation between measurements within subjects, an analysis of variance for repeated measurements was used to see whether there was an overall difference between the two groups studied at two different occasions (NBW, LBW, control, and HFO diet). Paired Student t-tests were used to detect statistically significant differences within the NBW and LBW groups in response to HFO, and unpaired Student t-tests were used to detect differences between the birth weight groups. The Kolmogorov-Smirnov test was used to check for normality. Nonnormally distributed data were analyzed using nonparametric tests, whereas normally distributed data were analyzed using a parametric tests.

Values of P < 0.05 were considered statistically significant. Data are presented as means ± SD or SE. In a post hoc power calculation, we found that with our current study design there was a >80% chance of detecting a 20% change in peripheral insulin action in response to HFO.
RESULTS

Clinical characteristics of the study participants. The LBW subjects were shorter and had a more inappropriate body fat deposition, with more fat on the trunk and less on the legs. Furthermore, they had significantly lower HDL cholesterol levels (Table 1). Both findings are in accordance with previous reports (4, 14, 36).

There were no detectable changes in weight, BMI, or body composition after 5 days of HFO in either of the groups. HFO did not affect the total cholesterol level. However, both the NBW and the LBW subjects experienced significantly reduced fasting plasma triglycerides and VLDL cholesterol as well as increased plasma HDL cholesterol levels after the HFO diet (Table 1). Reduced plasma LDL cholesterol level was observed in the NBW subjects only. The fasting plasma concentration of the liver enzyme aspartate aminotransferase was increased significantly in the NBW subjects, whereas a tendency was seen in the LBW subjects after HFO.

Baseline and insulin-stimulated whole body glucose and insulin metabolism. On the control diet (CON), the LBW subjects had significantly higher fasting blood glucose, serum insulin, and C-peptide levels compared with the NBW subjects, as shown in Table 2 and as published previously (4). During insulin stimulation, there were no significant differences between the two groups, although a trend existed for a lower glycolytic flux (GF) and a higher endogenous glucose storage (EGS) in the LBW subjects. There were no other differences on the CON diet.

In response to HFO, the LBW subjects displayed an increased insulin-stimulated GF in the face of a significantly decreased rate of EGS. Furthermore, the higher degree of peripheral insulin resistance in the LBW subjects was also reflected in an increased rate of fat oxidation during insulin infusion compared with the NBW men (Table 2).

Fasting blood glucose levels increased significantly in both groups after 5 days of HFO (Table 2) (3). The NBW subjects had increased fasting C-peptide and insulin levels during the control diet, whereas no changes were observed in the LBW subjects (Table 2). Reduced plasma LDL cholesterol level was observed in the NBW subjects only. The fasting plasma concentration of the liver enzyme aspartate aminotransferase was increased significantly in the NBW subjects, whereas a tendency was seen in the LBW subjects after HFO.

Insulin secretion and disposition indices. During the IVGTT, the LBW subjects had significantly elevated area under the curve (AUC) glucose compared with NBW subjects after HFO, whereas there were no differences in AUC insulin or in AUC C-peptide between the two study groups (Table 2).

After HFO, AUC insulin increased significantly in both groups, and AUC C-peptide increased in the NBW group only. Similarly, both groups experienced an increase in the incremental first-phase insulin response in response to HFO (Table 2). When the disposition indices (DI) of insulin secretion were calculated for the two groups, the LBW subjects compensated appropriately for both peripheral as well as hepatic insulin action (Table 2). This is in contrast to the NBW subjects, who as reported previously (5) exhibited significantly increased compensation for the degree of peripheral insulin action during HFO but appropriate compensation for degree hepatic insulin action.

Hepatic insulin resistance and hepatic glucose production. The LBW subjects had a significantly higher degree of hepatic insulin resistance than NBW subjects on the control diet (102.3 ± 12.0 vs. 68.7 ± 6.7 mg·kg FFM⁻¹·min⁻¹, P = 0.02; Fig. 1B) (4). However, when exposed to HFO the LBW subjects main-

Table 1. Clinical characteristics of NBW and LBW participants before and after HFO

<table>
<thead>
<tr>
<th></th>
<th>NBW Subjects (n = 25)</th>
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<th>LBW Subjects (n = 18)</th>
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<th>P Value (ANOVA)</th>
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<tbody>
<tr>
<td>Body composition</td>
<td></td>
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<tr>
<td>Weight, kg</td>
<td>78.4 ± 9.3</td>
<td>78.6 ± 9.7</td>
<td>77.1 ± 11.3</td>
<td>77.1 ± 11.4</td>
<td>0.83</td>
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<tr>
<td>Height, m</td>
<td>1.83 ± 0.07</td>
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<td>1.77 ± 0.05</td>
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<td>0.02</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.3 ± 2.4</td>
<td>23.3 ± 2.5</td>
<td>24.6 ± 3.8</td>
<td>24.6 ± 3.8</td>
<td>0.65</td>
</tr>
<tr>
<td>Trunk FM/total FM, g (DEXA)</td>
<td>0.50 ± 0.05</td>
<td>0.50 ± 0.04</td>
<td>0.53 ± 0.04#</td>
<td>0.53 ± 0.04#</td>
<td>0.08</td>
</tr>
<tr>
<td>Leg FM/total FM, g (DEXA)</td>
<td>0.37 ± 0.04</td>
<td>0.37 ± 0.04</td>
<td>0.34 ± 0.04##</td>
<td>0.34 ± 0.04##</td>
<td>0.05</td>
</tr>
<tr>
<td>Trunk FM/leg FM (DEXA)</td>
<td>1.09 ± 0.19</td>
<td>1.08 ± 0.16</td>
<td>1.24 ± 0.17##</td>
<td>1.23 ± 0.18#</td>
<td>0.03</td>
</tr>
<tr>
<td>Lipid profile and ASAT</td>
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<tr>
<td>Triglycerides, mmol/l</td>
<td>0.92 ± 0.35</td>
<td>0.73 ± 0.35*</td>
<td>1.07 ± 0.37</td>
<td>0.72 ± 0.24**</td>
<td>0.0002</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.36 ± 0.83</td>
<td>4.18 ± 0.82</td>
<td>4.36 ± 0.78</td>
<td>4.27 ± 0.79</td>
<td>0.37</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>2.51 ± 0.72</td>
<td>2.28 ± 0.78*</td>
<td>2.69 ± 0.76</td>
<td>2.57 ± 0.80</td>
<td>0.05</td>
</tr>
<tr>
<td>VLDL cholesterol, mmol/l</td>
<td>0.42 ± 0.16</td>
<td>0.33 ± 0.16*</td>
<td>0.49 ± 0.18</td>
<td>0.32 ± 0.12**</td>
<td>0.0004</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.40 ± 0.22</td>
<td>1.56 ± 0.25**</td>
<td>1.19 ± 0.23##</td>
<td>1.38 ± 0.28**##</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ASAT, U/l</td>
<td>22.8 ± 6.7</td>
<td>27.0 ± 7.1*</td>
<td>22.9 ± 4.8</td>
<td>27.3 ± 7.7</td>
<td>0.05</td>
</tr>
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</table>

Data are means ± SD. NBW, normal birth weight; LBW, low birth weight; CON, control; HFO, high-fat overfeeding; BMI, body mass index; FM, fat mass; DEXA, dual-energy X-ray absorptiometry; ASAT, aspartate aminotransferase. NBW vs. LBW: #P ≤ 0.05, ##P ≤ 0.01. CON vs. HFO diet: *P ≤ 0.05, **P ≤ 0.01.
Table 2. Basal and insulin-stimulated data from the clamp examinations and from the IVGTT in NBW and LBW subjects before and after HFO

<table>
<thead>
<tr>
<th></th>
<th>NBW Subjects (n = 25)</th>
<th>LBW Subjects (n = 18)</th>
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<tbody>
<tr>
<td></td>
<td>CON</td>
<td>HFO</td>
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<tr>
<td>Baseline</td>
<td></td>
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</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>4.59 ± 0.47</td>
<td>5.05 ± 0.40***</td>
</tr>
<tr>
<td>Serum insulin, pmol/l</td>
<td>30.2 ± 14.7</td>
<td>43.4 ± 29.2*</td>
</tr>
<tr>
<td>Serum C-peptide, pmol/l</td>
<td>408 ± 146</td>
<td>529 ± 260**</td>
</tr>
<tr>
<td>FFA, µmol/l</td>
<td>334 ± 136</td>
<td>205 ± 82***</td>
</tr>
<tr>
<td>Glucose oxidation, mg·kg FFM⁻¹·min⁻¹</td>
<td>2.34 ± 0.76</td>
<td>2.43 ± 0.71</td>
</tr>
<tr>
<td>Fat oxidation, mg·kg FFM⁻¹·min⁻¹</td>
<td>1.00 ± 0.38</td>
<td>1.02 ± 0.33</td>
</tr>
<tr>
<td>EGS, mg·kg FFM⁻¹·min⁻¹</td>
<td>0.87 ± 1.83</td>
<td>1.98 ± 4.14</td>
</tr>
<tr>
<td>GF, mg·kg FFM⁻¹·min⁻¹</td>
<td>1.64 ± 1.96</td>
<td>2.19 ± 4.61</td>
</tr>
<tr>
<td>Insulin-stimulated</td>
<td></td>
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<tr>
<td>Blood glucose, mmol/l</td>
<td>5.11 ± 0.31</td>
<td>5.17 ± 0.30</td>
</tr>
<tr>
<td>Serum insulin, pmol/l</td>
<td>870 ± 232</td>
<td>867 ± 181</td>
</tr>
<tr>
<td>Serum C-peptide, pmol/l</td>
<td>406 ± 279</td>
<td>453 ± 195</td>
</tr>
<tr>
<td>FFA, µmol/l</td>
<td>9.29 ± 4.39</td>
<td>12.42 ± 6.43**</td>
</tr>
<tr>
<td>Glucose oxidation, mg·kg FFM⁻¹·min⁻¹</td>
<td>5.18 ± 0.82</td>
<td>5.04 ± 0.98</td>
</tr>
<tr>
<td>Fat oxidation, mg·kg FFM⁻¹·min⁻¹</td>
<td>0.01 ± 0.25</td>
<td>0.17 ± 0.33</td>
</tr>
<tr>
<td>EGS, mg·kg FFM⁻¹·min⁻¹</td>
<td>7.68 ± 2.80</td>
<td>8.38 ± 3.10</td>
</tr>
<tr>
<td>GF, mg·kg FFM⁻¹·min⁻¹</td>
<td>4.71 ± 2.17</td>
<td>3.47 ± 2.55**</td>
</tr>
<tr>
<td>Nonoxidative GF, mg·kg FFM⁻¹·min⁻¹</td>
<td>-0.02 ± 2.57</td>
<td>-1.15 ± 2.68</td>
</tr>
<tr>
<td>NOGM, mg·kg FFM⁻¹·min⁻¹</td>
<td>7.53 ± 2.88</td>
<td>7.18 ± 2.73</td>
</tr>
<tr>
<td>IVGTT (0-10 min)</td>
<td></td>
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<tr>
<td>AUC, glucose</td>
<td>125 ± 12.7</td>
<td>131 ± 19.4</td>
</tr>
<tr>
<td>AUC, insulin</td>
<td>2,229 ± 1,529</td>
<td>3,020 ± 1,884***</td>
</tr>
<tr>
<td>AUC, C-peptide</td>
<td>11,851 ± 4,673</td>
<td>13,768 ± 5,277**</td>
</tr>
<tr>
<td>Incremental PPFR</td>
<td>1,894 ± 1,431</td>
<td>2,604 ± 1,793***</td>
</tr>
<tr>
<td>DI (peripheral insulin action)</td>
<td>0.29 ± 0.19</td>
<td>0.35 ± 0.20*</td>
</tr>
<tr>
<td>DI (hepatic insulin action)</td>
<td>0.38 ± 0.36</td>
<td>0.25 ± 0.21</td>
</tr>
</tbody>
</table>

Data are means ± SD. IVGTT, intravenous glucose tolerance test; FFA, free fatty acids; EGS, endogenous glucose storage; FFM, fat-free mass; GF, glucolytic flux; NOGM, nonoxidative glucose metabolism; AUC, area under the curve; PPFR, first-phase insulin response; DI, disposition index. NBW vs. LBW: #P = 0.05; ##P = 0.01. CON vs. HFO diet: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

Discussion

Despite the development of overt peripheral insulin resistance and impaired expression of key OXPHOS genes, in vivo mitochondrial capacity for ATP synthesis as measured in two muscle groups was not reduced in the LBW subjects when challenged with HFO (Table 3). In fact, the total content of ATP as well as PCr in the resting state actually increased significantly in the leg muscles of the LBW but not the NBW
subjects. High-fat diet-induced insulin resistance in the LBW subjects was due primarily to an impairment of endogenous glucose storage, possibly reflecting reduced muscle glycogen synthesis (Table 2). In addition, insulin resistance in the LBW subjects was associated with an increased rate of insulin-stimulated fat oxidation relative to NBW controls (Table 2). Although LBW subjects had a reduced insulin-stimulated glycolytic flux rate compared with NBW subjects during the isocaloric control diet, they responded to HFO with a paradoxical increased glycolytic flux, representing a possible compensatory mechanism to cope for the high-fat overfeeding challenge in the short term (Table 2). Finally, the LBW subjects responded to HFO with lower or no increments in the fasting plasma levels of leptin, GIP, and PP compared with controls, which may contribute to the development of peripheral insulin resistance and reduced satiety as well as potentially to a reduced increase in insulin secretion to compensate for insulin resistance among LBW subjects.

Because diets rich in fat and calories have been linked to mitochondrial dysfunction (6, 24, 37), we investigated the effect of short-term, high-fat overfeeding in young and healthy LBW subjects with a known increased risk of type 2 diabetes and found that, despite development of peripheral insulin resistance and impaired OXPHOS gene expression, in vivo muscle mitochondrial aerobic capacity for ATP synthesis was not reduced. If anything, mitochondrial function, reflected as the total content of ATP as well as PCr in resting leg muscle, was increased by HFO. The ratio of PCr/Pi in the arm appeared to be slightly lower in the LBW subjects (not statistical significant) when compared with the NBW controls after HFO. Given that the PCr/Pi ratio may be a more reliable measurement of mitochondrial function compared with the absolute areas of PCr and Pi, respectively, we cannot completely exclude the possibility that we may have overlooked a minor relative defect of mitochondrial function in the LBW subjects in response to HFO. Although we do acknowledge that some degree of mitochondrial dysfunction can be present in isolated mitochondria and that there can be regional anatomic differences in function, as suggested by Rabøl et al. (34), the demonstration of normal values of maximal capacity for aerobic ATP production (Vmax) represents clear evidence for preserved function of the muscle mitochondria at a whole body level in the LBW subjects during overfeeding. Defective mitochondrial function in isolated preparations studied in vitro, as indicated also by the impaired OXPHOS genes expression levels, could even be overcompensated in the intact muscle functioning in vivo by elevated substrate levels and altered metabolic hormones. However, our data do not altogether support the idea that high-fat diet may cause insulin resistance due to impaired mitochondrial function in prediabetic subjects with LBW or in healthy NBW controls. If anything, the causal relationship may be opposite, with peripheral insulin resistance preceding and potentially causing impaired OXPHOS gene expression and at a later stage mitochondrial dysfunction at the cellular level during the lifelong process of development of insulin resistance and overt T2D in LBW individuals. As for the impaired OXPHOS gene expression levels among the LBW subjects after HFO, it is important to emphasize that gene expression levels represent the balance between the rates of synthesis and breakdown of the mRNA sequences. Therefore, the reduced OXPHOS gene expression levels in the LBW subjects after HFO could in theory reflect a higher turnover rate of these genes due to increased as opposed to decreased in vivo mitochondrial capacity for ATP synthesis. Nevertheless, reduced OXPHOS gene expression could also reflect an acute metabolic stress response that could become permanent and potentially causing impaired OXPHOS gene expression and at a later stage mitochondrial dysfunction at the cellular level during the lifelong process of development of peripheral insulin resistance and overt T2D in LBW individuals. If anything, the causal relationship may be opposite, with peripheral insulin resistance preceding and potentially causing impaired OXPHOS gene expression and at a later stage mitochondrial dysfunction at the cellular level during the lifelong process of development of insulin resistance and overt T2D in LBW individuals. As for the impaired OXPHOS gene expression levels among the LBW subjects after HFO, it is important to emphasize that gene expression levels represent the balance between the rates of synthesis and breakdown of the mRNA sequences. Therefore, the reduced OXPHOS gene expression levels in the LBW subjects after HFO could in theory reflect a higher turnover rate of these genes due to increased as opposed to decreased in vivo mitochondrial capacity for ATP synthesis. Nevertheless, reduced OXPHOS gene expression could also reflect an acute metabolic stress response that could become permanent and affect in vivo metabolism in LBW subjects if an unhealthy diet was consumed for a longer period. As for the finding of increased resting ATP as well as PCr levels among the LBW subjects after high-fat overfeeding, this could in itself actually be considered as a trait central to the idea of the thrifty (energy conserving) and insulin-resistant phenotype of LBW subjects (9). In support of this, enhanced skeletal muscle mitochondrial

**Fig. 1.** A: insulin-stimulated glucose uptake (M value) for normal- (NBW; n = 25) and low-birth-weight subjects (LBW; n = 18) on the control diet (open bars) and the high-fat overfeeding (HFO) diet (black bars). B: hepatic insulin resistance indices for NBW (n = 25) and LBW subjects (n = 18) during the control diet (open bars) and the HFO diet (black bars). C: hepatic glucose production (HGP) measured in the basal state using tritiated glucose for NBW (n = 25) and LBW subjects (n = 18) during the control diet (open bars) and HFO (black bars) diet. Data are means ± SE. NBW vs. LBW: #P ≤ 0.05. Control vs. HFO diet: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.
capacity to produce ATP has been reported in severely insulin-resistant Asian Indians living in the US (25). The link between increased mitochondrial function and insulin resistance could be mediated via increased generation of reactive oxygen species (7), perhaps as a consequence of reduced state 4 respiration, as proposed in a recent study of sheep exposed to the combination of fetal under- and postnatal overnutrition (17).

The decrease in insulin action observed during HFO in the LBW subjects was relatively modest and lower than observed previously by Brehm et al. (2), using in vivo intralipid infusion. Given that ATP synthesis measured by magnetization transfer as in the study by Brehm et al. (2) may be somewhat more sensitive compared with the current methodology, our study does not allow the more general conclusion that lipid-induced insulin resistance is not associated with any degree of mitochondrial dysfunction. However, we measured mitochondrial function and ATP synthesis after exercise, where the ATP synthesis rate is multifold stimulated, whereas Brehm et al. (2) measured mitochondrial function in resting muscle, which is why it could be argued that our power to detect differences might be higher.

Although not overt among LBW subjects at a young age, when studied during isocaloric conditions, we did in fact find previously that LBW among twins is associated with a significant impairment of muscle glycogen synthesis becoming manifest with increased age (33). In this study, we found near-significant impairment of insulin-stimulated muscle glycogen storage among the LBW subjects during the HFO challenge, supporting the idea of LBW being associated with impaired muscle glycogen synthesis during some metabolic conditions. The increased insulin-stimulated rate of GF among the LBW subjects may in this context be seen as a compensatory phenomenon preventing a more severe degree of insulin resistance, at least during a short-term perspective, and may furthermore explain why the impaired muscle glycogen storage among the LBW subjects during the HFO challenge, supporting the idea of LBW being associated with impaired muscle glycogen synthesis during some metabolic conditions. The increased insulin-stimulated rate of GF during HFO does not seem to have increased lactate levels and muscle acidification, as evidenced by the NMR data.

Despite similar plasma FFA levels in the two groups during the clamp, the LBW men showed significantly increased insulin-stimulated fat oxidation after HFO (Table 2). Increased fat oxidation is a hallmark of T2D and has long been thought to
play a primary role in the development of insulin resistance (20). However, the finding of simultaneous normal plasma FFA levels, glucose oxidation rates, and in vivo mitochondrial function in the LBW subjects during high-fat feeding questions two of the currently most prominent hypotheses linking increased fat oxidation with insulin resistance, including the classic Randle cycle (35) as well as the "mitochondrial dysfunction hypothesis" (22, 31). Instead, the increased fat oxidation rate may contribute to or explain the increased content of the most important energy-dense substrates, including ATP and PCr, in the LBW subjects during overfeeding. Increased muscle energy content, including intramyocellular lipids, is commonly associated with insulin resistance (21), and the surprisingly improved lipid profile after overfeeding (Table 1) could indicate increased intracellular lipid deposition (5).

Unlike the NBW subjects, the LBW subjects were unable to increase plasma leptin levels in response to overfeeding, due primarily to the fact that the leptin level tended to be elevated already with the control diet (Fig. 2). Elevated plasma leptin levels have been observed previously in LBW, obese, and T2D subjects (11, 40) and are generally thought to represent leptin resistance. Nevertheless, the fact that LBW subjects were unable to increase the plasma leptin levels when exposed to overfeeding suggests that they did not experience the same degree of appetite reduction as the NBW subjects after overfeeding. Furthermore, the LBW subjects also displayed a reduced rise of the plasma levels of the satiety-regulating hormone PP in response to HFO compared with the NBW controls, supporting the notion of reduced suppression of appetite among the LBW subjects during the HFO diet.

### Table 3. Effects of HFO on in vivo mitochondrial function in the forearm flexor muscles and tibialis anterior of the leg measured by $^{31}$P-MRS at rest and after energy-depleting exercise

<table>
<thead>
<tr>
<th></th>
<th>NBW Subjects (n = 19)</th>
<th>LBW Subjects (n = 15)</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>HFO</td>
<td>CON</td>
</tr>
<tr>
<td><strong>Rest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP total, mM/s</td>
<td>0.72 ± 0.37</td>
<td>0.59 ± 0.30</td>
<td>0.59 ± 0.21</td>
</tr>
<tr>
<td>PCR, mM</td>
<td>21.96 ± 2.17</td>
<td>21.45 ± 2.20</td>
<td>22.17 ± 2.07</td>
</tr>
<tr>
<td>PCR/Pi</td>
<td>8.31 ± 2.00</td>
<td>7.80 ± 1.37</td>
<td>7.57 ± 1.18</td>
</tr>
<tr>
<td>pH</td>
<td>7.01 ± 0.03</td>
<td>7.02 ± 0.02</td>
<td>7.01 ± 0.03</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR, mM</td>
<td>9.15 ± 3.25</td>
<td>9.67 ± 3.96</td>
<td>10.46 ± 3.17</td>
</tr>
<tr>
<td>PCR/Pi</td>
<td>1.59 ± 1.03</td>
<td>1.7 ± 1.80</td>
<td>1.28 ± 0.70</td>
</tr>
<tr>
<td>pH</td>
<td>6.32 ± 0.32</td>
<td>6.35 ± 0.27</td>
<td>6.35 ± 0.25</td>
</tr>
<tr>
<td>Vmax, m/s</td>
<td>0.34 ± 0.08</td>
<td>0.30 ± 0.09</td>
<td>0.34 ± 0.10</td>
</tr>
<tr>
<td>Pcr1/2, s</td>
<td>68.0 ± 25.1</td>
<td>69.6 ± 24.9</td>
<td>70.5 ± 21.1</td>
</tr>
<tr>
<td>Pymi1/2, s</td>
<td>44.3 ± 18.0</td>
<td>47.8 ± 20.5</td>
<td>44.0 ± 14.9</td>
</tr>
</tbody>
</table>

Data are means ± SD. PCR, phosphocreatine; Vmax, maximum velocity. CON vs. HFO diet: *P ≤ 0.05.

### Table 4. Gene expression measured as fold mRNA changes in NBW and LBW subjects after 5 days of HFO in the basal and insulin-stimulated state

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Insulin Stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBW HFO (n = 21)</td>
<td>LBW HFO (n = 13)</td>
</tr>
<tr>
<td><strong>PPARGC1A</strong></td>
<td>0.47 ± 0.10</td>
<td>0.34 ± 0.09</td>
</tr>
<tr>
<td><strong>ATP5O</strong></td>
<td>1.13 ± 0.25</td>
<td>0.84 ± 0.23#</td>
</tr>
<tr>
<td><strong>COX7A1</strong></td>
<td>0.65 ± 0.14</td>
<td>0.50 ± 0.14</td>
</tr>
<tr>
<td><strong>NDUFB6</strong></td>
<td>1.56 ± 0.34</td>
<td>1.25 ± 0.35</td>
</tr>
<tr>
<td><strong>UQCRB</strong></td>
<td>1.06 ± 0.23</td>
<td>0.80 ± 0.22#</td>
</tr>
</tbody>
</table>

Data are means ± SE. PPARGC1A, peroxisome proliferator-activated receptor-γ coactivator-1α; ATP5O, ATP synthase, H+ transporting mitochondrial F1 complex, O subunit; COX7A1, cytochrome c oxidase subunit VIIA polypeptide 1; NDUFB6, NADH dehydrogenase p 1β subcomplex 6; UQCRB, ubiquinol-cytochrome c reductase. NBW vs. LBW: #P ≤ 0.05.
lack of a rise in plasma leptin levels could also indicate subtle

differences in fat deposition, which is consistent with a reduced
capacity for depositing lipid in subcutaneous adipocytes, re-
sulting in increased fat oxidation and ectopic fat deposition.

Taken together, these conditions may promote further over-
feeding and eventually obesity, insulin resistance, and overt
T2D in LBW subjects. Adiponectin is generally considered to
be a marker of insulin sensitivity, and although the plasma
levels of adiponectin increased in both groups after the HFO
diet, possibly representing a compensatory mechanism, this
was not sufficient to prevent the development of insulin resis-
tance in the LBW subjects. As for the remaining adipokines
and appetite-regulating as well as gut incretin hormones mea-
sured in this study, the most interesting observation may be
that the LBW subjects increased fasting plasma GIP levels to a
significantly lesser extent than did the controls (Fig. 2). This
finding is in line with the insignificant increase in fasting
plasma insulin and C-peptide levels in the LBW subjects after
overfeeding. Given that impaired insulin secretion is a cardinal
defect in T2D (18), our data suggest that one among many
different mechanisms by which LBW predisposes to T2D
operates via a reduced capability to increase plasma GIP
secretion and subsequently insulin secretion when challenged
by HFO.

Both groups experienced a somewhat unexpected fall in
plasma FFA concentrations at baseline. This could be a result of
an increased uptake into the adipose tissue caused by
stimulation of lipoprotein lipase by the increased fasting insu-
lin levels during the HFO diet and could therefore reflect an
increased clearance and/or increased capacity for lipid storage.
Furthermore, the decreased plasma FFA levels may be ex-
plained by an increased capacity to oxidize fat on the HFO diet,
as evidenced by unaltered lipid oxidation rates in the presence
of lower plasma FFA levels.

Although fasting glucose, insulin, and C-peptide levels (Ta-
ble 2) as well as the degree of hepatic insulin resistance (Fig.
1B) were increased significantly in the LBW subjects on the
control diet as shown previously (4), these key metabolic
variables converged toward similar levels in the two groups
during HFO, indicating that the LBW subjects are closer to
developing manifest T2D, as characterized by development of
both hepatic and peripheral insulin resistance, whereas in the
control subjects the HFO diet selectively caused hepatic insulin
resistance without influencing peripheral insulin action. Simi-
larly, in vivo insulin secretion in the absolute sense or ex-
pressed as peripheral or hepatic insulin action disposition
indices was identical in the two groups during overfeeding
(Table 2). Thus, the most important adverse differential re-
ponse to the HFO challenge in the LBW subjects was the
development of peripheral insulin resistance. From a more
teleological and hypothetical perspective, the adverse and dif-
ferential metabolic profile of the LBW subjects and the notion
that the NBW subjects developed hepatic insulin resistance and
elevated fasting blood glucose levels after overfeeding suggest
that the metabolic defects seen in the LBW subjects may reflect
a relative imbalance between energy intake and metabolism.

The overfeeding diet of the current study is comparable with
commonly occurring feast periods in most societies, so these
novel findings draw attention to the significant deleterious
effects of such a diet even during short-term exposure in
individuals at risk of developing T2D.

In conclusion, the young men in the current study with LBW
exhibit normal or, if anything, increased and not decreased in
vivo muscle mitochondrial function contrasting the devel-
opment of peripheral insulin resistance and reduced muscle
OXPHOS gene expression in response to a short-term HFO
challenge. Reduced increments of fasting plasma leptin, PP,
and GIP levels may contribute to insulin resistance, lower
satiety and impaired insulin secretion, and, therefore, ulti-
ately T2D in LBW subjects when exposed to HFO.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.B., A.A., B.Q., and A.V. did the conception and design of the research;
the results of the experiments; C.B. prepared the figures; C.B. drafted the
and revised the manuscript; C.B. and A.V. approved the final version of the
manuscript.

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