Diabetologia (2008) 51:836–845
DOI 10.1007/s00125-008-0956-5

ARTICLE

The intrauterine metabolic environment modulates the gene expression pattern in fetal rat islets: prevention by maternal taurine supplementation

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Received: 6 November 2007 / Accepted: 11 January 2008 / Published online: 3 March 2008

Abstract
Aims/hypothesis Events during fetal life may in critical time windows programme tissue development leading to organ dysfunction with potentially harmful consequences in adulthood such as diabetes. In rats, the beta cell mass of progeny from dams fed with a low-protein (LP) diet during gestation is decreased at birth and metabolic perturbation lasts through adulthood even though a normal diet is given after birth or after weaning. Maternal and fetal plasma taurine levels are suboptimal. Maternal taurine supplementation prevents these induced abnormalities. In this study, we aimed to reveal changes in gene expression in fetal islets affected by the LP diet and how taurine may prevent these changes.
Methods Pregnant Wistar rats were fed an LP diet (8% [wt/wt] protein) supplemented or not with taurine in the drinking water or a control diet (20% [wt/wt] protein). At 21.5 days of gestation, fetal pancreases were removed, digested and cultured for 7 days. Neoformed islets were collected and transcriptome analysis was performed.
Results Maternal LP diet significantly changed the expression of more than 10% of the genes. Tricarboxylic acid cycle and ATP production were highly targeted, but so too were cell proliferation and defence. Maternal taurine supplementation normalised the expression of all altered genes.
Conclusions/interpretation Development of the beta cells and particularly their respiration is modulated by the intrauterine environment, which may epigenetically modify expression of the genome and programme the beta cell towards a pre-diabetic phenotype. This mis-programming by maternal LP diet was prevented by early taurine intervention.

Keywords Diabetes · Early programming · Fetal islets · Maternal low-protein diet · Rats · Taurine · Transcriptome

Abbreviations

EST	expressed sequence tag
LP	low protein
LPT	low-protein diet supplemented with taurine
SAM	significance analysis of microarrays
TCA	tricarboxylic acid

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Electronic supplementary material The online version of this article (doi:10.1007/s00125-008-0956-5) contains supplementary material, which is available to authorised users.

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Introduction

The growth and development of the fetus is a highly controlled process encoded in its genetic potential. This process is highly susceptible to changes in the intrauterine environment. Epidemiological studies have suggested an association between poor fetal or infant growth and increased risk of developing glucose intolerance and metabolic syndrome later in life [1, 2]. A recent review study suggested that inadequate maternal nutrition might disturb the development of the fetus, which must adopt strategies to ensure survival that will programme its future health [3]. In experimental animal models, alteration of the intrauterine environment induced, for example, by gestational diabetes [4], placental insufficiency [5, 6] or poor maternal nutrition [7–11] compromise the development of endocrine pancreas in the progeny and impact on its future health even in the subsequent generation [4, 6, 12]. Reduction of food intake by 50% in rats during gestation reduced the beta cell mass in offspring at birth by 30% [9]. When maternal diet was reduced in protein content during gestation, a number of findings were observed: reduction in beta cell mass due to diminished proliferation [8, 13], enhanced apoptosis [11, 13], reduced insulin secretion in vitro from isolated islets [14], impaired islet vascularisation [8, 15] and enhanced sensitivity to cytotoxic effects of cytokines in vitro [11]. Increased susceptibility to cytokine cytotoxicity and lower insulin secretion persisted in adulthood despite limiting the low-protein (LP) diet to the period of gestation and lactation [7, 12]. Maternal and fetal plasma glucose levels were not altered by maternal malnutrition, but the plasma amino acid profile was perturbed both in the mother and their fetuses [16]. The concentration of taurine, a sulphur amino acid that does not participate in protein synthesis, was decreased in maternal and fetal plasma. Taurine is important during development [17]. Supplementation of the maternal LP diet with taurine to normalise the fetal plasma level corrected pancreas development, the insulin secretory deficiency and susceptibility to cytokines and toxins [11, 12, 15, 18, 19].

Previously, we have used proteomics to study fetal rat islet protein expression in animals fed a control or a LP diet, demonstrating that expression of proteins involved in a number of different biological pathways was modified by a maternal LP diet [20]. The present study aimed: (1) to determine the changes in gene expression that are induced by the maternal LP diet and may favour a ‘prediabetic phenotype’ and (2) to investigate to what extent and by which pathways maternal taurine supplementation restores the normal phenotype.

Methods

Animals Out-bred virgin female Wistar rats (Janvier, Le Genest Saint Isle, France) aged 3 months were caged overnight with male rats for fertilisation. Copulation was verified the next morning by detection of a vaginal plug. The animals were housed at 25°C with 10 h dark–14 h light cycle. One group of pregnant rats was fed a normal control diet (20% protein content), a second group an isoenergetic LP diet (8% [wt/wt] protein content) and a third was fed the LP diet supplemented with taurine (LPT) (2.5% [wt/wt]; Sigma Chemical, Brussels, Belgium) in the drinking water. The composition of both diets has been described previously [8]. Diets were purchased from Hope Farms (Woerden, The Netherlands). They were similar in fat content and rendered isoenergetic by addition of carbohydrate to the LP diet. The rats were given free access to their respective diets. Pregnant rats in all three groups ate the same quantity of food and drank the same volume of water. At day 21.5 of gestation, dams and their fetuses were killed by decapitation and the fetal pancreases were removed for islet isolation.

All animal experiments were carried out according to national and international law and ethical standards. The experiments were approved by the Animal Ethics Committees of the Université Catholique de Louvain, Belgium.

Isolation, culture and labelling of islets for microarray analysis Briefly, the pancreases were removed from 21.5-day-old control, LP and LPT fetuses, digested with collagenase (specific activity 381 U/ml; Sigma, St Louis, MO, USA) and cultured for 7 days in RPMI 1640+10% (vol./vol.) fetal bovine serum and antibiotics (one pancreas/2 ml medium). From day 2 of culture, the medium was replaced every 24 h. After 7 days of culture, neoformed islets were handpicked as described elsewhere [14]. These cultures provided islets (90–95% of beta cells) that aggregated progressively on the layer of non-endocrine cells.

For microarray analyses as well as for RT-PCR and ATP analyses, 15–19 pregnant rats, 200 fetuses, 12 cultures and 10,000 neoformed islets were used per experimental group. Batches of 500 neoformed fetal islets were handpicked and total RNA was extracted by Trizol method and stored until use.

Gene expression analysis The following procedures were all performed according to Affymetrix (Santa Clare, CA, USA) standard procedures. Briefly, 5 μg total RNA was used as starting material for the target preparation. First and

second strand cDNA synthesis was performed using the SuperScript II System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions except for the use of an oligo-dT primer containing a T7 RNA polymerase promoter site. Labelled aRNA was prepared using a labelling kit (BioArray High Yield RNA Transcript; Enzo, New York, USA) that used biotin-labelled CTP and UTP (Enzo) in the reaction together with unlabelled NTPs. Unincorporated nucleotides were removed using RNeasy columns (Qiagen, New York, USA). cRNA (15 μg) was fragmented, loaded onto the Affymetrix Rat Genome RAE 230A probe array cartridge and hybridised for 16 h. The arrays were washed and stained in the Affymetrix Fluidics Station and scanned using a confocal laser-scanning microscope (Scanner 3000 System with workstation and autoLoader; GeneChip; Iobion, La Jolla, CA, USA). The raw image files from the quantitative scanning were analysed by Affymetrix Gene Expression Analysis Software (MAS 5.0) resulting in cell files containing background-corrected probe values. Five independent array experiments were used for each of the three experimental conditions.

The expression data were normalised using the sequence-enhanced Robust Multi-Array Average (GC-RMA) algorithm [21] implemented in ArrayAssist Software (Iobion). The data were filtered to remove probe sets below the detection limit (absent according to Affymetrix detection algorithm in MAS 5.0), thereby reducing the dataset from 15,866 to 10,346 probes (annotated genes and expressed sequence tags [ESTs]) with detection in at least three of five arrays (Table 1).

Table 1 General data from the Affymetrix microarray analyses

	RAE 230A probe array
Probes on array	15,866
Annotated genes on array	4,699
ESTs on array	11,167
Probes present in islets[a]	10,346
Annotated genes in islets	3,871
Annotated ESTs in islets	6,475
C vs LP SAM analysis in total *	1,124
Annotated in SAM analysis	459
ESTs in SAM analysis	665
C vs LPT SAM analysis in total *	0

C, control
[a] On minimum of three of five chips
*$p<0.05$

Significance analysis was performed using significance analysis of microarrays (SAM), which assigns a score to each gene on the basis of change in gene expression relative to the standard deviation of repeated measurements. For genes with scores greater than an adjustable threshold, SAM uses permutations of the repeated measurements to estimate the percentage of genes identified by chance, as described by Tusher et al. [22]. Multiclass response was used and considered genes with q values below 5% to be significantly differentially expressed.

In a first step, biological pathways targeted by the maternal LP diet were identified using the Affymetrix tool browsing the genes identified as significantly changed. In a second step, genes were assigned to functional groups by database searches on PubMed [23] and SOURCE (http://source.stanford.edu/cgi-bin/source/sourceSearch), which compile information from UniGene [24], Swiss-Prot [25], GeneMap99 [26], RHdb [27] and LocusLink [28] websites.

Quantitative real-time gene expression To confirm our results from the microarray analysis, quantitative real-time PCR analysis was performed on selected genes that were significantly changed by LP diet and restored by taurine. We chose genes involved in cellular defence (*Sod3*, *Hspa1a* [heat shock protein 1A, which corresponds to the heat shock 70 kDa protein 1A in humans; also known as *Hsp70-3* or *Hsp70.3*] and *Prdx3*), proliferation (*Igf2* and *Igf1r*) and metabolism (*Mdh1*). Five to eight RT-PCR analyses were performed per experimental group.

Total RNA was extracted from isolated neoformed islets from control, LP and LPT fetuses. Cells were lysed in a 1% (vol./vol.) β-mercaptoethanol-containing buffer obtained from an RNA extraction kit (Macherey Nagel, Hoerdt, France); RNA was extracted as described by the manufacturer. All RNA used for quantitative real-time gene analysis met the minimum requirement of at least a 1.8 ratio of 18S:28S rRNA. The RNA aliquots were stored at −80°C before use.

The cDNAs were synthesised from 1 μg DNA-free total RNA. Reverse transcription was performed as described by the manufacturer (Amersham Biosciences, Orsay, France) by adding 0.5 μg OligodT_{20} (Invitrogen, Merelbeke, Belgium). Products were incubated for 1 h at 37°C to allow retro-transcription. The final reaction mixture was divided into aliquots and stored at −20°C until used.

The mRNA was monitored in 96-well plates by quantitative RT-PCR of the target fragments and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) housekeeping gene sequence as internal standard. Amplification was achieved with specific primers of the target sequence (details, see Electronic supplementary material [ESM] Table 1).

The template concentration per reaction represented one-tenth of the cDNA reaction performed on 1 μg total RNA. Amplification was achieved in 20 μl reaction mixture containing 2 μl cDNA, 25 pmol/l of each oligonucleotide primer and 2× Sybr Green Master Mix (Westburg, Leusden, The Netherlands).

After activation of the hot-start DNA polymerase for 15 min at 95°C, solutions underwent 40 cycles of amplification in a sequence detection system (ABI PRISM 7000; Applied Biosystems, Lennik, Belgium). Amplification parameters included 15 s denaturation at 94°C and a 1 min annealing and extension step at 60°C. Direct detection of PCR products was monitored by measuring the increase in fluorescence caused by the binding of SYBR Green to double-stranded DNA. RT blank control PCRs showed no product amplification for all genes examined in this study (data not shown).

All quantifications were achieved with the comparative Ct method and normalised to the endogenous control *Gapdh* mRNAs as described by the manufacturer. Each sample was analysed in duplicate.

Primers were designed using the software Primer Express (Applied Biosystems). For each gene of interest, at least three couples of primers were tested for efficacy. Primers were selected only when their efficacy, calculated as described by the manufacturer, exceeded 95%.

For each gene of interest, samples of islet preparations in each condition were amplified in 96-well plates concomitantly with specific primers for the gene of interest and for *Gapdh* as internal standard. As described by the manufacturer, quantification requires the calculation of: (1) the mean Ct value of the replicate wells for each sample; (2) the difference between the mean Ct values of the samples in the wells containing the gene of interest and those of the internal standards (ΔCt); and (3) the difference between the mean ΔCt values of the samples and the mean ΔCt value of the control sample (ΔΔCt). The relative quantity value was expressed as $2^{-\Delta\Delta Ct}$.

ATP measurement Islets collected after 7 days of culture were washed in KRB and incubated in KRB without glucose at 37°C in 5% CO_2/O_2 air for 60 min. Islets were then divided into batches of 100 islets and transferred into dishes containing KRB with 3.3 or 16.7 mmol/l glucose. After 2 h incubation, islets were transferred in cold PBS and lysis buffer for ATP extraction was added. Islets were sonicated for 30 s. Samples were immediately placed on ice. Islet lysate ATP content was measured using ATP luminescent assay kit (Promega, Madison, WI, USA) using 50 μl in duplicate. ATP was calculated per μg protein in the islet homogenate. Five to seven independent experiments were performed for each experimental group.

Table 2 Genes identified as being involved in cellular respiration, glycolysis, TCA cycle and electron transport, and seen to be significantly changed by the maternal LP diet

Gene ID	Gene name	Gene symbol	Fold change[a]
Glycolysis			
1367743_at	Phosphofructokinase, liver, B-type	*Pfkl*	0.64
1386961_at	Phosphofructokinase, muscle	*Pfkm*	1.8
1371392_at	Glucose phosphate isomerase	*Gpi*	0.70
1386998_at	Aldolase C, fructose biphospate	*Aldoc*	0.61
TCA cycle			
1367589_at	Aconitase 2, mitochondrial	*Aco2*	1.08
1367642_at	Succinate-CoA ligase, GDP-forming, alpha subunit	*Suclg1*	1.18
1367670_at	Fumarate hydratase 1	*Fh1*	1.26
1369967_at	Citrate synthase	*Cs*	1.65
1370865_at	Isocitrate dehydrogenase 3, gamma	*Idh3g*	1.14
1371296_at	Succinate dehydrogenase complex, subunit D, integral membrane protein	*Sdhd*	1.28
1388294_at	Succinate dehydrogenase complex, subunit D, integral membrane protein	*Sdhd*	1.16
1371311_at	Succinate dehydrogenase complex, subunit C, integral membrane protein	*Sdhc*	1.15
1372123_at	Succinate dehydrogenase complex, subunit B, iron sulphur (Ip) (predicted)	*Sdhb* [b]	1.17
1372790_at	Malate dehydrogenase 1, NAD (soluble)	*Mdh1*	1.40
Ions transport			
1368648_at	Cytochrome C oxidase subunit IV isoform 2	*Cox4i2*	0.37
1398857_at	Surfeit 1	*Surf1*	1.30

Numbers below 1 are downregulated and numbers above 1 are upregulated genes in low protein islets
[a] LP vs control ($p<0.05$)
[b] Predicted

Table 3 Genes identified as being involved in beta cell proliferation and cell cycle, and seen to be significantly changed by the maternal LP

Gene ID	Gene name	Gene symbol	Fold change[a]
1367571_at	Insulin-like growth factor 2	*Igf2*	0.8
1386878_at	IGF binding protein 2	*Igfbp2*	0.73
1368123_at	IGF-1 receptor	*Igf1r*	0.75
1369735_at	Growth arrest gene 6	*Gas6*	0.77
1386884_at	Protease, serine, 11 (Igf binding)	*Prss11*	0.60
1367631_at	Connective tissue growth factor	*Ctgf*	0.34
1370789_at	Prolactin receptor	*Prlr*	2.10
1370345_at	Cyclin B1	*Ccnb1*	1.33
1388154_at	E2F transcription factor 5	*E2f5*	1.26
1370699_at	EGF receptor	*Egfr*	1.34
1369699_at	GLP-1 receptor	*Glp1r*	1.22
1368947_at	Growth arrest gene	*Gadd45*	1.42
1367927_at	Prohibitin	*Phb*	1.24
1371949_at	Basic leucine zipper and W2 domains	*Bzw1*	1.39
1388305_at	A-Raf proto-oncogene serine/threonine-protein kinase	*Araf1*	1.32
1388105_at	D123 gene product	*D123*	1.26
1388629_at	Inosine-5′-monophosphate dehydrogenase 2	*Impdh2*	1.22
1367715-at	Tumour necrosis factor receptor super family	*Tnfrsf1a*	0.80

Numbers below 1 are downregulated and numbers above 1 are upregulated genes in low protein islets

[a] LP vs control ($p<0.05$)

Results

Microarray analysis Analysing the expressed mRNA in the fetal islets collected after 7 days of culture, we found that of 15,866 RNAs probes, 10,346 were detectable in the islets (Table 1). Of the 10,346 probes present, 3,871 probes represented annotated genes and 6,475 ESTs. Maternal LP diet significantly changed the expression ($p<0.05$) of 1,124 genes and ESTs, of which 74.4% (836) were upregulated and 25.6% (288) downregulated. The maximally increased or decreased expression level was threefold (Tables 2, 3 and 4). Every gene modified by the LP diet was restored by taurine, since no significantly different gene expression levels were identified when comparing control and LPT islets.

First, we identified biological pathways targeted by the maternal LP diet using the Affymetrix tool, NetAffx Analysis Center, browsing the genes annotated that were significantly ($p<0.05$) changed ($n=459$ genes; Table 1). This analysis strongly suggested that the metabolism of the fetal beta cell was dramatically altered (Fig. 1). Maternal LP diet affected 5.1% of genes known to be involved in cellular metabolism pathways (256/5,019 genes). One of the most affected pathways was cellular respiration (Table 2), in which 12 genes were significantly (χ^2 test=98.6) modulated by the LP diet (Fig. 1). Eleven were upregulated and one, cytochrome C oxidase subunit IV isoform 2, was downregulated. Among these 12 genes, ten contribute to the tricarboxylic acid (TCA) cycle and were all significantly (χ^2 test=88.96) upregulated (Fig. 1, Table 2). In addition, more than 10% of the 459 genes identified as significantly changed in the LP diet islets encoded for mitochondrial proteins.

Control as well as LP and LPT fetal islets expressed transcriptional factors involved in beta cell differentiation. *Pdx1*, *Hnf6*, *Hnf1*, *Ngn3*, *Nkx6*, *Pax4* and *Pax6* genes were all present and similarly expressed in the three groups. In

Table 4 Identified genes involved in the beta cell defence significantly changed by the maternal LP diet

Gene ID	Gene name	Gene symbol	Fold change[a]
1370912_at	Heat shock protein 1A	*Hspa1a*	0.36
1368247_at	Heat shock protein 1B	*Hspa1b*	0.34
1367577_at	Heat shock protein 1	*Hspb1*	0.36
1368322_at	Superoxide dismutase 3	*Sod3*	0.54
1372727_at	Suppressor of cytokine signalling 2	*Socs2*	0.57
1367591_at	Peroxiredoxin 3	*Prdx3*	1.21
1367903_at	Haem oxygenase 2	*Hmox2*	1.33
1369994_at	Calcitonin gene related peptide receptor component protein	*Crcp*	1.38
1368311_at	Methylated DNA protein methyl transferase	*Mgmt*	1.23
1374911_at	Oxidative stress responsive 1	*Osr1*	1.59
1368354-at	Glutathione *S*-transferase theta	*Gstt1*	1.3

Numbers below 1 are downregulated and numbers above 1 are upregulated genes in low protein islets

[a] LP vs control ($p<0.05$)

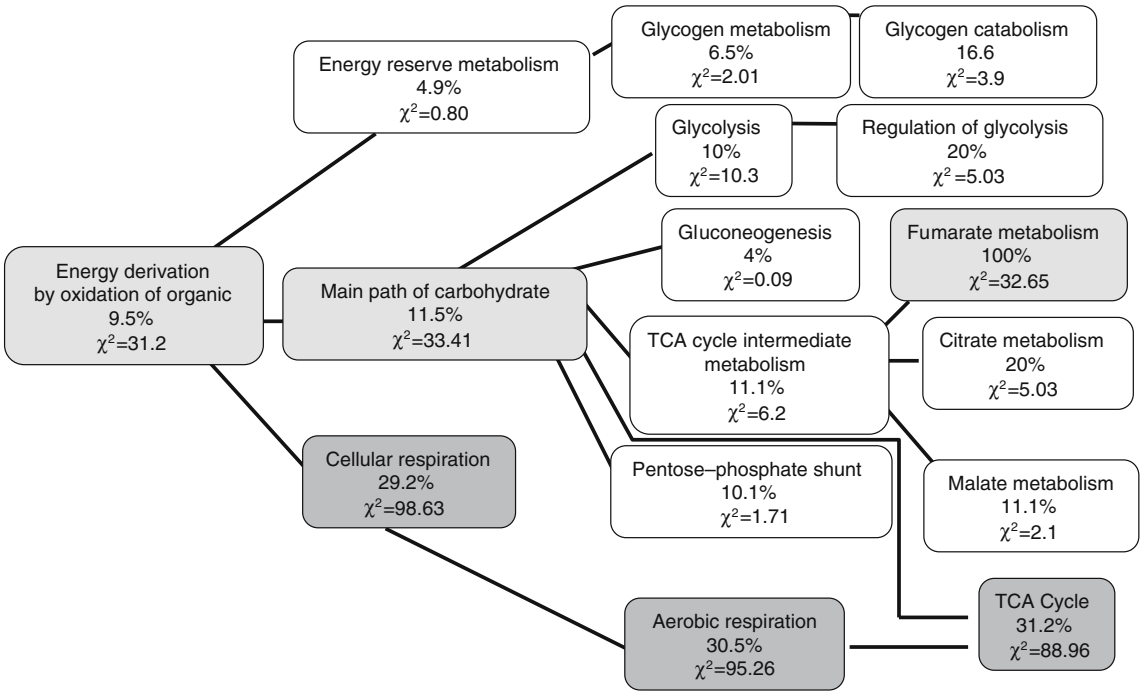

Fig. 1 Networks identified by Affymetrix analyses showing that maternal LP targeted the cellular respiration and the TCA cycle. %, percentage of genes involved the pathway and significantly changed by the LP diet. Of the genes involved in the TCA cycle, 31% (ten of 32) were altered by the LP diet

addition, *Ins1*, *Ins2*, *Gcg*, *Sst* and *Slc2a2* (also known as *Glut2*) were also equally expressed in the three groups. In contrast, many genes involved in cell cycle and cell proliferation were changed by the LP diet (Table 3) and restored by taurine. The genes encoding some growth factors, their receptors and their binding proteins such as *Igf2*, *Igfbp2*, *Igf1r*, *Glp1*, *Egfr* and *Ctgf* were significantly downregulated, while others favouring growth arrest were upregulated in LP islets (Table 3).

Of genes involved in cellular protection, LP diet reduced expression of *Hspa1a* and *Hspa1b*, *Hspb1* [also known as *Hsp27*]*)* by 65% compared with controls. *Sod3* and *Socs2* were also 50% downregulated. Other genes coding for antioxidant enzymes such as *Prdx3*, *Hmox2* and *Gstt1* were significantly upregulated by the LP diet, as also was *Osr1* (Table 4).

RT-PCR gene expression Maternal LP diet significantly reduced the expression of *Sod3* and *Hspa1a* ($p<0.05$) mRNA and maternal taurine supplementation restored these levels (Fig. 2a). An increase in the expression of *Prdx3* (Fig. 2a) and a decrease in that of *Igf2* and *Igf1r* (Fig. 2b) were observed in LP islets compared with control islets, but the difference was not significant. Compared with LP islets, taurine supplementation increased *Igf2* and *Igf1r* expression (Fig. 2b). *Mdh1* mRNA expression was increased by the LP diet and restored with taurine although the difference was not significant (Fig. 2c).

ATP production When fetal islets were incubated for 2 h in 3.3 mmol/l glucose, LP islets produced significantly ($p<0.05$) more ATP than control and LPT islets. No difference was observed between control and LPT islets. Protein content per 100 islets was 17.45±0.41, 17.26±0.33 and 16.45±0.22 μg in the control, LP and LPT groups respectively; it was similar in the three groups. When control and LPT islets were stimulated with 16.7 mmol/l glucose, they increased their ATP production by 300% ($p=0.07$), while no significant increase was observed in LP islets (Fig. 3).

Discussion

By microarray analyses, we demonstrated that maternal LP diet altered more than 10% of the gene expression in fetal islets. This was seen despite the islets being maintained in culture for 7 days. The TCA cycle seemed to be a major target. We further showed that taurine supplementation to the maternal LP diet prevented these changes.

Maternal LP diet induced up to threefold changes in the relative expression level of several genes, a finding which was also observed in other gene expression analyses of diet manipulation [29]. These changes are smaller than those observed in islets exposed to acute stress, e.g. by cytokines [30] or to high glucose levels [31]. However, significant

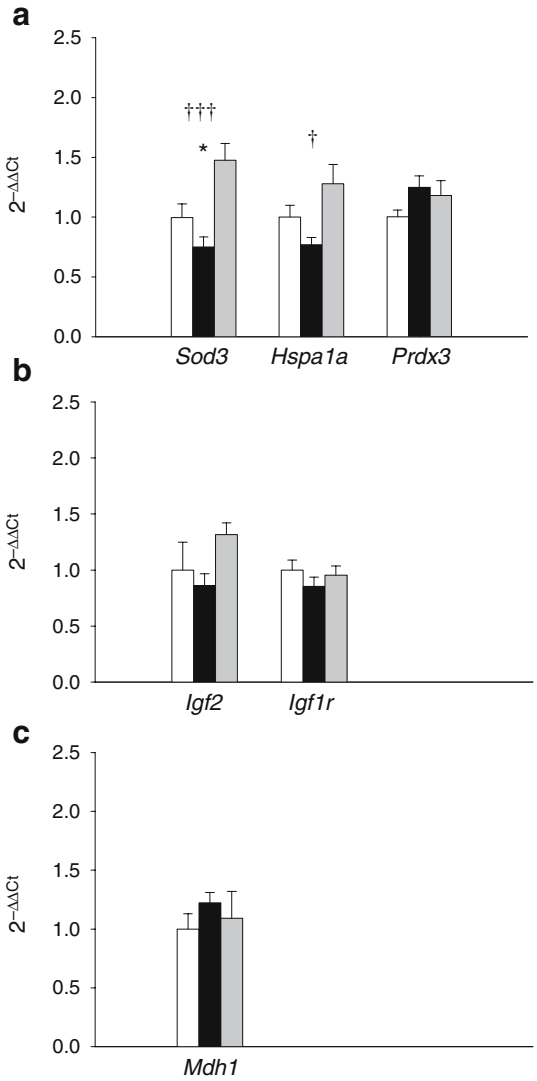

Fig. 2 Levels of *Sod3*, *Hspa1a* and *Prdx3* (**a**), *Igf2* and *Igf1r* (**b**), and *Mdh1* (**c**), measured by RT-PCR in control (white bars), LP (black bars) and LPT (grey bars) fetal islets. Data are means±SEM, n=5–8. *p<0.05 control vs LP; †p<0.05, †††p<0.001 LP vs LPT

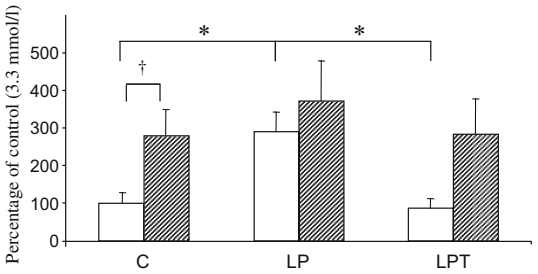

Fig. 3 Level of ATP produced by control (C), LP and LPT fetal islets in 3.3 mmol/l (white bars) and in 16.7 mmol/l (hatched bars) glucose. Data are means±SEM, n=5–7, expressed as percentage of controls. *p<0.05 LP vs C and LPT; †p=0.07

changes in the expression levels of 10% of islet genes seem sufficient to modify the fetal beta cell phenotype. This emphasises the significance of maternal diet manipulation as an epigenetic phenomenon. The study design, however, does not allow any discrimination to be made between primary causative or secondary changes in gene expression leading to the phenotype modification.

Normal beta cells have aerobic metabolism at least threefold higher than in most other cell types and may therefore be more susceptible to nutritional environment [32, 33]. First, we identified that the lack of protein in the diet targeted cellular respiration mechanism, especially the TCA cycle, since ten of the 32 genes involved were upregulated in the LP fetal islets. ATP production measured in LP islets cultured in low glucose concentration (3.3 mmol/l) confirmed this. LP islets secreted less insulin than control already in basal condition [14]. It may be difficult to reconcile higher ATP production and low insulin release in LP islets, but we have clearly demonstrated that the alteration was located at the level of exocytotic events [14]. Compared with control and LPT islets, LP islets were unable to enhance their ATP production when stimulated with glucose. This may explain the lower insulin secretion in response to secretagogues reported in LP islets and its prevention by maternal taurine supplementation [19]. Further experiments measuring glucose oxidation and utilisation would have given insight into the altered beta cell metabolism of the LP islets. In addition we found that among the 459 annotated significantly changed genes, 10% are genes coding for mitochondrial proteins.

It is known that reduction of the placental blood flow during the last 3 days of gestation or high-fat diet during gestation in rats induced adulthood glucose intolerance, insulin resistance and abnormal mitochondrial function in the offspring [34, 35]. Given the link between reduced mitochondrial DNA content and development of type 2 diabetes [36], re-programming of mitochondrial function has been proposed as a key adaptation of the fetus to survive in utero in an altered nutritional environment [34, 35].

In a second step, we searched one by one among the 459 altered annotated genes for those that could explain features of the fetal beta cell phenotype previously demonstrated to be induced by maternal LP diet. Reduction in beta cell mass previously reported in LP diet fetuses most likely cannot be attributed to a diminished neogenesis and endocrine differentiation as proposed in the event of maternal caloric restriction [9], since the expression of important transcription factor genes involved in pancreas, islet and beta cell development (*Pdx1*, *Hnf6*, *Hnf1*, *Ngn3*, *Nkx6*, *Pax4* and *Pax6*) was affected neither by the maternal LP diet nor by taurine supplementation. The same was true for the two insulin genes and the glucagon, somatostatin and glucose transporter genes. In contrast, many genes involved in cell

cycle and cell proliferation were changed by the LP diet and their expression restored by taurine. This may fit in with the lengthening of the beta cell cycle and the lower proliferation rate that we previously described in fetuses from dams fed an LP diet [8, 13, 37].

Evidence for the involvement of the IGF gene/protein family in beta cell development is abundant. IGFs are mitogenic for beta cell and act as cell survival factors by inhibiting apoptosis [38]. IGFs are perturbed in plasma and hepatocytes of fetus from LP-fed mothers [39]. The pancreatic expression of *Igf2* mRNA and protein was lower in LP fetus and neonates [13]. By microarray analyses, we found that *Igf2*, *Igf1r* and *Igfbp2* were downregulated in LP islets and restored by taurine. IGF-2 exerts its mitogenic and antiapoptotic action via its binding to the IGF-1 receptor. IGF-1 receptor knockout mice have 50% reduction in their beta cell mass [40] and deletion of the IGF-1 and insulin receptors in the beta cell induced reduced beta cell mass and increased apoptosis and default in insulin secretion, three alterations that were also observed in the LP fetal islets [8, 11, 14].

Normal beta cells are equipped with lower antioxidant defences than others cell types [41] and are therefore more vulnerable to reactive oxygen, nitrogen species and cytokines. Beta cells from LP fetuses feature an increased rate of apoptosis after exposure to interleukin 1β and nitric oxide [11]. This might be caused by reduction in expression of *Hspa1a*, *Hspa1b*, *Hspb1* and *Sod3* as observed in the microarray, *Hspa1a* being confirmed by RT-PCR. The increased expression of *Osr1* could contribute to the higher vulnerability. Ten proteins involved in protein folding and chaperoning were found to be altered by the LP diet in proteomic analyses [20]. The microarray analysis also revealed that increased expression of *Hmox2*, *Prdx3* and *Mgmt* involved cellular defence and DNA repair. *Crcp*, which is known to be upregulated in response to inflammation, was upregulated in the LP islets. This may be taken as proxy of increased oxidative stress in these islets. Interestingly, islets from adult offspring of mothers fed an 8% protein diet secrete more nitric oxide then control islets, even in absence of cytokines [42]. These findings suggest that the maternal LP diet promotes a fetal beta cell phenotype with increased susceptibility to cytokines, beta cell toxins and oxidative stress.

The simple supplementation of maternal diet with taurine abolished the effects of the LP diet on gene expression. We have previously shown that maternal taurine supplementation of the LP diet preserved the normal fetal beta cell phenotype [11, 12, 15, 18, 19]. No single gene or specific pathways responsible for the taurine effect could be identified, since every gene had an expression level similar to that in control islets. Furthermore, the preventive effect of taurine supplementation cannot be accounted for by a change in the expression of *Slc6a6,* the gene encoding for the taurine transporter, since it was present but not changed either by the LP diet or by taurine.

Hence, we have no explanation for the preventive action of taurine. Taurine prevented the changes in expression of genes involved in metabolism and the TCA cycle, and ATP production was completely normalised. Taurine might be incorporated into mitochondria through a putative mitochondrial taurine transporter [43]. In beta cells overexpressing uncoupling protein 2, taurine increased mitochondrial Ca^{2+} influx through the Ca^{2+} uniporter, thereby enhancing mitochondrial metabolic function and increasing the ATP/ADP ratio [44]. It is important to note that addition of taurine to the LP diet normalised islet ATP production in the presence of low and high glucose concentration. It has been shown by others that increased reactive oxygen species production in hyperglycaemic adult rats infused with glucose may be prevented by taurine co-infusion [45]. Therefore, the taurine effects we reported might be mediated by normalisation of mitochondrial metabolism. Interestingly, a recent report suggested that taurine could critically affect mitochondrial function. Yasukawa et al. [46] found two novel taurine-containing modified uridines in human mitochondrial DNA. When the uridine modifications were not present, defective mitochondrial function occurred and diseases such as mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MERRF) syndrome and myoclonic epilepsy with ragged red fibres (MELAS) syndrome may ensue.

In conclusion, this study demonstrates that reduction of protein intake during pregnancy has considerable impact at the level of the fetal insulin-secreting cell and affects expression of more than 10% of its genes. Cellular respiration seems to be the major target. Our finding that expression changes persist in culture after exposure to abnormal maternal metabolic environment supports the notion that the intrauterine programming of gene expression is involved. Importantly, LP diet-induced changes in gene expression can be fully prevented by adding taurine to the maternal LP diet. This observation emphasises that taurine plays an important role in the development of the endocrine pancreas and needs to be further investigated. One question that remains is: is the altered phenotype that we observed the consequence of few modifications of gene expression leading to many beta cell secondary effects, or does it result from induction of a wide network of relatively minor imbalances?

Acknowledgements This work was supported by the European Commission (NUTRIX QLK1–2000–00083, 5th Frame Programme), the Parthenon Trust (London, UK), Fonds de la Recherche Scientifique (FNRS), The Danish Diabetes Association, Novo Nordisk A/S, Denmark, the Michaelsen Foundation and University of Copenhagen.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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