Adipose Tissue Inflammation: Developmental Ontogeny and Consequences of Gestational Nutrient Restriction in Offspring

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Increasing adiposity predisposes to the development of the metabolic syndrome, in part, through adipose tissue dysregulation and inflammation. In addition, offspring nutrient-restricted (NR) in utero can exhibit an increased risk of early-onset insulin resistance and obesity, although the mechanisms remain unclear. We aimed to: 1) define adipose tissue ontogeny of key proinflammatory and endoplasmic reticulum stress gene expression from late fetal to early adult life and 2) examine the impact on these genes in gestational nutrient restriction. Pregnant sheep were fed 100% (control) or 50% (NR) of their nutritional requirements between early to mid (28–80 d, term ~147 d) or late (110–147 d) gestation. In control offspring, toll-like receptor 4 (TLR4), and the macrophage marker CD68, peaked at 30 d of life before declining. IL-18 peaked at 6 months of age, whereas the endoplasmic reticulum chaperone glucose-regulated protein 78 peaked at birth and subsequently declined through postnatal life. TLR4 and CD68 positively correlated with relative adipose tissue mass and with each other. Early to midgestational NR offspring had decreased abundance of IL-18 at 6 months of age. In late gestational NR offspring, CD68 was significantly lower at birth, a pattern that reversed in juvenile offspring, coupled with increased TLR4 abundance. In conclusion, the in utero nutritional environment can alter the adipose tissue inflammatory profile in offspring. This may contribute to the increased risk of insulin resistance or obesity, dependent on the timing of nutrient restriction. Establishing the optimal maternal diet during pregnancy could reduce the burden of later adult disease in the offspring. (Endocrinology 150: 3913–3920, 2009)
fetal to early adulthood, of adipose tissue inflammation and how
maternal nutrient restriction during discrete fetal developmental windows can impact it. We hypothesized that the expression of key proinflammatory mediators would rise with increasing relative fat mass and that, in NR offspring, there would be further dysregulation of these genes. Therefore, in the present study, we aimed to: 1) determine the early life ontogeny of key proinflammatory genes (TLR4, IL-18, and the macrophage marker CD68) and early ER stress (GRP78) in perirenal adipose tissue (PAT); 2) investigate the impact of maternal nutrient restriction, during early to mid and late gestation, on the PAT mRNA abundance of these key genes in the offspring; and 3) explore further inflammatory-related genes within PAT of the juvenile late NR offspring known to be glucose intolerant and have increased visceral adiposity. The PAT depot was chosen as it represents about 80% of total fat mass in the newborn sheep (24).

Materials and Methods

Ontological gene expression study

To establish the ontogeny of the genes of interest, we used a mixture of Welsh Mountain and Border Leicester Swaledale sheep. We previously demonstrated there are no significant differences of gene expression between breeds of the same developmental age (24, 25). PAT was sampled from fetuses at 140 d gestation (term = 147 d) and sheep at 1 d, 30 d, 6 months and 1 yr of age (total of 32 sheep, n = 5–8 per sampling age). These sampling points were chosen to represent important human equivalent developmental periods in early life, i.e. late fetus, newborn, infancy, prepubertal, and young adult, respectively. All animals were humanely euthanized with an overdose of pentobarbital sodium (100 mg/kg; Euthatal; RMB Animal Health, Stoke, UK). PAT was rapidly dissected, weighed, and flash frozen in liquid nitrogen before storage at −80 °C until analysis. All animals were born naturally after a normal pregnancy during which all mothers received 100% of their metabolizable energy (ME) throughout pregnancy, allowing for their own maintenance requirements plus that of the growing fetus according to the Agricultural Research Council recommendations (26).

Maternal nutrient manipulation studies

As previously discussed, fetal organ development can be nutritionally targeted, depending on the timing of the nutrient restriction undertaken in the mother (19). Therefore, we compared two periods of nutrient restriction.

Study 1: early to midgestational nutrient restriction (d 28–80)

The timing of nutrient restriction occurs before the period of maximal adipose tissue deposition in the fetus. In the sheep fetus, PAT mass increases 25-fold from 80 d gestation through to term (24). The experimental model and nutrient restriction protocol have previously been described in detail (25). Briefly, 23 pregnant Welsh Mountain sheep of a similar age and weight were individually housed from 28 d gestation. Pregnant sheep were randomly assigned to one of two nutritional groups. Control sheep were fed to appetite (100% of ME), whereas NR sheep were fed 50–60% of their ME from d 28 to 80. Food intakes were based on recommended amounts as defined by the Agricultural Research Council (26) and tailored for the growing fetus. After 80 d gestation, all sheep were offered 100% of their ME; this was again increased according to the gestation to account for the additional needs of the growing fetus. Offspring of control and NR pregnant sheep were then randomly allocated to have PAT sampling at 140 d gestation or at 6 months postnatal age (n = 5–7/group). Animals randomized to sampling at 140 d gestation were humanely euthanized (as described earlier) and the fetus rapidly...
dissected and weighed. PAT was dissected, weighed, and snap frozen as described earlier. The remaining offspring were reared as normal with their mothers until weaning (3 months) when they were put out to pasture. At 6 months of age, they were humanely euthanized and PAT sampled as described.

**Study 2: late gestational nutrient restriction (d 110 to term)**

This period of nutrient restriction was chosen to coincide with the period of maximal fat deposition in the growing fetus. After mating, 24 Border Leicester Swaledale sheep were individually housed and randomly assigned to one of two diets. Control animals were fed as per the control group in the early to midgestation study described earlier. Late NR pregnant sheep were fed as per the control group until d 110 gestation when they were fed 50–60% of their ME according to their maintenance requirements and that of the growing fetus. All offspring were born naturally and reared with their mother until time of tissue sampling or weaning at 3 months of age. After weaning, offspring were reared at pasture. Offspring (n = 4–8/group) were humanely euthanized at the time of tissue sampling, i.e. 1 d, 30 d, and 1 yr of age and PAT obtained (as described earlier).

All procedures were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986, and approved by the local ethics committee of the University of Nottingham.

**Laboratory procedures**

**RNA extraction**

Total RNA was extracted from a representative area of PAT (−1 g) using TRI-reagent (Sigma, Poole, UK). To verify the quantity and quality of the RNA extracted, we used spectrophotometric analysis (Nanodrop; Thermo Scientific Inc., Wilmington, DE). For each animal, 1 μg of RNA was reverse transcribed (RT) using reverse transcriptase and a Touchgene thermocycler (Techne; Barloworld Scientific Ltd., Stone, UK) for 40 cycles. We excluded negative controls. For each experiment, a 10-fold dilution standard curve was included as well as reference sample for standardization. qPCR was performed in 96-well plates using the Techne Quantica 14 real-time thermocycler (Techne; Barloworld Scientific) for 40 cycles. All data were analyzed using the ΔΔct method (27) and expressed as a ratio to the 1-yr-old control animals.

**Quantitative real-time PCR (qPCR)**

The mRNA abundance of the genes of interest was measured using qPCR. For each gene we created a standard curve, thus ensuring uniformity, efficiency and accuracy for each 96-well plate. DNA for each of the standard curves was made after DNA extraction (QIAquick gel extraction kit, 28704; QIAGEN Ltd., Crawley, UK) of the final product of PCR, run with 2% agarose gel electrophoresis. In addition, correct product validation of all primers were confirmed by product size on gel electrophoresis and, when necessary, gene sequencing with the results checked against known sequences on the GenBank database. To establish the mRNA abundance, we performed qPCR using 20 μl reactions consisting of 1 μl of cDNA, 1× SYBR Green master mix (QIAGEN) and forward and reverse ovine-specific oligonucleotide primers (Sigma-Aldrich, Guildingham, UK) (Table 1). Samples were run in duplicate and included negative controls. For each experiment, a 10-fold dilution standard curve was included as reference sample for standardization. qPCR was performed in 96-well plates using the Techne Quantica 14 real-time thermocycler (Techne; Barloworld Scientific) for 40 cycles. We excluded and repeated any experiments if the standard curve analysis demonstrated an R² < 0.983 or if the efficiency varied by greater than 5%. 18s rRNA was used as a housekeeping gene for the normalization of mRNA expression (21). Data were analyzed using the ΔΔct method (27) and expressed as a ratio to the 1-yr-old control animals.

**Statistical analysis**

Data were assessed for normality using Kolmogorov-Smirnov test followed by appropriate parametric or nonparametric analysis. Ontogeny data were analyzed using ANOVA with post hoc Bonferroni correction for multiple tests. Independent Student’s t test or Mann-Whitney test were used to compare between control and NR groups as appropriate. Correlations were determined by the Spearman rank order test. All data were analyzed using SPSS software (version 14.0; Chicago, IL) and expressed as means ± SEM with significance set at P < 0.05.

**Results**

**Ontogeny of inflammation and ER stress**

Both TLR4 and CD68 demonstrate similar ontological development patterns in PAT. The mRNA abundance of both TLR4 and CD68 are low in the late fetus and early newborn period peaking at 30 d before significantly declining through adolescence and into young adult life (Fig. 1, A and B). IL-18

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**TABLE 1.** Ovine-specific oligonucleotide forward and reverse primers used for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR-4 (44)</td>
<td>Forward, TGCTGCTGTTAATAATG</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>Reverse, CCCCCTGTTAAGGAGGAGC</td>
<td>177</td>
</tr>
<tr>
<td>CD68</td>
<td>Forward, GTCTTCATCCACACACCAGT</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Reverse, GCTGAGGACCTATTACCC</td>
<td>171</td>
</tr>
<tr>
<td>IL-18</td>
<td>Forward, ACGACGATTTTCTCTCCATTGC</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>Reverse, TGGGAAATTCCTCCAGAAGG</td>
<td>171</td>
</tr>
<tr>
<td>GRP78</td>
<td>Forward, GCCTGATTCTTCAAGACCATCCT</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Reverse, GCCCTCTGGAAGGCTTTT</td>
<td>150</td>
</tr>
<tr>
<td>MCP-1 (45)</td>
<td>Forward, CTTCCCGAGCATCTACCTTTA</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>Reverse, TACGAGACGACGAGAGGAG</td>
<td>136</td>
</tr>
<tr>
<td>CCR2</td>
<td>Forward, GTGGCTCTGACCTCTCCTC</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>Reverse, GAAACAGGCGCTGTTGAAAG</td>
<td>198</td>
</tr>
<tr>
<td>TNFα (46)</td>
<td>Forward, ATGACAGACGACCTCTCTG</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>Reverse, GGCGATGAACCTTTTGTAGA</td>
<td>198</td>
</tr>
</tbody>
</table>

**Forward, **

**Reverse, **

**Product size (bp),**
follows a similar pattern although the time of maximal expression is delayed, occurring at 6 months before declining in young adults (Fig. 1C). GRP78 peaks on the first day of life before a stepwise reduction through adolescence and into adulthood (Fig. 1D).

Increasing adiposity is positively associated with increasing adipose tissue inflammation. Therefore, we explored correlations of relative PAT mass, for all control animals and observed significant positive associations with mRNA abundance of TLR4 (R² = 0.63, P < 0.0001) and CD68 (R² = 0.58, P < 0.0001) in PAT (Fig. 2, A and B). Similarly, there was a positive correlation between relative PAT mass and TLR4 mRNA abundance (R² = 0.70, P < 0.0001) in control (Fig. 2C).

**Effects of early to midgestational nutrient restriction on PAT inflammation and ER stress**

Early to midgestational nutrient restriction in the 140-d fetus and 6-month adolescent had no effect on the PAT mRNA abundance of TLR4, CD68, or GRP78 (Fig. 3, A, B, and D). We observed a significant reduction (2.3-fold) in PAT IL-18 of 6-month NR offspring (Fig. 3C).

**Effects of late nutrient restriction on PAT inflammation and ER stress**

Control and late NR offspring had a similar mRNA abundance of TLR4 at 1 and 30 d of age (Fig. 4A). However, in the late NR offspring, there was a significant increase in TLR4 abundance (4.3-fold) between 1 and 30 d of age with no significant
difference observed in the control offspring. Subsequently at 1 yr of age, NR offspring have a significantly greater abundance (3-fold) of TLR4 compared with control offspring. On d 1 of life in late NR offspring, there was a significant reduction in the expression of CD68 compared with controls (Fig. 4B); however, this pattern is reversed at 1 yr with a 3.5-fold increase in the NR offspring. No differences were noted in IL-18 or GRP78 (Fig. 4, C and D), although the latter did show a trend ($P = 0.07$) toward an increased abundance in the late NR offspring at 1 yr of age.

We next explored important proinflammatory genes in the BAT of offspring at 1 yr of age (Fig. 5). Monocyte chemoattractant protein (MCP)-1, a potent macrophage signaling cytokine, and TNFα were not different between the groups. Chemokine receptor-2 (CCR2), whose main ligand is MCP-1, was significantly up-regulated (32-fold) in the late NR offspring. In addition, the abundance of uncoupling protein (UCP)-2, a mitochondrial protein known to decrease insulin signaling in adipose tissue (28), was also significantly increased (8-fold) in the late NR offspring. In keeping with the ontological development, irrespective of nutritional group, TLR4 and CD68 mRNA abundance in the 1-yr-old animals were positively correlated ($R^2 = 0.82$, $P < 0.001$).

**Discussion**

The present study is the first to describe the early life ontogeny, from fetus to young adult, of key genes implicated in inflammation and ER stress resulting in adipose tissue dysfunction. Furthermore, we demonstrate that maternal diet during pregnancy can influence the expression of these genes, within PAT, potentially contributing to the development of an early insulin-resistant phenotype in young adults.

**Ontogeny of TLR4, CD68, IL-18, and GRP78**

Both TLR4 and CD68 mRNA abundance follow a similar developmental pattern peaking at 30 d of age before declining to low adult levels. The peak at 30 d may represent a period whereby the transition from brown adipose tissue (BAT) to white adipose tissue (WAT) has occurred and therefore reflect tissue remodeling (29). TLR4 can be activated by FFAs (4). During early infancy, there is rapid growth and fat deposition (29). The greater abundance of TLR4, during this period of life, may represent the increased storage of lipids within the adipose tissue. This is supported by the strong correlation between TLR4 and relative PAT mass. The elevated FFA delivery to the adipose tissue may simulate increased TLR4 abundance, which may in turn alter the differentiation of preadipocytes and their subsequent proinflammatory properties (30). Activation of TLR4 will induce proinflammatory pathways within the adipose tissue and potentially recruit macrophages to the tissue. We used CD68 abundance as a relative marker of macrophage number because we and others (21, 31) have found it correlates well with adipose tissue macrophage content. There were strong positive correlations with CD68 and both relative PAT mass and TLR4 abundance. It is therefore plausible that fat mass, TLR4, and macrophage abundance are closely linked, even in lean individuals.

IL-18 expression in adipose tissue and plasma concentrations are significantly higher in obese, compared with lean individuals (12), as well as in individuals with type 2 diabetes (32). Our PAT IL-18 ontological data demonstrate a significant increase from the perinatal period into adolescence before declining in early adult life. Maximal IL-18 abundance occurs some time after that of TLR4 and CD68. In addition, relative PAT mass and IL-18 abundance did not correlate. The role of IL-18 in type 2 diabetes and obesity requires further clarification, although our data suggest that its abundance in PAT may not be as important as other proinflammatory genes such as TLR4. This may explain why IL-18 abundance in adipose tissue does not change in obese individuals after weight loss, unlike plasma concentrations, which decrease significantly (32). IL-18 may be more important in energy homeostasis and appetite regulation (11) than local adipose tissue inflammation and seems unlikely to be an important local mediator of insulin resistance.

During late fetal and early postnatal life, as seen in humans, sheep have significant amounts of BAT for nonshivering thermogenesis (29). After the early neonatal period, this BAT is replaced by WAT with very little BAT remaining after infancy. GRP78, an ER chaperone crucial for protein synthesis, peaks around birth, and is likely to represent the high metabolic rate within the BAT. The stepwise decline through the postnatal period could reflect the replacement of BAT with the less metabolically active WAT and therefore a reduction in protein synthesis. Total adult adipocyte number can be set in early childhood, occurring at an earlier age in obese compared with lean children (33). ER stress occurs in adipocytes of obese individuals and contributes to the inflammatory state within the tissue. The significantly greater abundance of GRP78 in early life, compared with adulthood, could represent a sensitive window whereby ER function in obese children is adversely altered.

**Impact of early to mid gestational nutrient restriction on inflammatory and ER stress pathways**

Using an ovine model, previous work by our group and others has demonstrated that early to mid gestational nutrient restriction results in increased adipose tissue mass in the late fetus (34). Furthermore, at 4 and 8 months of age, offspring have increased adiposity coupled with perturbed glucose/insulin handling (35). We therefore selected time points similar to these (late fetus and 6 months of age) to establish the effects in early to midgestation
NR offspring. Early to midgestational nutrient restriction did not alter PAT abundance of TLR4, CD68, or GRP78 in either the late fetus or adolescent offspring. Human epidemiological data from the Dutch famine suggest offspring exposed to poor maternal diet in early or midgestation were more likely to become obese or have obstructive airways disease, respectively (18). Our experimental intervention spans the boundaries of both of these time frames, so we might not expect to see significant differences in our young lean offspring. However, we recently demonstrated that there is a marked up-regulation of TLR4, CD68, and GRP78 in obese early to midgestational NR offspring at 1 yr of age compared with obese controls. It is therefore plausible that only with a subsequent second hit, that of early-onset obesity, do we observe the adverse PAT inflammatory manifestations of prior early to midgestation nutrient restriction in young adults (22).

IL-18 abundance in early to midgestational NR offspring is down-regulated at 6 months but not in the near-term fetus. The relative abundance of BAT compared with WAT in the late fetus may mask any significant differences at this time point. Epidemiological studies of human populations exposed to early gestational undernutrition demonstrate an increased risk of obesity in the offspring (18). Furthermore, IL-18−/− mice develop hyperphagia, obesity, and insulin resistance (11). The significant reduction of IL-18 observed in the adolescent NR offspring may be one potential mechanism explaining the increase risk of obesity in later life. Decreased IL-18 in the NR offspring may reduce appetite suppression and so increase food intake, promoting a positive energy balance and ultimately obesity. Clearly, further work establishing IL-18 receptor abundance and distribution as well as IL-18 plasma concentrations will help define the importance of these findings.

Impact of late gestational nutrient restriction on inflammatory and ER stress pathways

Offspring exposed to late gestational undernutrition are significantly more likely to become glucose intolerant (18). The late NR fetus has a reduced adipose tissue mass compared with controls (36). We have previously shown, using an ovine model, that late gestational NR juvenile offspring are significantly more glucose intolerant and insulin resistant and have a greater relative PAT mass (20), although the mechanisms causing this remain unclear. This discrepancy between reduced fetal adiposity and increased juvenile adiposity in late NR offspring may suggest that the early postnatal period switch from BAT to WAT may be an important window in which later fat mass is set (23). Therefore, the focus of the late nutrient restriction model was that of early life adipose tissue development during this transition phase (d 1 and 30 of life).

The present study demonstrates that late gestational nutrient restriction can impact on the expression of TLR4 and CD68 in PAT of offspring. There are no differences between control and late NR offspring for TLR4 abundance at 1 or 30 d. However, the significant increase observed for TLR4 between 1 and 30 d in the NR group is not evident in the control group. These findings are interesting when coupled with the decreased abundance of CD68 on d 1 of life in the NR offspring, a pattern that is reversed in later life. As discussed earlier, white adipocyte precursors are committed during prenatal or early postnatal life (23) and preadipocytes can be inhibited by macrophage secreted factors, therefore suppressing conversion into mature adipocytes (9). The reduction in macrophage content on d 1 of life could have important implications for early adipogenesis. Between d 1 and 30 of life, the adipose tissue undergoes significant remodeling (29) because BAT is replaced by WAT. This potentially represents a sensitive period during which the prenatal programmed reduction in macrophage content, and hence macrophage secreted factors, may allow more preadipocytes to undergo maturation. It would thus increase the adipocyte pool and may partly explain the greater PAT mass in juvenile late NR offspring. Subsequently with increasing adiposity, the marked rise in TLR4 could trigger proinflammatory pathways, most notably the nuclear factor-κB and c-Jun NH2-terminal kinase pathways (3, 37), increasing the macrophage content in juvenile late NR offspring. The increased macrophage content in the PAT may result in adipocyte and macrophage cross talk leading to a down-regulation of glucose/insulin signaling pathways. Lumeng et al. (38) demonstrated that both macrophage to adipocyte interaction and macrophage secreted factors resulted in a down-regulation of the glucose transporter GLUT4, in adipose tissue. Taken together, these findings may partly explain the reduced amounts of GLUT4 protein observed in the PAT of late gestational NR juvenile offspring (20). Future work should aim to identify whether the source of the TLR4 up-regulation is from the macrophages or adipocytes because this could have an important bearing on the mechanisms of type 2 diabetes.

Late gestational nutrient restriction had no effect on IL-18 or GRP78 abundance at any postnatal sampling point. IL-18 may be an important appetite suppressor (11), so it is not surprising that there are no differences in offspring after late nutrient restriction, a period known to have a greater influence over glucose metabolism rather than obesity (18). NR offspring have similar GRP78 expression to the control offspring suggesting that early ER stress is not apparent in our model. Our data suggest that it may be the inflammatory process that occurs before ER stress in this model. Further work investigating the impact of NR in older animals may better define this. In addition, the relative importance of other tissue depots needs to be explored.

To further investigate the potential mechanisms by which juvenile late gestational NR offspring are glucose intolerant and insulin resistant (20), we explored other key metabolic and inflammatory genes in these animals. MCP-1 abundance was not different between the groups, suggesting it is not the main recruitment signal for macrophages in our model, and this is in keeping with obesity data we previously published (22). Indeed, the role of MCP-1 in obesity-related macrophage signaling is controversial and other monocyte chemokines may be equally important (37). There is, however, a marked increase in CCR2, which could potentially represent a phenotypic switch by resident macrophages or an influx of nonresident macrophages; the increase in CD68 abundance supports the latter. Previous studies demonstrate that such macrophages are potent proinflammatory cells, unlike those already resident within adipose tissue and are able to promote insulin resistance within the tissue (39). TNFα abundance is not significantly different between the groups and,
at this early stage of inflammation, is unlikely to be an important adipose tissue cytokine in our model. However, TNFα may have a different expression pattern in other tissues, such as liver and muscle, thereby affecting insulin and glucose homeostasis. There is a significant increase in UCP-2 abundance in the late gestational NR offspring, which could be an adaptive response to counteract the increase in reactive oxygen species known to occur with insulin resistance (40). However, the increase in UCP-2 could also inhibit insulin signaling within adipose tissue (28) and potentially accelerate the onset of type 2 diabetes.

The present study does not address the potential role of epigenetics and the influence of promoter regions associated with inflammatory genes. A growing body of evidence now highlights the important role of the prenatal diet and how this can alter epigenetic mechanisms (41). These findings are interesting in the context of the recent work of Li et al. (42) demonstrating that promoter regions, within monocytes, can alter the proinflammatory properties of the cell potentially influencing the development of insulin resistance. Our data further strengthen the hypothesis that the in utero environment can influence the risk of disease in later life and that this may occur via pathways involved in adipogenesis and inflammation. Complementary work is needed to clearly identify the mechanisms involved along with any epigenetic interactions.

Although the focus of the present study is on gene expression, it would also be interesting to confirm that protein abundance matches these data. Although not feasible in this study, we recently demonstrated that, in PAT of sheep, both GRP78 and CD68 mRNA correlate with the protein abundance and macropage content (as identified by crown-like structures), respectively (22). Furthermore, in humans, both adipose tissue mRNA and protein abundance for TLR-4 and IL-18 seem to correlate (12, 43).

In conclusion, this is the first study to demonstrate, in nonobese individuals, that adipose tissue inflammation can be altered by the in utero nutritional environment and that this may contribute to the early abnormal glucose/insulin signaling disturbances observed in the metabolic syndrome. It is possible that the mechanisms involved are related to macrophage and adipocyte interactions from an early age, although further work is needed to clarify this. These findings may partly explain why human populations exposed to a suboptimal nutritional environment in late gestation are more likely to develop glucose intolerance. Additional work aimed at establishing what constitutes optimal maternal nutrition during pregnancy may allow us to reduce the global burden of the metabolic syndrome.

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