Effect of maternal nutrient restriction from early to midgestation on cardiac function and metabolism after adolescent-onset obesity


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Chan LL, Sébert SP, Hyatt MA, Stephenson T, Budge H, Symonds ME, Gardner DS. Effect of maternal nutrient restriction from early to midgestation on cardiac function and metabolism after adolescent-onset obesity. Am J Physiol Regul Integr Comp Physiol 296: R1455–R1463, 2009. First published February 25, 2009; doi:10.1152/ajpregu.91019.2008.—Maternal nutrient restriction (NR) from early to midgestation has marked effects on endocrine sensitivity and organ function of the resulting offspring. We hypothesized that early NR may reset the expression profile of genes central to myocardial energy metabolism, influencing ectopic lipid deposition and cardiac function in the obese adult offspring. NR offspring were exposed to an “obesogenic” environment, and their cardiac function and molecular indexes of myocardial energy metabolism were assessed to explore the hypothesis that an obese individual’s risk of heart disease may be modified after maternal NR. Pregnant sheep were fed 100% (control) or 50% (NR) energy requirement from days 30 to 80 of gestation and 100% energy requirement thereafter. At weaning, offspring were exposed to an obesogenic environment or remained lean. At ~1 yr of age, the hemodynamic response of these offspring to hypotension, together with left ventricular expression profiles of fatty acid-binding protein 3 (FABP3), peroxisome proliferator-activated receptor-γ (PPARγ) and its coactivator (PGC)-1α, acetyl-CoA carboxylase (ACC), AMP-activated protein kinase (AMPK)-α2, and voltage-dependent anion channel 1 (VDAC1), was determined. Obesity produced left ventricular hypertrophy in all animals, with increased ectopic (myocardial) lipid in NR offspring. Obesity per se significantly reduced myocardial transcript expression of PGC-1α, AMPKα2, VDAC1, and ACC and increased expression of PPARγ and FABP3. However, although NR animals were similarly obese, their transcript expression of ACC, PPARγ, and FABP3 was similar to that of lean animals, indicating altered cardiac energy metabolism. Indeed, blunted tachycardia and an amplified inotropic response to hypotension characterized cardiac function in obese NR offspring. The results suggest that maternal NR during early organogenesis can precipitate an altered myocardial response to hypotension and increased myocardial lipid deposition in the adult offspring after adolescent-onset obesity, potentially rendering these individuals more at risk of early heart failure as they age.

lipid infiltration; hypotensive challenge; myocardial energy metabolism; ectopic lipid deposition; insulin resistance

OBESITY IS A SIGNIFICANT RISK factor for diabetes, hypertension, cardiovascular disease, and dyslipidemia (56). Sedentary lifestyle, together with increased caloric intake, is the main cause of obesity and related onset of the metabolic syndrome (27, 42). Obesity is well known to increase the risk of a broad spectrum of noncommunicable diseases, including renal (11) and cardiovascular disease, through changes in sympathetic activation (2), an upregulated renin-angiotensin system (40), and cardiac hypertrophy, atherosclerosis, and local inflammatory responses (1, 28). Specifically, obesity has been shown to have a substantial inhibitory effect on cardiac function, partly as a consequence of excess fat accumulation in and around the heart (29). Increased peri- and epicardial fat is associated with a fetal pattern of myocardial energy utilization, i.e., a predominantly glucose, rather than fatty acid, dependence and changes in expression of the key intracellular molecular energy “switches,” e.g., peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α (31), acetyl-CoA carboxylase (ACC), and AMP-activated protein kinase (AMPK)-α2 (30). At the same time, obesity results in significant changes in left ventricular (LV) and diastolic function (13) that are exacerbated by the concomitant sympathetic overdrive (24, 25), leading to a myocardium that is refractory to β-adrenergic signaling (8).

Global changes in maternal macronutrient intake targeted at defined stages of pregnancy can have pronounced effects on cardiovascular control in the resulting offspring (21). Such adaptations are usually accompanied by significant changes in the density of expression of organ receptor populations and relevant enzyme activities and, hence, endocrine responsiveness and substrate transport and utilization (for review see Ref. 34). For example, maternal nutrient restriction in early pregnancy is associated with increased tissue-specific glucocorticoid receptor (GR) expression in the newborn (52), which results in increased blood pressure later in life, but only in a prefeeding basal state (23). Maternal nutrient restriction coincident with early heart development, however, has no obvious long-term effects on cardiac function when the offspring are maintained under a natural “free-living” environment (22, 23). Importantly, the long-term programming outcomes appear to be amplified when the offspring are exposed to an obesogenic environment (44, 45).

The extent to which the prenatal nutritional environment may influence the expression of genes associated with cardiac hypertrophy has been examined previously (26), but potential effects on energy metabolism in the heart, especially with an early onset of obesity, have not been studied. This is important, since cardiac hypertrophy is associated with suppressed insulin-dependent GLUT4 expression and heart-type fatty acid-binding protein (FABP)-3 (43) but enhanced insulin-independent GLUT1 transport (1) and ectopic lipid accumulation (47). These changes in myocardial energy metabolism with obesity may also impact other “nutrient sensors” within cardiac tissue, such as expression of PPARγ and its coactivator (PGC-1α)
(14). Adaptations of this type could also determine, in part, progression toward heart disease with obesity, as a consequence of changes in myocardial substrate supply and utilization, particularly fatty acids, since these account for more than half of ATP production in the heart.

Although the prenatal nutritional environment has been shown in many studies to alter specific aspects of growth, development, and physiology per se, it is evident that the physical expression of a “programmed” end point is dependent, in part, on the postnatal environment. Specifically, the degree of physical activity, food intake, and energy density of the food, i.e., the degree of exposure and active engagement in an “obesogenic” lifestyle, may influence the levels of hypertension and/or obesity (39, 51, 53). In the present study, we hypothesized that programming by prenatal nutrient restriction may reset the expression profile of genes that are central to myocardial energy metabolism, influencing lipid deposition in this tissue and, ultimately, affecting cardiac function under duress. In allowing these “programmed” animals to become overweight, we sought to model a sedentary, Westernized culture, characterized by reduced physical activity and increased access to energy-dense food. The effects of obesity on myocardial function were then assessed in vivo during a hypotensive challenge followed by a molecular characterization of myocardial energy metabolism. This included a direct comparison of the effects of obesity per se by further comparison of cardiovascular function and cardiac outcomes between lean and obese individuals born to normally fed mothers.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986, and approved by the local ethics committee of the University of Nottingham at Sutton Bonington. At day 30 of gestation, 16 twin-bearing ewes were randomly allocated to receive a control [ ~ 7.8 MJ/day of metabolizable energy (ME), n 6] or nutrient-restricted (50% of control, n = 10) diet until day 80 of gestation. Thereafter, all sheep were fed to 100% calculated ME requirements to full term (12-13 MJ/day near to term) (2). Offspring were delivered spontaneously and reared by their mothers as singletons (i.e., 1 twin was euthanized) from day 7 to weaning (10 wk). There were three males in the control group and two males in the nutrient-restricted groups. After birth, all mothers were fed a diet of hay ad libitum, together with a fixed amount of concentrate pellets sufficient to fully meet their own ME requirements plus that needed to maintain lactation. All diets contained adequate minerals and vitamins. From weaning to 12 mo of age, all offspring were group housed in a barn (50 m^2; i.e., restricted activity) with ad lib access to hay and concentrate pellets (140 g/kg crude protein, 3% oil, 12.7 MJ/kg dry matter; Manor Farm Feeds) to promote increased fat deposition; these sheep were designated “obese” (O) or “nutrient-restricted obese” (NRO), as previously described (48). Another group of sheep (n = 8) was incorporated into the experimental design to control for the postnatal treatment structure, i.e., adolescent-onset obesity. These sheep were contemporaneous to control animals, i.e., the ewes were fed 100% ME requirements throughout gestation and gave birth to twin female offspring, with only one of each twin pair used for study. At weaning, however, these sheep lived in an environment that encouraged unlimited low-to-moderate physical activity (a field as opposed to a barn), with supplemental feed provided as required (53). These sheep were designated “lean” (L). At 1 yr of age, i.e., as young adults, arterial (carotid) and venous catheters (jugular) were inserted into all sheep. Before surgery, all food, but not water, was withdrawn from the animals for 24 h. Anesthesia was induced with propofol (Rapinovet; 6 mg/kg) and maintained with 3–4% isoflurane in 3–4 l/min O_2. All sheep received a course of antibiotic (10 mg/kg im procaine penicillin; Duphagen, Fort Dodge Animal Health, Southampton, UK) and analgesia (2 mg/kg im flunixin meglumine; Finadyne, Schering-Plough, Kenilworth, UK) for 3 days postoperatively. Catheter patency was maintained by daily flushing with heparinized saline (50 IU heparin/ml). All sheep had established normal feeding patterns within 1 h after surgery and showed no visible signs of discomfort for the duration of the experimental period.

Experimental Protocols

Cardiovascular experiments. No experiment was performed until 2–4 days after postoperative recovery. The investigator was blinded to the dietary origin of the sheep before any experiment was performed. All sheep were prehabituated to the experimental conditions before the same experiment was conducted with three different treatments on 3 separate days: 1) with a background infusion of saline, 2) with pretreatment and infusion of the muscarinic antagonist atropine sulfate, and 3) with pretreatment and infusion of the mixed β-antagonist propranolol.

Cardiovascular responses to sodium nitroprusside infusion. Sheep were habituated to a metabolic crate, and after ≥1 h, the arterial catheter was connected to calibrated pressure transducers (Sensor-Nor 840; S 4925) attached at heart level and linked to a data acquisition system (Po-Ne-Mah, version 3, Gould Instrument Systems), and baseline data were recorded over 1 h. Analog signals for real-time systolic, diastolic, and mean arterial pressure and heart rate were recorded at 1-s intervals, digitized, and then stored on an Excel spreadsheet for further analysis (18). Resting cardiovascular data (baseline systolic, diastolic, and mean arterial pressure and heart rate) for these animals has been described elsewhere (53). The first derivative of the positive and negative change in pressure associated with each heartbeat (+dP/dt and –dP/dt) was calculated automatically and taken to reflect the strength of myocardial contractility and relaxation, respectively. The rate-pressure product [[(mmHg min)$^{-1}$]/10] was used as an assessment of myocardial work.

Saline infusion. On a background of saline infusion (1 ml/min), the sheep were infused intravenously (2.5 μg·kg$^{-1}$·min$^{-1}$) with the endothelium-dependent vasodilator sodium nitroprusside (SNP; Abbott Laboratories, Maidenhead, UK) for 5 min, with a further 5-min recording of the recovery period.

Atropine infusion. On a separate day, the protocol for saline infusion was followed exactly, except the sheep received a bolus dose of atropine intravenously (2.4 mg) followed by a constant infusion of 1 mg/ml. Further bolus doses (1.2 mg) were given, and each failed to elicit an increase in heart rate, confirming complete muscarinic blockade, as previously described in detail (35).

Propranolol infusion. On a separate day, the protocol for saline infusion was again followed, except the sheep received a bolus dose of propranolol intravenously (20 mg) followed by a constant infusion of 1 mg·ml$^{-1}$·min$^{-1}$, as previously described in detail (55).

Blood and Molecular Analyses

Biochemical analyses. Whole blood was withdrawn from the jugular catheter into heparin tubes [50 μl of glutathione-EGTA solution (4.75 g of EGTA and 3.00 g of glutathione dissolved in 50 ml of deionized water)] heparinized tubes at 5 min before and 4 min into the SNP infusion with saline. Plasma concentrations of catecholamines were measured by HPLC with electrochemical detection, as previously described in detail (19). At the end of all experimental protocols, all sheep were humanely euthanized with electrocortical stunning and exsanguination. A representative sample of LV tissue was flash frozen in liquid nitrogen and stored at −80°C until analysis.

Lipid extraction and tissue triglyceride assay. Lipids in frozen LV (~500 mg) were first extracted with 1:1 chloroform-methanol and
dissolved in 1:1 (vol/vol) tert-butyl alcohol-Triton X-100 before enzymatic measurement (Infinity Triglycerides Liquid Stable Reagent, Thermo Electron) (12, 18).

Total RNA isolation and reverse transcription. RNA was extracted from a fixed quantity of the LV (∼100–200 mg) using TRIReagent (catalog no. T9424, Sigma). RNA concentration was measured spectrophotometrically, and its purity was confirmed by measurement of the ratio of absorbance at 260 nm to absorbance at 280 nm. RNA concentration was adjusted to 3 μg/μl using nuclease-free water (Ambion), and samples were stored at −80°C. First-strand cDNAs were reverse transcribed in a reaction containing 3 μg of total RNA, 200 U of SuperScript II reverse transcriptase (catalog no. 18064-014, Invitrogen), first-strand buffer [250 nM Tris-HCl (pH 8.3), 375 mM KCl, and 15 mM MgCl₂], 125 ng of pd(N)₉ random hexamer 5’-phosphate (catalog no. 27-2166-01, GE Healthcare), 10 mM dNTP mix (catalog no. 28-4065-64, GE Healthcare), and 40 U of RNaseOUT recombinant RNase inhibitor (catalog no. T9777-019, Invitrogen). The conditions of synthesizing cDNA were therefore in accordance with the manufacturer’s protocol.

PCR for standard curve generation. PCR was performed in the presence of Thermo-Start Master Mix (AB-0938-DC-MM, ABgene). The forward and reverse primers (Sigma Genosys) are listed in Table 1. The amplification parameters were as follows: 95°C (15 min); 35–40 cycles at 94°C (45 s), annealing temperature was 72°C for 15 min.

Quantitative real-time PCR assay. The relative abundance of each gene was measured by quantitative real-time PCR using the Quantica Real-Time Nucleic Acid Detection System (Technoe, Barloworld Scientific) with QuantiTect SYBR Green PCR Master Mix (catalog no. 204145, Qiagen), diluted RT reactions, and 10–15 pmol of the forward and reverse primers (Table 1). A reverse transcribed-negative control was used to ensure absence of genomic DNA contamination. The amplification parameters were as follows: 95°C (15 min); 45 cycles at 94°C (30 s), annealing temperature described above (see PCR for standard curve generation). Melt curve analysis was performed to ensure reaction specificity. Primers were designed to amplify small fragments (150–250 bp) and ensure ~98% amplification efficiency. 18S was chosen as the housekeeping gene, inasmuch as the alternatives (e.g., GAPDH and β-actin) are nutritionally sensitive (49).

Statistical Analyses

Data were first analyzed for an effect of treatment group (L, O, and NRO) by a univariate general linear model procedure with treatment and, where appropriate, sex as a fixed effect using SPSS version 14 (SPSS, Chicago, IL). Specific contrasts selected a priori to test for effects of postnatal obesity (L vs. O) or prenatal diet (O vs. NRO) were also examined. Estimated marginal means are presented, together with their respective SE, unless otherwise stated. For cardiovascular responses, data were analyzed as area under the response curve (Pulvinar version 5, GraphPad, San Diego, CA). For repeated measures (e.g., catecholamines before and after SNP), all data were compared by paired t-test followed by a repeated-measures general linear model to test for any treatment effects (SPSS version 14). For all comparisons, statistical significance was accepted when P < 0.05.

RESULTS

Body Composition and Metabolic Status

The overall effect of exposure to an obesogenic environment [∼65% reduced physical activity, ~30% increased food intake (53)] from after weaning (3 mo of age) to young adulthood (1 yr of age) has been reported previously (44, 53). Obese sheep were, by definition, significantly heavier [∼90 vs. 58 kg (± 1.8 SE)] and fatter [∼7.0 vs. 1.5 kg (± 0.3 SE) visceral fat mass] than lean sheep at 1 yr of age, with marginally increased fasting plasma glucose concentration [5.47 vs. 4.47 mmol/l (± 0.08 SE)] and significantly higher plasma nonesterified fatty acid [0.60 vs. 0.31 mmol/l (± 0.08 SE)], leptin [20 vs. 3 ng/ml (± 1.5 SE)], and insulin [1.34 vs. 0.56 ng/ml (± 0.20 SE)] concentrations.

Table 1. Summary of ovine specific oligonucleotide primers for myocardial genes determined using real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Forward Primer Sequence</th>
<th>Reverse Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₁</td>
<td>NM_174498</td>
<td>5'-ate cac acc atc tcc tct ag-3'</td>
<td>5'-tct cta aac ctc ctc g-3'</td>
</tr>
<tr>
<td>α₂</td>
<td>NM_174499</td>
<td>5'-ctg cac gtc ttc cat agt gc-3'</td>
<td>5'-tcc tgc tct tct tct ctg-3'</td>
</tr>
<tr>
<td>β₁</td>
<td>NM_194266</td>
<td>5'-gca tca cca acc tct tca tc-3'</td>
<td>5'-cac aca ggg tgt cca tgg gc-3'</td>
</tr>
<tr>
<td>β₂</td>
<td>NM_174231</td>
<td>5'-att gtc tcc tcc att tgt gc-3'</td>
<td>5'-cat ctc gct cca ctc gac gc-3'</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>NM_177945</td>
<td>5'-gct tgt gtt tgc ctt tgt gc-3'</td>
<td>5'-gcc tgg tgt tgt tgt gc-3'</td>
</tr>
<tr>
<td>AMPKα₂</td>
<td>NM_214266</td>
<td>5'-gtc gsa gta tta gaa gcg gc-3'</td>
<td>5'-cag cac ctc atc aat gcg gc-3'</td>
</tr>
<tr>
<td>ACC</td>
<td>NM_001099256</td>
<td>5'-gct atg gaa gtc ggc tgg gaag-3'</td>
<td>5'-cag cac ctc atc aat gcg gc-3'</td>
</tr>
<tr>
<td>GR</td>
<td>X70407.1</td>
<td>5'-act gcc cca agt gaa aac aga-3'</td>
<td>5'-atg aag aag aag gcg gag ttg-3'</td>
</tr>
<tr>
<td>GLUT1</td>
<td>U99029.1</td>
<td>5'-ggc gca gat gaa ggc gag cag c-3'</td>
<td>5'-agc gca cca cca agg aaa-3'</td>
</tr>
<tr>
<td>GLUT4</td>
<td>AB0005283.1</td>
<td>5'-agt atg tgg cgg aga atc tga ggc c-3'</td>
<td>5'-aat gaa agg aga gag gag-3'</td>
</tr>
<tr>
<td>IR</td>
<td>Y1557728.1</td>
<td>5'-ctg ctc cat cta cca cag aa-3'</td>
<td>5'-cgt aac ttc cgg aag aag ga-3'</td>
</tr>
<tr>
<td>FABP3</td>
<td>NM_174313</td>
<td>5'-aga tgg tgc aag gga aag ccc-3'</td>
<td>5'-cga aac cca cca agg aaa-3'</td>
</tr>
<tr>
<td>VDAC1</td>
<td>NM_00126352</td>
<td>5'-cct ccc acg cat gtg ctt ctc-3'</td>
<td>5'-aga ttc ggt aac ggc gag gg-3'</td>
</tr>
<tr>
<td>PPARγ</td>
<td>NM_001100921</td>
<td>5'-acg gaa aag acg cca gcg ctc c-3'</td>
<td>5'-cag tca ctc aat cca cca cag-3'</td>
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<tr>
<td>18S</td>
<td>NR_002170</td>
<td>5'-ctc ctt gct ggt gtc ccc ctc-3'</td>
<td>5'-agt gac gcc gcg gct ttc c-3'</td>
</tr>
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</table>

ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; FABP, fatty acid-binding protein; GR, glucocorticoid receptor; IR, insulin receptor; PGC, peroxisome proliferator-activated receptor (PPAR) coactivator; VDAC, voltage-dependent anion channel.
LV Triglyceride and Transcript Expression for Cardiac Energy Metabolism

The triglyceride content of the LV was unaffected by postnatal obesity per se but was significantly increased (~3-fold) in the nutrient-restricted obese offspring (Fig. 1A). Myocardial expression of FABP3 and PPARγ2 was raised by obesity, a response that was not seen in the nutrient-restricted obese offspring (Fig. 1, B and D). However, mRNA abundance for ACC was reduced with postnatal obesity, but this was not observed in nutrient-restricted obese offspring (Fig. 1C). Gene expression of PGC-1α, AMPKα2, and voltage-dependent anion channel 1 (VDAC1) were reduced by postnatal obesity per se, again an adaptation not observed in nutrient-restricted obese offspring (Table 2). Gene expression of AMPKα2 was positively correlated with triglyceride content in the nutrient-restricted obese offspring only ($r^2 = 0.57$, $P < 0.05$; Fig. 2).

There was no effect of obesity on gene expression for GLUT4, the insulin receptor (IR), or the GR, although GR and GLUT1 mRNA abundance were reduced in the nutrient-restricted obese offspring (Table 2). However, GLUT1 gene expression was raised in the obese group compared with the lean group (Table 2).

Plasma Catecholamines and Myocardial AR Gene Expression

Resting plasma catecholamines were almost twofold greater in obese than in lean animals, but because of individual variation, this difference did not achieve statistical significance (Fig. 3A). With hypotension, obese animals exhibited a significantly greater increment in total plasma catecholamines ($0.153 \pm 0.716, 0.538 \pm 1.523$, and $1.516 \pm 0.946$ nmol/l for L, O, and NRO, respectively, $P < 0.05$, L vs. O). Gene expression for the $\beta_1$- and $\beta_2$-ARs was reduced with obesity, but not in nutrient-restricted obese offspring (Fig. 3, B and C). There was no difference between dietary groups in the expression of mRNA for the $\alpha_1$- or $\alpha_2$-ARs (data not shown).

Resting Cardiovascular Status and Response to SNP

Saline infusion. Before saline infusion, mean arterial pressure was higher in obese (O and NRO) than in lean sheep.

Table 2. Mean expression of genes involved in cardiac energy metabolism in LV of lean, obese, or prenatally nutrient-restricted obese offspring

<table>
<thead>
<tr>
<th>Gene</th>
<th>mRNA Abundance in LV, AU</th>
<th>L (n = 8)</th>
<th>O (n = 6)</th>
<th>NRO (n = 11)</th>
<th>P</th>
</tr>
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<tr>
<td>PGC-1α</td>
<td></td>
<td>1.00±0.08</td>
<td>0.55±0.10</td>
<td>0.62±0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AMPKα2</td>
<td></td>
<td>1.00±0.25</td>
<td>0.44±0.10</td>
<td>0.56±0.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VDAC</td>
<td></td>
<td>1.00±0.19</td>
<td>0.59±0.14</td>
<td>0.36±0.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GR</td>
<td></td>
<td>1.00±0.14</td>
<td>1.46±0.52</td>
<td>0.52±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>GLUT1</td>
<td></td>
<td>1.00±0.37</td>
<td>1.21±0.49</td>
<td>0.13±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>GLUT4</td>
<td></td>
<td>1.00±0.19</td>
<td>0.65±0.25</td>
<td>0.35±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>IR</td>
<td></td>
<td>1.00±0.39</td>
<td>0.63±0.14</td>
<td>0.45±0.08</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. AU, arbitrary units; LV, left ventricle; L, lean; O, obese; NRO, nutrient-restricted obese; NS, not significant.
(97 ± 2 and 99 ± 2 mmHg in O and NRO, respectively, vs. 89 ± 1 mmHg in L, P = 0.03 by 1-way ANOVA), with no interaction with prenatal diet. These results are similar to those published previously for systolic and diastolic pressures in these animals (53). Resting rate-pressure product was significantly higher in obese sheep [8.62 ± 0.87, 10.07 ± 0.66, and 11.96 ± 0.82 (mmHg·min⁻¹)/10⁻³ in L, O, and NRO, respectively], but +dP/dt (800 ± 117, 739 ± 113, and 803 ± 103 mmHg/s in L, O, and NRO, respectively) and −dP/dt (545 ± 74, 328 ± 72, and 458 ± 66 mmHg/s in L, O, and NRO, respectively) were similar in obese and lean sheep. SNP infusion elicited a rapid and significant decline in blood pressure (Fig. 4A), provoking a significant increment in heart rate (Fig. 4B) during the challenge that was significantly greater (0 to 5 min; P < 0.05, F = 4.25) in lean than in obese sheep (33 ± 8, 12 ± 8, and 12 ± 7 beats/min in L, O, and NRO, respectively). Although there was no effect of obesity per se on +dP/dt (Fig. 4C), there was a trend (P = 0.09, F = 3.16) for the increment to be greater in the nutrient-restricted obese group [2,689 ± 2,008 and 7,208 ± 1,555 area-under-the-curve (AUC) units in O and NRO, respectively]. In addition, there was a trend (P = 0.09, F = 3.15) for the rate of cardiac relaxation (−dP/dt) to be slower in obese sheep (6,935 ± 1,819 and 3,232 ± 1,017 AUC units in L and O, respectively; Fig. 4D). During the 5-min recovery period, the return of diastolic pressure (−137 ± 47 and −362 ± 26 AUC units in L and O, respectively, P = 0.001, F = 17.2) and +dP/dt (−487 ± 1,228 and 3,113 ± 686 AUC units in L and O, respectively, P = 0.01, F = 6.54) toward baseline was significantly blunted in obese compared with lean sheep. There were no effects of prenatal diet on the recovery of cardiac function in obese sheep.

**Atropine infusion.** There were no significant effects of obesity or prenatal diet on the cardiac response to SNP during atropine infusion (Fig. 5); however, the differences in response during saline infusion were abolished when the experiment was conducted on a background of muscarinic blockade (Fig. 4A vs. Fig. 5A). Overall, the cardiac response to SNP as reflected in heart rate (Fig. 5B), +dP/dt (Fig. 5C), and −dP/dt (Fig. 5D) after atropine was similar to that observed during saline infusion (i.e., a comparison of equivalent results presented in Figs. 4 and 5) in all groups.

**Propranolol infusion.** There were no significant effects of obesity or prenatal diet on the cardiac response to SNP during propranolol infusion (Fig. 6A). As expected, propranolol significantly blunted the increment in heart rate (Fig. 6B) and cardiac contractility (+dP/dt; Fig. 6C) and relaxation (−dP/dt; Fig. 6D) in all groups relative to results observed during saline infusion (i.e., a comparison of equivalent results presented in Figs. 4 and 6). Consistent with the response observed during saline infusion, the recovery of diastolic pressure after withdrawal of SNP tended to be blunted in obese compared with lean sheep (Fig. 6A).
The major finding of the present study is that fetal exposure to maternal nutrient restriction during early gestation increased ectopic lipid deposition in the LV and reset cardiac energy metabolism when the offspring became obese. This was particularly striking, since levels of myocardial ectopic lipid levels in contemporaneous nutritional controls, which were equally obese, were as low as those in lean animals. Myocardial lipid infiltration is normally related to current diet, aging, and diastolic dysfunction and clinically is often seen in heart failure patients with diabetes or obesity (5, 29, 47). Our findings in prenatally programmed animals thus suggest that they are potentially more at risk from earlier heart failure. However, despite having higher blood pressure (53), they did not show any obvious clinically significant metabolic or other health

Fig. 4. Cardiovascular response to sodium nitroprusside (SNP) during saline infusion in lean (n = 8), obese (n = 6), and obese offspring born to mothers nutrient restricted between early and midgestation (n = 10) at 1 yr of age. +dp/dt and −dp/dt, 1st derivative of positive and negative change in blood pressure. Values are means ± SE. *P < 0.05, L vs. O.

Fig. 5. Basal blood pressure response to SNP during atropine infusion in lean (n = 8), obese (n = 6), and obese offspring born to mothers nutrient restricted between early and midgestation (n = 10) at 1 yr of age. Values are means ± SE.
problems at \( \sim \) 1 yr of age (44). Therefore, the molecular changes that accompanied raised lipid within the hearts of these in utero nutrient-restricted obese animals may provide important early evidence of the developmental mechanisms by which cardiac function can become impaired with obesity.

Obesity is the most significant and potentially preventable risk factor for coronary events, increasing the incidence of other significant risk factors such as hypertension (35, 36, 50). Defined obesity is increasingly observed in childhood and, subsequently, tracks into adulthood, giving these individuals an exacerbated cardiovascular risk (4). In the present study, juvenile-onset obesity per se led to LV hypertrophy (LVH), most likely through increased afterload, myocardial workload, and increased peripheral vascular resistance (49). Indeed, in the present study, rate-pressure product was significantly greater in obese animals, indicative of increased myocardial functional stress, which, together with the increase in LV wall thickness, but without clinical symptoms, suggests that these offspring are at increased risk of heart failure (1, 5, 41, 54). Functionally, in all obese sheep, the heart was compromised, as indicated by delayed myocardial relaxation after a hypotensive stimulus. Although it is accepted that the greater increment in plasma catecholamines during hypotension in obese animals may, in part, contribute to this response, the failure of propranolol infusion to eliminate the effect suggests deficits in cardiac contractility per se as the primary mechanism.

Chronic sympathetic activation by systemic \( \beta \)-AR agonist administration results in cardiac hypertrophy, blunted parasympathetically mediated cardiovascular reflexes, and suppressed \( \beta \)-AR-mediated inotropic responses and sensitivity (10, 37, 38). We also report reduced gene expression of \( \beta_1/\beta_2 \)-AR (but not \( \alpha_1/\alpha_2 \)-AR) in hypertrophied myocardium with obesity. Desensitization of adrenergic signaling occurs in chronic heart failure that is characterized by a rapid increase in sympathetic activation and a decrease in \( \beta_1 \)-AR abundance (7, 20). In the present study, there was a nonsignificant trend for higher circulating catecholamine concentrations in obese than in lean animals and, in the obese group only, downregulated \( \beta_1/\beta_2 \)-AR. Maternal nutrient restriction combined with postnatal obesity, however, resulted in protected \( \beta_1/\beta_2 \)-AR gene expression and, consequently, a strong and sustained increase in systolic contractility with hypotension that was abolished by pretreatment with propranolol.

In combination with reduced \( \beta_1/\beta_2 \)-AR signaling in the obese heart, we also observed significant changes in the molecular machinery influencing myocardial energy metabolism, as reflected by lower transcript levels of \( \beta_1/\beta_2 \)-AR, AMPK\( \alpha_2 \), ACC, and PGC-1\( \alpha \). Myocardial energetic deficiency is the likely mechanism responsible for an apparent inability to increase cardiac work under physiological stimuli, such as hypoxia, sympathetic activation, and ischemia (3). Myocardial PGC-1\( \alpha \) has been shown to be downregulated by obesity induced through consumption of a high-fat diet and to underpin the cardiac dysfunction in this model (15) and in an LVH model unrelated to obesity (31). Thus, in the present study, we propose that low PGC-1\( \alpha \) in both groups of obese animals is a major factor contributing to LVH (53). In addition, further myocardial energetic controls are mediated by AMPK, of which \( \alpha_2 \) is the primary isoform mediating its catalytic activity in the heart (9). AMPK\( \alpha_2 \) is activated by a high AMP-to-ATP ratio, which enables phosphorylation of ACC, which, in turn, favors myocardial fatty acid utilization. Again, we have confirmed that obesity downregulates AMPK\( \alpha_2 \) and ACC mRNA abundance. However, importantly, in prenatally nutrient-restricted obese animals (NRO group), there was dissociation between the two enzymes, i.e., low AMPK\( \alpha_2 \) but normalized ACC. This suggests that early-life exposure to a reduced maternal intake of a balanced diet entrains a myocardial ener-

Fig. 6. Basal blood pressure response to SNP during propranolol infusion in lean (n = 8), obese (n = 6), and obese offspring born to mothers nutrient restricted between early and midgestation (n = 10) at 1 yr of age. Values are means ± SE.
getic imbalance, when coupled with excess weight gain post-natally. Therefore, based on these data, we speculated that the myocardium of nutrient-restricted obese animals was more susceptible to fatty acid infiltration. Indeed, this proved to be the case. Triglyceride content was nearly threefold greater in the hearts of prenatally nutrient-restricted, but postnatally over-nourished, animals than in contemporaneous overweight controls, in which myocardial triglyceride content was similar to that in lean animals.

The primary change in oxidative metabolism in the failing heart is due to a reliance on the β-oxidation of nonesterified fatty acids to glycolysis for metabolic energy, i.e., a return to a fetal pattern of oxidative metabolism (6, 17). Indirect evidence that an adaptation of this type was beginning to occur in the previously nutrient-restricted obese offspring is provided by the relative changes in transcript expression for the enzymes governing myocardial energy metabolism (ACC, AMPKα2, and FABP3). Maintenance of elevated ACC in conjunction with reduced AMPKα2 in the hearts of offspring born to nutrient-restricted mothers could be indicative of a shift away from fatty acids to glycolysis for metabolic energy, i.e., a return to donor for substrate metabolism in the obes phenotype in adipose tissue (16, 48). Clearly, future studies should be focused on examining the regulation of cardiac energy metabolism in nutritionally programmed animals that subsequently become obese.

**Perspectives and Significance**

We have shown, for the first time in a relevant large-animal model, that maternally nutrient-restricted offspring, as obese adults, develop dysfunction in their myocardial energy metabolism, resulting in a tendency for elevated ectopic lipid deposition in their myocardial tissue. It is hypothesized that these individuals would therefore have an increased likelihood of heart failure as they age, an adverse outcome that is likely exacerbated by their accompanying insulin resistance (44) and changes in adipose tissue biology (45).

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