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Delivery by Caesarean section, rather than vaginal delivery, promotes hepatic steatosis in piglets

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ABSTRACT

There has been a marked increase in the number of babies born by elective CS (Caesarean section). Following CS, the lack of normal stimuli that occur at birth alters the thermogeneic response, but any effects on hepatic metabolism have not been identified. In the present study, we compared the effect of delivery on hepatic metabolism in piglets, born either by CS or VD (vaginal delivery) and fed by TPN (total parenteral nutrition), by measuring lipid metabolism and enzyme activity coupled with metabolomic and genomic approaches. Hepatic lipid in the CS piglets at 7 days post-partum was in excess of 5 mg/g of liver consistent with hepatic steatosis, whereas in the VD piglets the amount of lipid was markedly lower (3 mg/g of liver) and below the threshold for a diagnosis of steatosis. Metabolomic analysis indicated that CS resulted in higher hepatic glycerol and lower glycerol phosphate dehydrogenase activity, suggesting that CS causes a decrease in hepatic gluconeogenesis from glycerol. CS also resulted in altered cholesterol handling and gene expression, despite the same dietary intake for 7 days post-partum. Furthermore, the CS piglets had a lower expression of interferon-responsive genes, but a higher expression of markers of immature hepatocytes. In conclusion, the results suggest that VD promotes normal liver maturation and hepatic metabolism, thereby reducing the accumulation of hepatic lipid.

INTRODUCTION

The number of babies born by elective CS (Caesarean section) has increased dramatically in the past 25 years, exceeding 25% of births in the U.K. in 2007 [1] and 30% in Brazil [2]. Despite the increasing rate of CS deliveries, little is known about the health implications for the offspring. Recently, epidemiological research has

Key words: birth, Caesarean section, liver, metabolic syndrome, steatosis, total parenteral nutrition (TPN).

Abbreviations: ACAT, acetyl-CoA acetyltransferase; ADRP, adipose differentiation-related protein; CS, Caesarean section; dpf, days post-fecundation; GALP, galanin-like peptide; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; IFN, interferon; IRF, IFN regulatory factor; KC, Kupffer cell; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acid; NK, natural killer; NKT cell, NK T-cell; PEG, paternally expressed; PEPCK, phosphoenolpyruvate carboxykinase; PID1, phosphotyrosine interaction-domain-containing 1; PLS-DA, partial least squares-discriminant analysis; qRT-PCR, quantitative real-time PCR; SCD1, stearoyl CoA desaturase 1; StAR, steroidogenic acute regulatory protein; T_3, 3,3′,5-tri-iodothyronine; TAG, triacylglycerol; TPN, total parenteral nutrition; VD, vaginal delivery.

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indicated that CS is a risk factor for Type 1 diabetes [3], asthma [4–6] and elevated BMI (body mass index) [7] in later life, when compared with children born by VD (vaginal delivery). Although CS is known to result in significant changes in both the hormonal milieu and the consequent plasma metabolite profile immediately post-partum [8], little is known about any long-term effects on metabolism due to programming in the perinatal period.

Marked changes in metabolism have to occur at birth to allow the newborn to adapt to extra-uterine life. Pre-partum, fetal blood glucose levels are regulated maternally; fetal glucose supply is continuous and insulin operates as a growth factor. Following birth, glucose supply is discontinuous, and the neonate must regulate its own blood glucose concentration via the insulin–glucagon axis. Consequently, at birth there is a marked decrease in circulating plasma insulin, whereas both plasma glucagon concentration and the number of glucagon receptors increase dramatically [9,10]. These changes result in a low insulin/glucagon ratio, which promotes glycogenolysis and gluconeogenesis to maintain plasma glucose concentrations.

The processes that reduce insulin secretion and promote glucagon production are thought to be transient hypoglycaemia following cessation of umbilical blood flow, the surge in stress hormones accompanying VD (predominantly adrenaline and cortisol) and the onset of enteral feeding [11]. During elective CS, at least one of the main triggers for altered insulin function is absent, and CS has been shown to modify the thermogenic response of both humans [12] and sheep [13]. These observations raise the question of whether CS results in altered glucose metabolism after birth, particularly in the liver.

Long-term abnormalities in glucose regulation can have serious consequences, and insulin resistance is linked to the development of obesity, NAFLD (non-alcoholic fatty liver disease) and the metabolic syndrome [14] with its resultant cardiovascular disease, all of which are significant and growing problems worldwide [15] in both children and adults [16]. Although increased energy intake and a more sedentary lifestyle contribute to insulin resistance, other factors, particularly perinatal programming events (e.g. disproportionate growth *in utero*), are also involved [17]. Insulin resistance is marked by enhanced net liver glucose production and decreased tissue glucose uptake, resulting in elevated blood glucose levels, and is associated with obesity and the accumulation of hepatic lipid [18,19].

Neonatal NAFLD is associated with TPN (total parenteral nutrition) [20], and we have shown previously [21] that the neonatal piglet is a useful model to study perinatal factors influencing the development of hepatic lipid accumulation. Given the changes in metabolism that occur at birth, we used this model to test the hypothesis that CS delivery would promote the accumulation of hepatic lipid in response to feeding by TPN. We therefore compared the metabolic and transcriptomic response of TPN-fed piglets that were born either by CS or VD.

MATERIALS AND METHODS

Ethical approval

Experimental protocols were carried out as defined by the regulations of the Animals (Scientific Procedures) Act 1986, followed local ethical standards and were approved by the Home Office (U.K.).

Experimental design

Four nulliparous sows (25 % Meishan, 12.5 % Duroc and 62.5 % Large White × Landrace) were artificially inseminated three times in a 36-h period with pooled semen from multiple boars. This method maximizes the genetic variation of the piglets without recourse to large numbers of sows entering the study and allows accurate estimation of the date of parturition. As described previously [21], piglets from two sows were delivered by CS at 112 dpf (days post-fecundation). Two sows delivered piglets by VD at term (mean, 114 dpf). Piglets were selected to balance experimental groups for gender, body weight at birth and maternal influences. All piglets received a 10 ml bolus feed of sow's milk prior to surgical implantation of bilateral jugular vein catheters, approx. 3 h post-partum. A 3 cm catheter (0.96 mm outer diameter, 0.58 mm inner diameter; Portex) was inserted into the jugular vein, under 0.5–2 % (v/v) isoflurane (Schering-Plough Animal Health) anaesthesia and tunnelled subcutaneously to the back of the neck, where it was stitched into the trapezius muscle on the shoulder and exteriorized. Analgesia [0.05 ml of 4 % (w/v) tolfenamic acid/kg of body weight; Vetoquinol] was administered to the piglets and they were housed in individual open-top cages.

Five CS piglets (three from one sow and two from the other) and four VD piglets (two from each sow) were fed parenterally with TPN solution (1690 J/ml; 99.34 mg/ml glucose and 17.89 mg/ml amino acids; Portsmouth Hospital NHS Trust, Portsmouth, U.K.) plus 20 % Intralipid® (8.36 MJ/l; Fresenius Kabi) administered as described previously [21] for 7 days (final rates of 5.8 ml $\cdot$ h^{-1} $\cdot$ kg^{-1} of body weight for the TPN solution and 1.0 ml $\cdot$ h^{-1} $\cdot$ kg^{-1} of body weight for Intralipid®).

Some of the piglets from the same litters (six CS and six VD) were killed humanely using sodium pentobarbitone (Euthatal; Rhone Merieux) at birth, and their livers were immediately excised, frozen in liquid nitrogen and stored at −80 °C to provide baseline data. At 7 days, the piglets fed parenterally were killed humanely and samples of liver, muscle and plasma were taken for subsequent analysis, as described previously [21].

Laboratory analysis

Total liver lipid content, together with fatty acid profiles of the liver TAG (triacylglycerol) and total liver lipid, were determined as described previously [21]. Hepatic TAG, phospholipid, cholesterol, NEFAs (non-esterified fatty acids) and cholesterol esters were analysed as described previously [22]. Enzyme activity was determined using assays described previously for glycerol phosphate dehydrogenase [23], PEPCK (phosphoenolpyruvate carboxykinase) [24], succinate dehydrogenase [25] and malate dehydrogenase [26]. Hepatic glycerol was measured according to the method described by Casado et al. [27] utilizing the free glycerol reagent (Sigma–Aldrich). Plasma hormones and metabolites were determined using commercially available kits, according to the manufacturer's instructions [insulin, Mercodia porcine insulin ELISA (Diagenics); glucagon, Linco Research glucagon RIA kit (Millipore); T_3 (3,3′,5-tri-iodothyronine), T_3 RIA kit (MP Biomedicals); haptoglobin, Phase Range™ haptoglobin kit (Biognosis); glucose, Infinity Glucose Oxidase reagent (Thermo); and plasma cholesterol, total cholesterol kit (Thermo)].

Metabolomic analysis

High-resolution ^{1}H NMR spectra were acquired from aqueous liver metabolites and blood plasma on a DRX Bruker Avance II+ spectrometer as described previously [27a].

Spectra were processed in ACD 1D NMR Manager (version 11; Advanced Chemistry Development), zero-filled to twice the number of points and multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz. The spectra were Fourier-transformed, phased, baseline-corrected and referenced to the TSP (trimethylsilyl-2,2,3,3-tetradeuteropropionic acid) peak (at 0.00 p.p.m.). The spectra were divided into 224 buckets each of 0.04 p.p.m. between 0.2 and 9.4 p.p.m. [excluding the region of water resonance (4.65–4.89 p.p.m.)], and the spectral area of each bucket was integrated. To correct for any variation in sample volumes, the integral for each bucket was normalized to the total integral for the spectrum. The compounds detected in the spectra were identified by comparison with entries in the Human Metabolome Database (www.hmdb.ca; [28]) and spiking known standards into samples. To quantify individual components, the peaks were identified and integrated manually.

Gene expression analysis

Gene expression analysis was carried out using a GeneChip® Porcine Genome Array (Affymetrix). RNA was extracted from liver using an RNeasy® kit (Qiagen), and was run on a bioanalyser (Agilent 2100 BioAnalyser) to ensure an RIN (RNA integrity number) value above 8.0. Labelling and hybridization of the RNA was carried out by the MRC Clinical Sciences Centre/Imperial College Microarray Centre (Hammersmith Hospital, London, U.K.) using the Affymetrix GeneChip® platform. The samples were stained using SAPE (streptavidin conjugated to R-phycoerythrin). A biotinylated anti-streptavidin antibody was additionally used to amplify the signal. The arrays were scanned on an Affymetrix 3000 7G scanner.

The expression data were analysed using Resolver 7.1 (Rosetta Biosoftware). The genome array data were analysed to obtain a signature set of genes which showed a fold change between the two modes of delivery of greater than 2 and were significantly different between the treatments ($P < 0.01$) as determined by a two-way ANOVA, followed by Benjamini–Hochberg (FDR) multiple test correction. The genes were identified by a BLAST search of the accession number of the probe against the nr and est databases.

To confirm the gene array findings, qRT-PCR (quantitative real-time PCR) was performed. RNA was extracted as described above, and first-strand cDNA was generated using an Omniscript® Reverse Transcription kit (Qiagen). DNA primers were designed from the *Sus Scrofa* gene sequences obtained from NCBI (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov) over intron–exon boundaries to prevent amplification of genomic DNA (see Supplementary Table 1 at http://www.ClinSci.org/cs/118/cs1180047add.htm). The qRT-PCR analysis was carried out as described previously [29] in duplicate on each cDNA sample using RPLP0 (acidic ribosomal phosphoprotein P0) as a reference; the annealing temperature was optimized for each individual pair of primers (see Supplementary Table 1).

Table 1 Mode of delivery alters the lipid composition of the liver at 7 days post-partum

Values are means ± S.E.M., and are expressed as mg/g of liver. Piglets were born by CS ($n = 5$) or VD ($n = 4$) and fed parenterally for 7 days. Hepatic lipid composition was determined by TLC, followed by densitometry.

	Birth by		
Lipid class	CS	VD	*P* value
Phospholipid	14.32 ± 2.97	17.46 ± 2.48	0.390
Cholesterol	1.05 ± 0.17	1.35 ± 0.19	0.233
NEFA	0.28 ± 0.14	0.05 ± 0.02	0.142
TAG	8.98 ± 1.99	0.75 ± 0.79	0.007
Cholesterol ester	1.81 ± 0.22	0.68 ± 0.19	0.003

Statistical analysis

Statistical analysis was carried out in Minitab 14. Values are presented as means ± S.E.M. Data were shown to be normally distributed using an F test ($P < 0.05$), and an ANOVA general linear model was used to test for statistical differences.

RESULTS

Growth and phenotype

The birthweight of piglets entered into the present study was similar, regardless of whether they were born by CS or, on average, 2 days later by VD (1447 ± 82 and 1643 ± 238 g for CS and VD piglets respectively). Likewise, there were no significant differences between the CS and VD piglets in final body weight (1539 ± 116 and 2005 ± 267 g) or growth velocities (62 ± 11 and 65 ± 9 g/day).

Gut weight both as an absolute weight and expressed as a percentage of total body weight was significantly ($P = 0.034$) lower in CS piglets compared with VD piglets (3.29 ± 0.33 and 4.86 ± 0.46 % of total body weight respectively). However, there was no difference in the relative or absolute liver weight based on mode of delivery in CS piglets compared with VD piglets (3.29 ± 0.28 and 3.29 ± 0.02 % of total body weight respectively).

Glucose homoeostasis

Immediately after birth, the plasma glucose concentration was lower ($P = 0.001$) in the CS piglets compared with the VD piglets (1.92 ± 0.17 and 3.13 ± 0.22 mmol/l respectively). The higher plasma glucose concentration in the VD piglets was accompanied by an elevated ($P < 0.001$) plasma glucagon concentration (62.13 ± 3.59 compared with 124.4 ± 18.91 pg/ml respectively), but there was no difference in the plasma insulin concentration between the CS and VD piglets (9.88 ± 1.10 and 8.65 ± 0.90 pg/ml respectively). The glucagon/insulin ratio of the piglets was lower ($P = 0.024$) in the CS piglets at birth than the VD piglets (6.69 ± 0.37 and 10.08 ± 0.90 respectively).

After 7 days of parenteral feeding, plasma glucose concentrations tended to be lower in CS piglets than the VD piglets (3.80 ± 0.16 and 5.13 ± 0.76 mmol/l respectively; $P = 0.178$) and, although there was no difference in the plasma glucagon concentration (105.33 ± 45.54 and 153.69 ± 78.29 pg/ml respectively), the CS piglets were hyperinsulinaemic compared with the VD piglets (56.79 ± 11.77 and 23.55 ± 9.71 pg/ml respectively; $P = 0.037$). There was no difference in the glucagon/insulin ratio of the piglets on day 7 (2.17 ± 1.03 and 7.82 ± 5.65 in the CS and VD piglets respectively; $P = 0.212$).

Thyroid hormones

The plasma concentration of T_3, a potent mediator of metabolism post-partum, was determined immediately after birth and after 7 days. Plasma T_3 was significantly lower at birth in the CS piglets than the VD piglets (1.43 ± 0.09 and 3.75 ± 1.78 nmol/l respectively; $P = 0.003$). After 7 days, there were no differences in plasma concentration of T_3 (0.63 ± 0.18 and 0.84 ± 0.15 nmol/l in the CS and VD piglets respectively; $P = 0.417$).

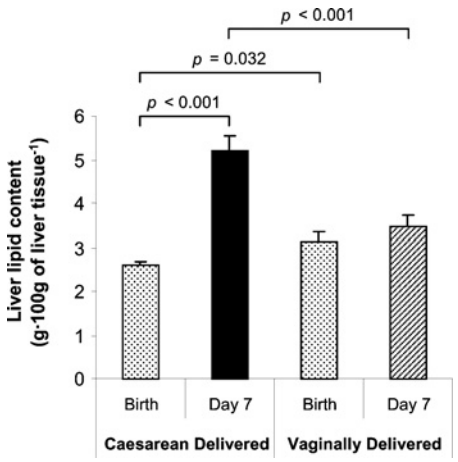

Figure 1 Mode of delivery alters lipid and carbohydrate storage in the liver

Comparison of the hepatic lipid content of animals born by either CS (112 dpf; $n = 6$) or VD (114 dpf; $n = 6$) at birth and after 7 days of TPN (CS; $n = 5$, VD; $n = 4$). Values are means ± S.E.M.

Hepatic lipid metabolism

Consistent with a role for mode of delivery in the development of hepatic steatosis in response to TPN, CS piglets accumulated more hepatic lipid, 7 days after birth, than VD piglets (Figure 1). Indeed, the amount of lipid in the liver of CS piglets was 5.2 mg/g of tissue, above that for a clinical definition of steatosis. In contrast, for the VD piglets it was 3.5 mg/g of tissue, which is significantly below the clinical definition of steatosis, and none of the VD piglets had a hepatic lipid in excess of 5 mg/g of tissue measured. Comparison of the hepatic lipids showed no difference in phospholipid, cholesterol or NEFA levels, but markedly higher TAG and cholesterol esters in the CS piglets (Table 1). In addition to elevated hepatic cholesterol esters, plasma cholesterol was lower in the CS piglets compared with the VD piglets (1.04 ± 0.25 and 1.73 ± 0.32 mmol/l respectively; $P = 0.046$), suggesting that the mode of delivery affected cholesterol metabolism. In spite of the elevated hepatic TAG in the CS piglets, there was no difference in plasma TAG levels between the piglet groups.

It was possible that differences in hepatic lipid content at birth were responsible for the difference in lipids on day 7. A comparison of the hepatic lipid content of the CS and VD piglets within 1 h of birth showed that the livers of CS piglets contained less lipid than VD piglets at this time point (Figure 1), consistent with the possible accumulation of fuel stores just prior to delivery. Therefore, over the 7 days of TPN, CS piglets accumulated more hepatic lipid than VD piglets.

Despite being on the same diet, the liver fatty acid profiles were substantially different between CS and VD piglets (Table 2). The proportion of the different fatty acids present in the livers of the CS piglets was similar

Table 2 Mode of birth significantly alters the fatty acid profile of the liver at 7 days post-partum in piglets fed parenterally with TPN compared with the fatty acid composition of the diet

Values are means ± S.E.M.

	Amount of fatty acid present (g/100 g of total fatty acids)			
Fatty acid	Diet	Birth by CS ($n = 5$)	Birth by VD ($= 4$)	P value
$C_{14:0}$	0.72	0.62 ± 0.10	0.28 ± 0.05	0.007
$C_{16:0}$	15.75	16.67 ± 0.53	17.24 ± 0.52	0.207
$C_{16:0n-7}$	2.03	1.90 ± 0.37	1.20 ± 0.40	0.096
$C_{17:1}$	0.16	0.15 ± 0.03	0.00 ± 0.00	0.001
$C_{18:0}$	9.82	9.69 ± 0.77	20.21 ± 0.95	<0.001
$C_{18:1n-9}$	19.65	18.15 ± 0.33	14.17 ± 1.38	0.009
$C_{18:2n-6}$	36.63	36.69 ± 1.39	23.46 ± 0.51	<0.001
$C_{18:3n-6}$	0.58	0.45 ± 0.01	0.35 ± 0.13	0.216
$C_{18:3n-3}$	2.13	2.66 ± 0.25	1.14 ± 0.14	<0.001
$C_{20:1n-9}$	0.39	0.13 ± 0.15	0.00 ± 0.00	0.178
$C_{20:2n-6}$	1.33	1.48 ± 0.13	0.84 ± 0.29	0.02
$C_{20:3n-6}$	0.98	0.93 ± 0.02	0.80 ± 0.10	0.085
$C_{20:4n-6}$	5.05	5.30 ± 0.58	11.63 ± 0.43	<0.001
$C_{20:5n-3}$	0.36	0.32 ± 0.06	0.72 ± 0.08	0.002
$C_{22:1n-9}$	0.20	0.03 ± 0.02	0.03 ± 0.03	0.443
$C_{22:4n-6}$	0.83	0.88 ± 0.16	0.99 ± 0.26	0.340
$C_{22:3n-3}$	0.45	0.45 ± 0.21	0.25 ± 0.13	0.196
$C_{22:5n-3}$	0.34	0.43 ± 0.17	0.00 ± 0.00	0.012
$C_{22:6n-3}$	1.73	2.46 ± 0.19	5.66 ± 0.37	<0.001

to that of the TPN solution, but markedly different from that in the livers of VD piglets. These findings suggest that the metabolism of lipids in the livers of the CS piglets was relatively passive, whereas there was marked fatty acid modification in the livers of the VD piglets. For example, the proportion of total saturated fatty acids was the same in the diet and the livers of CS piglets, but there was a marked increase in the proportion of saturated fatty acids in the livers of the VD piglets (26.29 g/100 g of total fatty acids in the diet, 26.98 g/100 g of total fatty acids in the CS piglets, and 37.73 g/100 g of total fatty acids in the VD piglets). Delivery by CS also resulted in a smaller stearic acid ($C_{18:0}$)/palmitic acid ($C_{16:0}$) ratio compared with VD (0.58 ± 0.02 and 1.17 ± 0.06 respectively; $P < 0.001$). Similarly, the oleic acid ($C_{18:1n-9}$)/stearic acid ($C_{18:0}$) ratio, which has been used previously as an index of Δ^9-desaturase activity [30], in CS livers was the same as the composition of the diet (1.90 ± 0.19 in the CS piglets and 2.00 in the diet). However, this ratio was significantly lower in the livers of VD piglets (0.71 ± 0.11; $P = 0.003$). As hydrogenation of oleic acid is not known to occur *in vivo*, these results suggest a reduced elongation of palmitic acid and higher Δ^9-desaturase activity in CS piglets.

Analysis of the polyunsaturated fatty acids was also consistent with reduced elongase activity in the CS piglets. For example, the proportions of $C_{20:4n-6}$ and $C_{22:6n-3}$ were much lower in the CS piglets, whereas their respective essential precursors ($C_{18:2n-6}$ and $C_{18:3n-3}$) were significantly higher. Furthermore, the ratios of both $C_{20:4n-6}/C_{18:2n-6}$ and $C_{22:6n-3}/C_{18:3n-3}$ were much lower in CS piglets than in VD piglets (0.14 and 0.50, and 0.92 and 4.96 respectively). These findings indicate that, in addition to lower elongase activity, the Δ^6- and Δ^5- desaturases are also less active in the CS piglets.

Liver metabolomics

To determine the effects of delivery mode on carbohydrate and amino acid metabolism, we compared the metabolic profiles of aqueous liver extracts by ^{1}H NMR spectroscopy. Multivariate statistical analysis by PLS-DA (partial least squares-discriminant analysis) of these results showed a clear separation of metabolic profiles between the CS and VD groups (Figure 2A). Analysis of the loading plots identified succinate, aspartate, oxaloacetate, α-ketoglutarate, glucose and glutamate as contributors to the separation. Independent integration of the peaks corresponding to these metabolites revealed higher (approx. 50%) oxaloacetate, aspartate, α-ketoglutarate and glutamate, and significantly lower glucose and succinate, in the livers of CS piglets fed for 7 days by TPN (Figures 2B and 2C).

Hepatic enzyme activity

To determine whether the changes in metabolite levels were accompanied by changes in hepatic enzyme

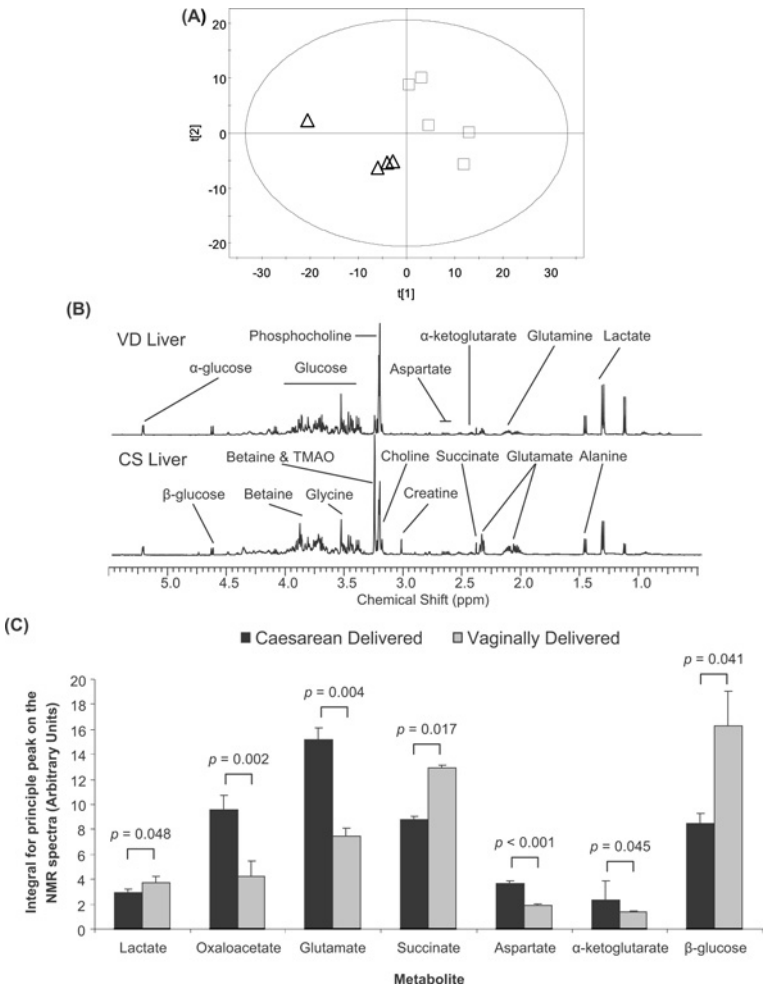

Figure 2 Mode of delivery modifies metabolomic profile 7 days post-partum in piglets fed by TPN

(A) PLS-DA analysis of ^{1}H NMR spectra of aqueous metabolites from parenterally fed piglets born either by CS (□; $n = 5$) or VD (△; $n = 4$). (B) Representative ^{1}H NMR spectra of aqueous liver extracts from piglets fed by TPN and born either by CS or VD. (C) Relative integral values for the peaks on the ^{1}H NMR spectra of aqueous liver extracts at 7 days post-partum from piglets born either by CS or VD and fed by TPN for 7 days. Peaks were individually integrated: lactate, 1.31–1.35 p.p.m.; glutamate, 2.34–2.35 p.p.m.; glutamate/glutamine, 2.35–2.37 p.p.m.; succinate, 2.39–2.41 p.p.m.; aspartate, 2.62–2.68 p.p.m.; α-ketoglutarate, 3.00–3.02 p.p.m.; and glucose, 3.24–3.25 p.p.m.. Values are means ± S.E.M.

activity, the activity of succinate dehydrogenase, malate dehydrogenase and PEPCK was measured. Delivery by CS resulted in lower succinate dehydrogenase activity and higher malate dehydrogenase activity, but did not alter PEPCK activity (Figure 3A). The lack of change in PEPCK activity suggests that the mode of delivery does not alter the use of oxaloacetate for gluconeogenesis.

Glycerol derived from glyceride metabolism is an alternative source of carbon for gluconeogenesis, and the lower levels of hepatic lipid in the VD piglets indicated its use for gluconeogenesis in these animals. The carbon for gluconeogenesis from lipids only comes from glycerol. We measured both the glycerol content and glycerolphosphate dehydrogenase activity in liver extracts. Hepatic glycerol content was lower (Figure 3B) and glycerolphosphate dehydrogenase activity was higher (Figure 3A) in VD piglets. These results suggest that glycerol in the VD piglets was converted into glucose, whereas in the CS piglets it was converted into lipid. There is very little adipose tissue in the neonatal piglet making adipose tissue an unlikely source of the glycerol and suggesting that the glycerol is produced by hydrolysis of the TAG provided in the lipid emulsion during TPN.

Liver gene expression

As the animals received the same diet for the 7 days it appeared likely that the differences in metabolism were underpinned by gene expression patterns that resulted from the mode of delivery. We therefore compared gene expression using RNA microarrays, and found that 7 days after delivery 118 genes were expressed at least 2-fold lower (32 were expressed more than 4-fold lower)

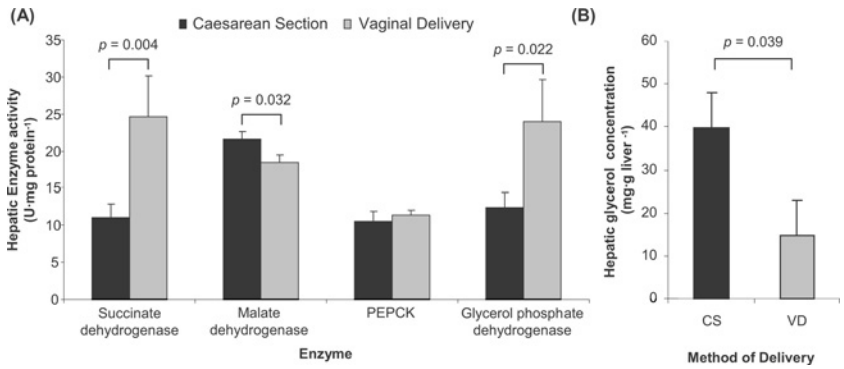

Figure 3 Mode of delivery modifies glucose homoeostasis

(A) Mode of delivery modifies tricarboxylic acid and gluconeogenic enzyme activities at 7 days post-partum in piglets fed by TPN (CS, $n = 5$; VD, $n = 4$). (B) Mode of delivery modifies liver glycerol content in the same animals. Values are means $\pm$ S.E.M.

and 243 genes were expressed at least 2-fold higher in the livers of the CS piglets (46 more than 4-fold higher) (see Table 3 and Supplementary Tables 2 and 3 at http://www.ClinSci.org/cs/118/cs1180047add.htm).

Twenty genes expressed at lower levels in the CS piglets were associated with inflammation. Of these, 14 were readily identifiable as IFN (interferon)-inducible genes, and these constituted three out of the four most markedly reduced genes. Furthermore, IFNγ was suppressed (2.1-fold) in the CS piglets, an observation consistent with previous demonstrations of reduced IFNγ in CS compared with VD piglets [31] and in response to CS delivery compared with normal or assisted delivery in humans [32]. The fourth gene in this group of most highly suppressed genes (approx. 30-fold) encoded the orexigenic peptide GALP (galanin-like peptide). Analysis of a 2 kb fragment of the promoter of this gene using TESS (Transcription Element Search System; http://www.cbil.upenn.edu/tess) showed that it contained a consensus sequence for the IFN-responsive transcription factors IRF1 (IFN regulatory factor 1) and IRF2, implying that it too is an IFN-responsive gene. Similarly, expression of another orexigenic peptide, angiopoietin-like 4, was lower in CS piglets, and promoter analysis showed that it also contained IRF1/2-binding sites. Among the changes in inflammatory gene expression, we also observed a lower expression of markers of the innate immune system, including the p80 NK receptor in the CS piglets. To identify any inflammatory conditions in the VD piglets, we measured the haptoglobin concentration (a marker of the immune response [33]) in the plasma on day 7, but found no significant difference between the groups (1.21 ± 0.01 and 0.95 ± 0.18 ng/ml in the CS and VD piglets respectively).

Twenty genes reduced in the CS piglets were associated with metabolism, of which five were involved in lipid metabolism. These genes encoded apolipoprotein A1, PID1 (phosphotyrosine interaction-domain-containing 1), lipoprotein lipase H and START {StAR (steroidogenic acute regulatory protein)-related lipid transfer protein; a cholesterol-responsive protein involved in cholesterol and lipid trafficking [34]}. These observations suggest modified cholesterol handling by the CS piglets and, consistent with this, expression of HMG-CoA (3-hydroxy-3-methylglutaryl CoA) synthase, determined by qRT-PCR, was lower in CS piglets compared with VD piglets (9.18 ± 3.30 and 45.87 ± 8.34 respectively; $P = 0.005$).

Of the genes more highly expressed in the CS piglets, levels of genes encoding calbindin and myo-inositol synthetase 1 were 100- and 20-fold higher respectively, than in VD piglets. Eight genes associated with inflammation, including five antimicrobial peptides, were also more highly expressed in the livers from CS piglets. There was higher expression of genes described previously as markers of hepatocyte stem cells [e.g. genes encoding ORM1 (orosomucoid 1), PEG3 (paternally expressed 3), PEG10 (paternally expressed 10), NOPE (neighbour of Punc E), DUSP9 (dual-specificity phosphatase 9), MAGED1 (melanoma antigen family D1), MAGED4 (melanoma antigen family D4) and AFP (α-fetoprotein)] [35], suggesting that the livers of CS piglets were less differentiated than those of VD piglets. The livers of the CS piglets also had a higher expression of genes associated with lipid accumulation, including those encoding ADRP (adipose differentiation-related protein), hepatic lipase, two acyl-CoA synthetases, ACAT2 (acetyl-CoA acetyltransferase 2), sterol C5 desaturase and SDR7 (short-chain dehydrogenases/reductase 7).

In addition to the inflammatory and lipid metabolism changes, there were significant differences in the expression of components of the activin signalling pathways. Expression of inhibin B was lower but that of the activin II receptor was higher in the CS piglets, implying a reduction in activin signalling after normal VD that did not occur or was reduced in response to delivery by CS. Previous studies using inhibin-deficient mice have shown that increased activin signalling in the liver results in liver damage [36]. A further marker of

Table 3 Genes which are significantly ($P < 0.01$) differentially expressed between the livers of CS and VD piglets fed parenterally for 7 days post-partum

Accession numbers are from the EST or GenBank® databases.

(A) Genes down-regulated by CS compared with VD

Gene function	Primary sequence name	Our assignment	Fold change
Metabolism			
CO953054	Ssc.27557.1.S1_at	Phosphotyrosine interaction-domain-containing 1 (PID1)	8.10
BX915625	Ssc.21663.1.A1_at	Transcribed locus lipase, endothelial (LIPG)	6.22
CF176316	Ssc.17934.1.S1_at	Steroidogenic acute regulatory protein (StAR)	2.71
BQ604621	Ssc.6240.1.A1_at	Apolipoprotein A1 (APOA1)	2.28
Immune response			
NM_213825.1	Ssc.4875.1.S1_at	Galanin-like peptide (GALP)	27.16
NM_214061.1	Ssc.221.1.S1_at	Interferon-inducible protein p78	17.47
BI403807	Ssc.12825.1.A1_at	NK P80NK rec	8.79
AW313822	Ssc.2641.1.S1_at	ISG ligase UbcH8	8.23
CK465051	Ssc.12504.1.A1_at	ISG12a	6.91
BI119523	Ssc.30752.2.A1_at	Interferon induced with tetratricopeptide repeats	6.41
NM_213826.1	Ssc.336.1.S1_at	ISG15-specific protease	5.48
CF789161	Ssc.21162.1.S1_s_at	Interferon-regulator factor 7 (IRF7)	3.29
CO953941	Ssc.29329.1.A1_at	Programmed death ligand 1 (activated by IFN)	2.68
AY293733.1	Ssc.4093.1.A1_at	Interferon γ (IFNG)	2.06
BI183736	Ssc.8980.1.A1_at	Angiopoietin-like 4 (ANGPTL4)	2.58

(B) Genes up-regulated by CS compared with VD

Gene function	Primary sequence name	Our assignment	Fold change
Ion transport			
L13068.1	Ssc.429.1.A1_at	Calbindin D-9k	100.00
Metabolism			
CN157463	Ssc.15723.1.S1_at	Myo-inositol 1-phosphate synthase A1	13.35
BI403313	Ssc.12695.1.S1_at	Acyl-CoA synthetase long-chain family member 4 (ACSL4)	3.98
BQ604949	Ssc.21999.1.S1_a_at	Aldehyde dehydrogenase 3 family member B1 (ALDH3B1)	3.96
BQ604008	Ssc.4426.1.S1_at	Acetyl-CoA acetyltransferase 2 (ACAT2)	3.55
AU058728	Ssc.6441.2.S1_at	SDR family member 7 (DHRS7)	2.94
BX675010	Ssc.19105.1.S1_at	Lipase hepatic (LIPC)	2.79
BF710977	Ssc.7534.1.A1_at	γ-Glutamyltranspeptidase	2.69
AY550037.1	Ssc.6323.1.S1_at	adipose differentiation-related protein 1 (ARFP)	2.19
CK450664	Ssc.10695.2.S1_at	Acyl-CoA synthetase short-chain family member 3 (ACSS3)	2.01
Antimicrobial			
CK464499	Ssc.20938.1.S1_at	Azurocidin 1 p	10.84
NM_214450.1	Ssc.27609.1.S1_at	PR39 protegrin family peptide	8.37
M81327.1	Ssc.13769.1.S1_at	Lactotransferrin (LTF)	5.01
L24745.1	Ssc.420.2.S1_x_at	Homologue of proline/arginine-rich antibacterial peptides	4.92
NM_213863.1	Ssc.420.1.S1_a_at	Antimicrobial peptide precursor (PG-2)	4.86
NM_213863.1	Ssc.420.1.S1_x_at	Antimicrobial peptide precursor (PG-2)	4.40
L39641.1	Ssc.420.4.S1_a_at	Antibacterial peptide precursor (PMAP37)	4.19
Cell cycle and signalling			
AF324155.1	Ssc.3831.1.S1_at	Corticosteroid-binding globulin (SERPINA6)	4.55
Markers of fetal hepatocytes and paternally expressed genes			
CN162607	Ssc.13476.1.A1_at	Paternally expressed 10 (PEG10)	7.04
CK458546	Ssc.25001.1.A1_at	Dual specificity phosphatase 9 (DUSP9)	7.04
CK456429	Ssc.2397.1.A1_at	Neighbour of Punc E 11 (NOPE11)	5.54
CN165575	Ssc.3014.1.S1_at	Melanoma antigen family D 4 (MAGED4)	3.48
NM_001001860.1	Ssc.22086.1.A1_at	Melanoma antigen family D 1 (MAGED1)	3.44
CK453813	Ssc.22623.2.S1_at	Paternally expressed 3 (PEG3)	2.99
NM_214317.1	Ssc.7131.1.A1_at	α-Fetoprotein (AFP)	2.98
BF711624	Ssc.7669.1.A1_at	Orosomucoid 2 (ORM2)	2.47
BQ604762	Ssc.2112.1.S1_at	Putative dual specificity phosphatase 4 (DUSP4)	2.29

liver damage, γ-glutamyltransferase, was also elevated in livers from CS piglets.

The array data were confirmed using qRT-PCR to analyse the expression of a subset of the genes which showed significant differences. This analysis showed that changes in the array were supported by changes in the RNA expression, as measured by qRT-PCR, with the exception of PID1 (see Supplementary Table 4 at http://www.ClinSci.org/cs/118/cs1180047add.htm).

DISCUSSION

Factors underlying the hepatic phenotype

The present study establishes mode of delivery as an important additional contributor to the development of hepatic steatosis, with CS delivery resulting in the amount of hepatic lipid exceeding 5 mg/g of tissue (the threshold for defining NAFLD in man [37]) compared with 3.5 mg/g of tissue in VD piglets. One possible factor that could influence hepatic lipid accumulation is calorific intake and it is known that birth by CS can reduce the suckling reflex and milk intake over the first few days of life (for example, [38]). However, by using TPN-fed piglets it was possible for us to ensure that the dietary intake was the same in all animals, precluding differences in dietary intake as a factor influencing our findings. TPN is associated with an increased risk of steatosis in human babies and our results suggest that this response may be much larger in babies born by CS and fed by TPN than in those born by VD.

In determining the mechanism leading to hepatic steatosis in response to CS delivery, we found that CS also has a significant impact on metabolism and gene expression that persists beyond the immediate post-partum period. Analysis of the fatty acid profiles was consistent with higher Δ^9 desaturase activity [SCD1 (stearoyl CoA desaturase 1)] in the CS piglets. SCD1 has been implicated in the accumulation of lipid in a number of conditions, and mice that lack SCD1 have reduced lipogenesis and are resistant to hepatic steatosis [39]. The lipid analysis also suggested that normal VD was required to increase fatty acid elongase activity and the activity of Δ^5 and Δ^6 desaturases. However, the contribution of the failure to increase the activity of these enzymes to the accumulation of hepatic lipid in CS animals remains to be established. The alterations in aqueous metabolites and enzyme activities that occur alongside the decrease in lipid accumulation were consistent with reduced activation of gluconeogenesis from lipid-derived sources (i.e. glycerol) in CS-delivered piglets. Consequently, in these piglets, glycerol was diverted into hepatic lipid rather than glucose (Figure 4).

The proposed difference in gluconeogenic activity between the CS and VD piglets is consistent with the altered insulin/glucagon ratio at birth and the raised plasma insulin concentration observed after 7 days. Other reports in the literature indicate that differential insulin/glucagon ratio between CS and VD neonates may arise as a result of the increase in stress-related endocrine changes (increased catecholamine and cortisol production) associated with VD and which are absent during elective CS [8,9]. However, a normal action of insulin is to activate *de novo* fatty acid synthesis, but the similarity between the lipid profiles of the TPN solution and the lipid composition of the hepatic TAGs suggest that the increased lipid is the result of storage of the lipid supplied and not synthesis. Any role therefore for the persistent hyperinsulinaemia in the CS piglets in the development of steatosis is most likely to be due to the suppression of hepatic gluconeogenesis.

The metabolic changes are accompanied by changes in gene expression that are associated with fat accumulation. For example, increased hepatic ADRP expression is one of the earliest markers of adipose differentiation [40,41] and is associated with NAFLD [42]. Furthermore, antisense inhibition of ADRP has been shown previously to reduce hepatic steatosis and insulin resistance in *ob*/*ob* mice [42]. Increased expression of genes encoding ACAT, ACSL5 (acyl-CoA synthase long-chain family member 5) and sterol C5 desaturase have also been shown to increase in insulin-resistant mice with NAFLD [43], implying that the mechanisms resulting in hepatic lipid accumulation in our CS piglets may be similar to those found in NAFLD and the metabolic syndrome in humans.

Altered immune gene expression

In addition to altered metabolism, we also observed marked differences in the expression of genes associated with inflammation and activated by IFN in the CS compared with the VD piglets. A number of studies have shown that parturition itself is associated with a marked increase in inflammatory markers and leucocytes in the myometrium [44]. Furthermore, analysis of the fetal membranes has shown that, although there is no increase in leucocyte number, there is a marked acute inflammatory gene expression signature [45]. Previous studies have also shown decreased IFN production by white blood cells in humans born by CS [32] and reduced serum IFN levels in piglets 14 days after CS delivery [31]. In the present study, we have shown that the inflammatory gene signature is still present 7 days after birth in animals born by VD. However, our results on their own do not determine whether the lower expression of IFN and markers of the innate immune system in the CS piglets are the result of the increased lipid accumulation or a result of the birth process itself. Interestingly, CS has been shown to alter the activity of cell types associated with the innate immune system [46] and results in an increased risk of Type 1 diabetes [3] and asthma [6], also suggesting long-term changes in the immune system as a result of CS.

It is possible that the reduced expression of genes associated with the innate immune system is important

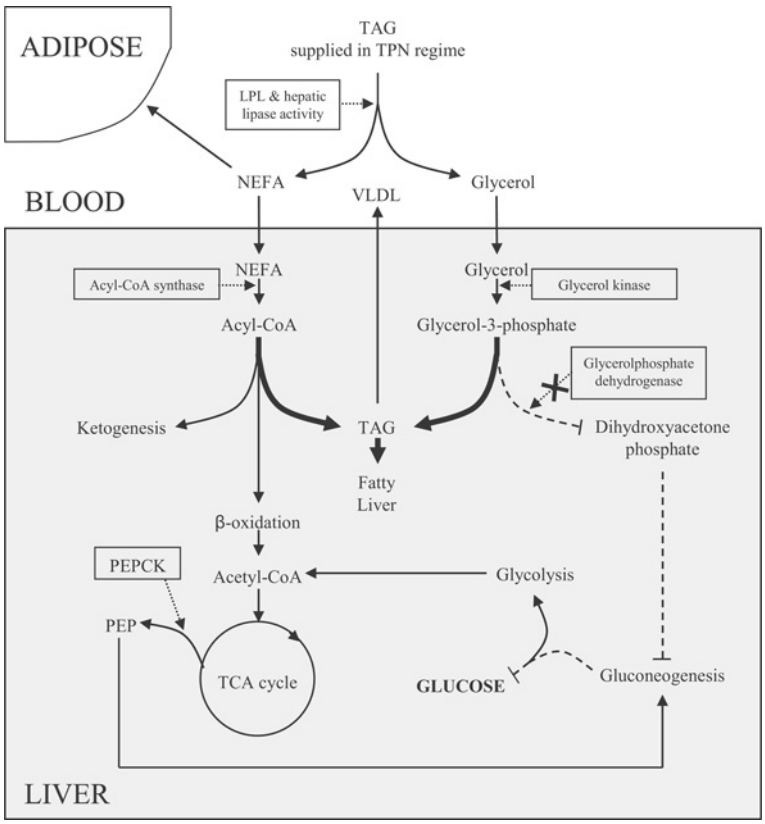

Figure 4 Proposed mechanism by which CS results in hepatic lipid accumulation

Following lipolysis of parenteral lipid, fatty acid and glycerol are taken up by the liver in the absence of mature adipose tissue in the CS neonate. Once in the liver, the decrease in glycerolphosphate dehydrogenase activity reduces the utilization of glycerol for gluconeogenesis, resulting in increased glycerol for TAG assembly and reduced glucose for export to the plasma. Little of the fatty acid is probably utilized in ketogenesis, as piglets (and human neonates) have very limited ketogenic activity [55], and the lower glucagon/insulin ratio in CS piglets also supports the possibility of reduced ketogenic activity. Although there was no measurable difference in PEPCK activity in CS compared with VD piglets, the hyperinsulinaemia is likely to reduce gluconeogenesis from phosphoenol pyruvate (PEP), thus the requirement for β-oxidation of NEFAs to provide energy for gluconeogenesis is likely to be small. Fatty acid and glycerol entering the liver are esterified to produce TAG which is stored in the liver, rather than being exported, resulting in fatty liver. LPL, lipoprotein lipase.

in the accumulation of lipid. The liver has a major role in the innate immune system, in particular the liver contains KCs (Kupffer cells; a class of hepatic macrophage), NK (natural killer) cells and NKT cells (NK T-cells) and, it has become evident that the innate immune system may play an important role in the development and progression of NAFLD [47]. In particular, studies using leptin-deficient (*ob*/*ob*) mice, which develop symptoms of dyslipidaemia, insulin resistance and NAFLD, have demonstrated that KC dysfunction may play a role in the onset of NAFLD [48,49].

It is not clear whether the changes in the expression of NK cell markers result from increased recruitment or differentiation of these cells. However, NKT cells express adrenergic receptors [50] and, in response to catecholamines, the number of NKT cells increases [47]. Thus lower secreted concentrations of noradrenaline during CS compared with VD [8] may result in lower NKT cell populations.

We also found marked elevation of two orexigenic peptides, GALP and angiopoietin-like 4, and the presence of IRF-binding sites in the promoters of their genes suggests that they may also be IFN-responsive genes in the liver. This observation implies that the IFN response that occurs at birth may contribute to the stimulation or regulation of appetite. The expression and activity of GALP has been analysed previously in the brain [51], but the role of the GALP expression that we have observed in the liver remains to be established. However, it is possible that this hepatic expression is part of the initial sensing of a requirement to feed orally. As our animals were fed by TPN any effect of this difference in GALP expression was not quantifiable by measuring feed intake; however, consistent with this suggestion, a reduced suckling reflex after CS delivery has been observed in humans [38] and rats [52]. It is not known whether normal levels of the peptide are established once the baby delivered by CS is fed enterally over an extended period of time or whether

VD is required to instigate normal appetite control. There is some evidence to suggest that babies delivered CS are more likely to become overweight in later life [7], favouring the hypothesis that VD is required for optimal appetite regulation. It is interesting that leptin, as with GALP and angiopoietin-like 4, appears to have a role in regulating the inflammatory response of the liver [47], strengthening the association between appetite regulation and the innate immune system.

It is possible that the differences in hepatic lipid accumulation, gene expression and metabolism observed between CS and VD piglets may be a direct effect of the procedures of CS delivery. Alternatively, the absence of both the hormonal and mechanical stimulation of VD in pre-labour CS-delivered infants results in abnormal patterns of post-partum metabolism and physiology that we observe. It appears most likely that the latter suggestion is the case and that the lack of exposure to the increase in stress hormones that accompanies normal VD is the primary cause of the observed differences. Consistent with this suggestion, stress hormones are known to be markedly lower following CS, and these hormones are known to modify metabolism and the differentiation or recruitment of innate immune cells in a manner consistent with our observations.

Maturation or mode of delivery?

In the final few days of *in utero* life, the fetus responds to changes in circulating concentrations of endogenous hormones, secreted from the maturing fetal endocrine glands, as well as exogenous maternal/placental hormones, which together act to increase fuel deposits and mature some of the organ systems to allow them to respond to the imminent change in environment at birth. It is possible that some of the differences we observe in our model are due to the lack of exposure to these changes in hormones. However, CS delivery was performed just 2 days prior to the expected delivery, as close to the window of natural birth as possible within our experimental system. By this time in the pig, cortisol levels are reported to have approximately doubled and have reached a plateau prior to the surge in cortisol that accompanies delivery [53]. It therefore appears unlikely that further maturation events will be initiated before the surge in stress hormones that accompanies birth and that it is this surge in hormones that is responsible for the changes in metabolism and gene expression we have observed. It should be noted that in human pre-labour CS there is a longer period prior to delivery date, suggesting that the livers of babies born by pre-labour CS will have experienced even less of the hormonal changes and may therefore be less mature than those of the piglets we studied.

Wider implications

The effects of mode of delivery on metabolism and gene expression that we observe are present 7 days after birth and suggest that VD is required for the liver to develop properly. As alluded to above, whether, given time, the liver will completely mature following CS remains to be established. However, it is possible that the lack of complete hepatic differentiation will lead to a propensity to develop NAFLD, insulin resistance and, thereby, a tendency to obesity and Type 2 diabetes. At the present time, there are no reported links between delivery by elective CS and Type 2 diabetes; however, there is an association between Type 1 diabetes and CS delivery that can be observed in humans [3]. As a number of risk factors associated with Type 1 diabetes and Type 2 diabetes are the same (e.g. high birthweight, obesity and catch-up growth [54]), it appears likely that CS delivery may also be associated with an increased risk of developing Type 2 diabetes.

In conclusion, our present results indicate that, in animals born by CS, there is a markedly lower level of glycerolphosphate dehydrogenase activity that reduces the entry of glycerol into the gluconeogenic pathway, as illustrated in Figure 4. The excess glycerol is then re-esterified with the acyl-CoA derived from the lipid supplied in the TPN solution facilitating fat storage and hepatic steatosis in the liver. Our results showing an association between mode of delivery and altered hepatic metabolism contributing to hepatic steatosis emphasize that the longer term consequences of CS on hepatic metabolism in the offspring now need to be tested.

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SUPPLEMENTARY ONLINE DATA

Delivery by Caesarean section, rather than vaginal delivery, promotes hepatic steatosis in piglets

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Table S1 Primer sequences, their melting temperature and the product length, used in the qRT-PCR measurements of hepatic gene expression

Gene name		Sequence (5′-3′)	Melting temperature (°C)	Product length (bp)
Acyl-CoA synthetase long-chain 4	Forward	CCGACCGAAGGGAGTGATGATG	66	280
	Reverse	CGGGACAGCTGCCATGAGTGTAG	66	
Alcohol dehydrogenase 5	Forward	CCCCTTTCTGGTGGATATTGTG	61	114
	Reverse	CCTGGGCAAACATTAGAAACTG	61	
Aldehyde dehydrogenase 3	Forward	GCTGGAGCACAAGTTTGACTACATC	62	207
	Reverse	GGGTCACAGTTGTCGTCCACGTAAC	66	
Angiopoietin-like 4	Forward	CCTGGAGAAGCAGCACTTGAGAATC	65	206
	Reverse	GCCGCTCTCCCTCTTCAAACAG	65	
Calbindin D-9k	Forward	GCTGCCGCACGACTGGAC	63	156
	Reverse	GGGGAATTCAGCCTGAATCAGTTG	66	
Cytochrome C (P450)	Forward	CCCTGCCCTGATTGACTATCTC	60	199
	Reverse	GCAGTGGCTATCAAGTTTTCAAGAG	61	
Galanin-like peptide	Forward	GGCTCTGACTGTCCCTCTGATCGTTC	68	191
	Reverse	CCAGGATCCCGAGGGCTGTCTTC	70	
HMG-CoA reductase	Forward	CTTGTAGAATGCCTTGTGATTG	61	76
	Reverse	AGCCAAAGCAGCACATAAT	60	
Phosphotyrosine interaction-domain-containing 1	Forward	CCAGTCTTAAGGCATGTAGAATAAG	58	158
	Reverse	CACTCCAGCTTTATTAATGCAAAC	59	
SRBP-cleavage-activating protein	Forward	GCCTGGTCCATGTGCACTTCAAG	66	126
	Reverse	CAGCCCCCACTTGGACTTGAC	64	

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Table S2 Genes which are significantly ($P < 0.01$) up-regulated in the livers of VD piglets compared with CS piglets fed parenterally for 7 days post-partum

Accession numbers are from the EST or GenBank® databases. EGF, epidermal growth factor; STAT, signal transducer and activator of transcription.

Gene function	Primary sequence name	Our assignment	Fold change
Ion transport			
BI403126	Ssc.2879.1.S1_at	Transcribed locus solute carrier family 25 (mitochondrial carrier; phosphate carrier)	−2.96
BQ604613	Ssc.24708.1.S1_at	Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 2 (SLC9A3R2)	−2.38
RNA and DNA metabolism			
BI184259	Ssc.9366.1.A1_at	CTD-binding SR-like protein rA9	−4.61
BX672562	Ssc.6433.1.S1_at	Zinc finger NFX1-type containing 1 (ZNFX1)	−2.64
CN156407	Ssc.26663.1.A1_at	SRY (sex determining region Y)-box 5 (SOX5)	−2.49
BQ604616	Ssc.13153.1.A1_at	Translation initiation factor 3	−2.33
Metabolism			
BX668048	Ssc.16261.1.S1_at	Cytochrome P450, family 2, subfamily C36	−8.28
CO953054	Ssc.27557.1.S1_at	PID1	−8.10
NM_214424.1	Ssc.190.1.S1_at	Cytochrome P450 4A24/cytochrome P450 4A21	−7.89
BX676000	Ssc.15363.1.S1_at	Methyltransferase-like 7B (METTL7B)	−7.88
BX915625	Ssc.21663.1.A1_at	Transcribed locus endothelial lipase (LIPG)	−6.22
NM_214270.1	Ssc.16302.1.S1_at	Urate oxidase (UOX)	−5.24
CF363330	Ssc.19465.1.A1_at	Glycine-N-acyltransferase (GLYAT)	−4.06
Z93098.1	Ssc.16124.1.S1_a_at	Cytochrome P450 C42	−3.92
NM_213932.1	Ssc.10993.1.S1_a_at	Urate transporter/channel protein, isoform (UATp,i)	−3.72
NM_214423.1	Ssc.204.1.S1_at	Cytochrome P450 3A29	−2.79
CF176316	Ssc.17934.1.S1_at	StAR	−2.71
CK461928	Ssc.4974.1.S1_at	Choline/ethanolamine phosphotransferase 1 (CEPT1)	−2.62
BM190132	Ssc.1206.1.A1_at	Glutaredoxin 1	−2.62
BX675338	Ssc.16234.1.S1_at	Haptocorrin	−2.50
CN153105	Ssc.5538.1.S1_at	*Homo sapiens* carbonic anhydrase II (CA2)	−2.42
BQ604612	Ssc.13849.1.S1_at	Branched-chain keto acid dehydrogenase kinase (BCKDK)	−2.38
BQ604620	Ssc.25198.1.A1_at	Membrane metalloendopeptidase	−2.31
BQ604621	Ssc.6240.1.A1_at	Apolipoprotein A1 (APOA1)	−2.28
	SscAffx.32.1.S1_at	NADH dehydrogenase subunit 4L	−2.24
Inflammation			
NM_213825.1	Ssc.4875.1.S1_at	GALP	−27.16
NM_214061.1	Ssc.221.1.S1_at	Interferon-inducible protein p78	−17.47
BI403807	Ssc.12825.1.A1_at	NK P80NK rec	−8.79
BI119523	Ssc.30752.2.A1_at	Interferon induced with tetratricopeptide repeats	−6.41
NM_214303.1	Ssc.1031.1.S1_at	2′,5′-Oligoadenylate synthetase 1 (OAS1)	−6.08
BX921277	Ssc.30752.1.S1_at	Interferon-induced protein with tetratricopeptide repeat	−5.42
AB105382.1	Ssc.13780.11.S1_x_at	MHC class I antigen	−5.29
AB005545.1	Ssc.16157.1.S1_at	C-reactive protein	−4.63
BX922784	Ssc.31140.1.S1_at	Interferon-induced tetratricopeptide	−4.30
NM_213817.1	Ssc.286.1.S1_at	Inflammatory response protein 6	−4.24
NM_214346.1	Ssc.17338.1.S1_at	Sialoadhesin (macrophage cell-surface protein)	−4.08
NM_214214.1	Ssc.657.1.A1_at	Monocyte chemoattractant protein 1 (CCL2)	−3.90
NM_213779.1	Ssc.23797.1.S1_at	Putative macrophage inflammatory protein (MIP)-1β protein	−3.80
BF078671	Ssc.19692.1.S1_at	Chemokine (C-X-C motif) ligand 3 (CXCL3)	−3.71
BX921052	Ssc.10588.1.A1_at	Interferon-induced protein 44 (IFI44) mRNA	−3.40
NM_213804.1	Ssc.15885.1.S1_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	−3.31
CF789161	Ssc.21162.1.S1_at	IRF7	−3.29
BP159940	Ssc.181.1.S1_at	T-cell receptor *g* chain	−3.03
CO937882	Ssc.6676.1.S1_at	Placenta-specific gene 8 protein	−2.92
CN154335	Ssc.4891.1.A1_at	1-Acylglycerol-3-phosphate O-acyltransferase 9 (AGPAT9)	−2.89
BI404223	Ssc.11048.1.S1_at	Placenta expressed gene 8 (PLAC8)	−2.73

Table S2 (Contd.)

Gene function	Primary sequence name	Our assignment	Fold change
CB288192	Ssc.10593.1.S1_at	Interferon-induced protein 44-like (IFI44L)	−2.71
AY643423.1	Ssc.30833.1.S1_at	Chemokine (C-C motif) ligand 3-like 1 (CCL3L1)	−2.70
CO953941	Ssc.29329.1.A1_at	Programmed death ligand 1 (activated by interferon)	−2.68
BI183736	Ssc.8980.1.A1_at	Angiopoietin-like 4 (ANGPTL4)	−2.58
BQ604619	Ssc.28384.1.A1_at	Tumour necrosis factor α (TNFα) receptor	−2.31
BQ604625	Ssc.13780.10.S1_x_at	UDP-N-acetyl-α-D-galactosamine	−2.25
AF464013.1	Ssc.13780.6.S1_x_at	MHC class I antigen	−2.19
AF464013.1	Ssc.13780.6.S1_a_at	MHC class I antigen	−2.15
AF464016.1	Ssc.13780.1.S1_x_at	MHC class I antigen	−2.14
AY293733.1	Ssc.4093.1.A1_at	Interferon *g*	−2.06
Antimicrobial			
NM_214117.1	Ssc.376.1.S1_at	Hepcidin antimicrobial peptide (HAMP)	−4.33
Other functions			
BI183574	Ssc.11557.1.A1_at	ISG15 ubiquitin-like modifier (ISG15)	−33.47
BX676643	Ssc.20101.1.S1_at	Protein 6-16	−9.30
AW313822	Ssc.2641.1.S1_at	ISG ligase UbcH8	−8.23
CK465051	Ssc.12504.1.A1_at	ISG12a	−6.91
NM_213826.1	Ssc.336.1.S1_at	ISG15-specific protease	−5.48
BQ604865	Ssc.13226.1.A1_at	Poly(ADP-ribose) polymerase 9 (PARP9)	−2.60
CA780016	Ssc.12918.1.S1_at	N-myc (and STAT) interactor (NMI)	−2.40
BX674279	Ssc.21917.1.S1_a_at	Ceroid-lipofuscinosis, neuronal 6, late infantile, variant (CLN6)	−3.68
BX915534	Ssc.25756.1.S1_at	Lipocalin 6 (LCN6)	−3.42
CF180854	Ssc.10931.1.S1_at	$\alpha\beta$ Crystallin	−3.39
CK457954	Ssc.1837.2.A1_at	Septin 7 (SEPT7)	−3.09
BX915975	Ssc.10391.2.S1_at	Melanoma cell-adhesion molecule (MCAM)	−2.99
AY264263.1	Ssc.26343.1.A1_at	Neuroglobin mRNA	−2.72
CN154817	Ssc.5421.1.S1_at	synaptotagmin XI (SYT11)	−2.62
BP445026	Ssc.10511.1.A1_at	F-box and leucine-rich repeat protein 5 (FBXL5)	−2.51
BX667123	Ssc.16729.1.S1_at	α-2-Glycoprotein 1, zinc-binding (AZGP1)	−2.45
AY034475.1	Ssc.15883.1.S1_at	Embryonic proteinase inhibitor-1 (EPI1)	−2.41
BQ603979	Ssc.13053.1.A1_at	Caspase 13	−2.41
CN162951	Ssc.2100.2.S1_at	PDZ-domain-containing 7 (PDZD7)	−2.41
BQ604614	Ssc.17987.1.A1_at	C9orf23	−2.36
BQ604618	Ssc.11219.1.S1_at	Fibulin 4	−2.31
BQ604624	Ssc.12446.1.A1_at	Ribosomal protein, large, P1 (RPLP1)	−2.25
BQ604626	Ssc.6433.2.S1_at	Ribosomal protein L36a (RPL36A)	−2.25
CK452619	Ssc.19186.1.S1_at	Myosin light chain 7, regulatory (MYL7)	−2.22
CK461642	Ssc.21805.1.S1_at	Complement component 4-binding protein β (C4BPB)	−2.08
NM_213977.1	Ssc.5062.1.S1_at	Selenoprotein W1	−2.06
CN158073	Ssc.20870.1.S1_at	Fibulin 1C	−2.02
CN165158	Ssc.20226.1.S1_at	Ring finger protein 167 (RNF167)	−2.00
Unknown			
BF709912	Ssc.11086.1.A1_at	Unknown	−5.51
BX923601	Ssc.26189.1.S1_a_at	Receptor (chemosensory) transporter protein 4 (RTP4)	−4.72
BI403077	Ssc.773.1.S1_at	Unknown	−3.05
CF367941	Ssc.19019.1.A1_at	Unknown	−2.85
CF363445	Ssc.9683.1.A1_at	Unknown	−2.82
BI346649	Ssc.6248.1.S1_at	Family with sequence similarity 57, member B (FAM57B)	−2.79
BQ602488	Ssc.13490.1.A1_at	Hypothetical mKIAA1486 protein	−2.53
AK230689	SscAffx.37.1.S1_at	Mitochondrial genome	−2.52
CO953600	Ssc.26509.1.A1_at	Hypothetical LOC529905	−2.42
BQ604611	Ssc.13167.1.A1_at	Hypothetical protein FLJ36031	−2.39

Table S2 (Contd.)

Gene function	Primary sequence name	Our assignment	Fold change
BQ604615	Ssc.2768.1.S1_at	Unknown	−2.34
CO947041	Ssc.29782.1.A1_at	Unknown	−2.20
CO989514	Ssc.22396.1.A1_at	Unknown	−2.15
Cell cycle and signalling			
BQ597478	Ssc.1093.1.A1_at	Frizzled 4	−8.82
CA779841	Ssc.15310.1.S1_at	G-protein αz polypeptide (GNAZ)-like	−5.21
CN162903	Ssc.18226.1.S1_at	Aurora kinase A-interacting protein 1 (AURKAIP1)	−4.83
CK455771	Ssc.24298.1.S1_at	Transmembrane protein with EGF-like and two follistatin-like domains 1 (TMEFF2)	−4.13
BI402878	Ssc.12578.1.A1_at	Insulin-like growth factor 1 (IGF1)	−3.72
BX916841	Ssc.773.2.S1_a_at	Histone H2B	−3.39
BI343951	Ssc.16526.2.S1_at	Rho guanine-exchange factor 16 isoform 1	−3.34
CN025467	Ssc.28340.1.A1_at	Protein kinase Cγ (PRKCG)	−3.20
U89949.1	Ssc.14544.1.S1_at	Folate-binding protein	−2.98
BI400976	Ssc.8909.1.A1_at	Inhibin βB precursor	−2.93
BQ600276	Ssc.9666.1.S1_at	BTG family, member 3 (BTG3)	−2.79
CO986932	Ssc.30169.1.A1_at	Sterile α motif domain-containing 9 (SAMD9)	−2.73
CF180335	Ssc.18010.1.A1_at	SH2 domain protein 1A (SH2D1A)	−2.41
BQ604617	Ssc.27305.1.S1_at	Transforming growth factor β2 (TGFB2)	−2.32
BQ604622	Ssc.28105.1.A1_at	Neuroblastoma, suppression of tumorigenicity 1 (NBL1), a bone morphogenetic protein (BMP) antagonist	−2.28
BQ604623	Ssc.2768.3.S1_at	Anti-oestrogen resistance 3 mRNA	−2.26
BF708594	Ssc.14764.1.A1_at	Growth-arrest and DNA-damage-inducible protein β (GADDβ)	−2.20
BI118962	Ssc.30871.1.A1_at	p53DINP1a	−2.14
BP167425	Ssc.2768.2.S1_at	Platelet-activating factor acetylhydrolase 2	−2.11

Table S3 Genes which are significantly ($P < 0.01$) up-regulated in the livers of CS piglets compared with VD piglets fed parenterally for 7 days post-partum

Accession numbers are from the EST or GenBank® databases.

Gene function	Primary sequence name	Our assignment	Fold change
Ion transport			
L13068.1	Ssc.429.1.A1_at	Calbindin D-9k	100.00
CO991860	Ssc.9517.1.A1_at	Solute carrier family 26, member 4 (SLC26A4)	18.30
BI402402	Ssc.2381.1.A1_at	S100 calcium-binding protein A9 (S100A9; calgranulin B)	4.16
CK450418	Ssc.3800.1.S1_at	Solute carrier family 7 member 9 (SLC7A9)	3.56
CN159136	Ssc.26732.1.S1_at	Chloride channel 5 (CLCN5)	3.33
BI399085	Ssc.6425.3.A1_at	ATP-binding cassette C member 5 (ABCC5)	2.48
RNA and DNA metabolism			
CK461628	Ssc.25090.1.S1_at	HIT type 4 (ZNHIT4)	5.59
AW486763	Ssc.2222.3.S1_at	Leucine-rich repeats and calponin homology (CH)-domain-containing 4 (LRCH4)	4.60
CF175661	Ssc.17874.1.A1_at	TOX high-mobility-group box family member 3 (TOX3)	3.57
CF792703	Ssc.2158.1.A1_at	Myeloid/lymphoid or mixed-lineage leukemia translocated to 3 (MLLT3)	3.34
CO942150	Ssc.31182.1.A1_at	Chemokine (C-C motif) receptor 4 (CCR4)-NOT transcription complex subunit 6-like (CNOT6L)	3.27
BE012932	Ssc.2496.2.S1_at	PHD1	3.26
BP172689	Ssc.24194.2.S1_a_at	Transcription elongation factor A (SII) 3 (TCEA3)	3.20
BP157849	Ssc.23809.2.S1_at	Trinucleotide repeat containing 6B (TNRC6b)	3.16
BP151992	Ssc.23811.1.S1_at	Zinc finger protein 64 homologue (ZFP64)	3.03
CO994053	Ssc.30573.1.S1_at	Zinc finger protein 207	2.79
CN159454	Ssc.6333.1.A1_at	Cleavage- and polyadenylation-specific factor 6 (CPSF6)	2.75
CF368920	Ssc.12117.2.A1_at	Nuclear localized factor 1 (NLF1)	2.58
BX675815	Ssc.20445.1.S1_at	Maintenance of chromosomes 2	2.58
BG896097	Ssc.6410.3.S1_at	Vestigial-like 4 (VGLL4) mRNA 3′ UTR	2.54
BG835188	Ssc.2544.1.S1_at	Hsal2	2.52
CN153725	Ssc.25044.3.A1_a_at	Chromodomain helicase DNA-binding protein 4 (Chd4)	2.50
CN153567	Ssc.1300.1.A1_at	Splicing factor, arginine/serine-rich 14 (SFRS14)	2.47
CF364289	Ssc.28690.3.S1_at	Histone deacetylase 6 (HDAC6)	2.37
CO986164	Ssc.14238.1.S1_a_at	Nucleosome assembly protein 1-like 1 (NAP1L1)	2.36
AF281156.1	Ssc.15962.1.A1_at	Homeobox protein A10	2.32
CK450853	Ssc.23872.1.A1_at	Transforming, acidic coiled-coil-containing protein 1 (TACC1)	2.23
BI341533	Ssc.24535.1.S1_at	Thyroid hormone receptor-associated protein 1 (TRA1)	2.16
CN157771	Ssc.17620.1.S1_at	ATP-dependent RNA helicase (DDX24)	2.06
BF708772	Ssc.8929.1.S1_at	Transcription elongation factor A (SII)-like 8 (TCEAL8)	2.05
CN155277	Ssc.27342.1.S1_at	One cut homeobox 2 (ONECUT2)	2.03
BG382145	Ssc.2489.2.S1_at	R3H domain and coiled-coil containing (R3HCC)	2.02
BI181396	Ssc.210.9.A1_at	CTD small phosphatase 2 (CTDSP2)	2.02
Metabolism			
CN157463	Ssc.15723.1.S1_at	Myo-inositol 1-phosphate synthase A1	13.35
CN166698	Ssc.2873.1.S1_at	PDZ-domain-containing 1-interacting protein 1 (PDZK1IP1)	12.27
CA778930	Ssc.15373.1.S1_at	Ankyrin repeat domain 43 (ANKRD43)	7.72
BI403313	Ssc.12695.1.S1_at	Acyl-CoA synthetase long-chain member 4	3.98
BQ604949	Ssc.21999.1.S1_a_at	Aldehyde dehydrogenase 3 family B1 (ALDH3B1)	3.96
CF177166	Ssc.26436.1.S1_at	Glycogen synthase kinase 3α (GSK3A)	3.67
BQ604008	Ssc.4426.1.S1_at	Acetyl-CoA acetyltransferase 2 (ACAT2)	3.55
CK463196	Ssc.2622.1.S1_at	Phosphoglycerate dehydrogenase (PHGDH)	3.52
CF789276	Ssc.21113.1.A1_at	Adenylate kinase 3-like 1 (AK3L1)	3.24
BQ600296	Ssc.295.2.A1_at	Glutaminase	3.16
CF364907	Ssc.18947.1.A1_at	COX15 homologue, cytochrome *c* oxidase assembly	3.01

Table S3 (Contd.)

Gene function	Primary sequence name	Our assignment	Fold change
AU058728	Ssc.6441.2.S1_at	SDR family member 7 (DHRS7)	2.94
BX675010	Ssc.19105.1.S1_at	Lipase hepatic (LIPC)	2.79
BF710977	Ssc.7534.1.A1_at	γ-Glutamyltranspeptidase	2.69
BX926752	Ssc.21858.1.A1_at	Hydroxysteroid dehydrogenase like 1 (HSDL1)	2.37
CO956776	Ssc.3410.2.S1_a_at	ATPase, Class II, type 9A (ATP9A)	2.34
AY550037.1	Ssc.6323.1.S1_at	Adipose differentiation-related protein (ADFP)	2.19
CF176222	Ssc.21842.1.S1_a_at	Named CTD-1, but probably an oxido reductase (ADFP)	2.05
CK450664	Ssc.10695.2.S1_at	Acyl-CoA synthetase short-chain family member 3 (ACSS3)	2.01
Inflammation			
AB105384.1	Ssc.18552.1.S1_at	SLA-2 mRNA for MHC class I antigen	13.74
CN154658	Ssc.7175.1.S1_at	Fetuin B (FETUB)	11.41
BP464815	Ssc.17204.1.S1_at	Cell differentiation protein (MAL)	6.21
AJ684641	Ssc.12313.1.A1_at	IL7b	2.61
AF248307.1	Ssc.16359.8.S1_at	VDJ region	2.53
CK457066	Ssc.24188.1.A1_at	Platelet factor 4 (PF4)	2.51
BX670708	Ssc.16359.2.S1_at	Artial VDJ heavy chain gene for immunoglobulin heavy chain	2.39
AB087953.1	Ssc.17783.2.S1_at	T-cell receptor α chain mRNA C-region 3′ end of cds	2.07
Antimicrobial			
CK464499	Ssc.20938.1.S1_at	Azurocidin 1p	10.84
NM_214450.1	Ssc.27609.1.S1_at	PR39 protegrin family peptide	8.37
M81327.1	Ssc.13769.1.S1_at	Lactotransferrin (LTF)	5.01
L24745.1	Ssc.420.2.S1_x_at	Homologue of proline/arginine-rich antibacterial peptides	4.92
NM_213863.1	Ssc.420.1.S1_a_at	Antimicrobial peptide precursor (PG-2)	4.86
NM_213863.1	Ssc.420.1.S1_x_at	Antimicrobial peptide precursor (PG-2)	4.40
L39641.1	Ssc.420.4.S1_a_at	Antibacterial peptide precursor (PMAP37)	4.19
Other functions			
BQ601666	Ssc.8177.1.A2_at	Proteolipid protein, lipophilin	12.11
BQ601157	Ssc.8177.1.A1_at	Proteolipid protein 1 (PLP1)	9.69
CF366016	Ssc.8925.1.S1_at	α Chain (α globin)	9.48
CK463769	Ssc.12561.1.A1_at	Branched-chain aminotransferase 1 (BCAT1)	9.34
CN029255	Ssc.5276.1.A1_at	F-box and leucine-rich repeat protein 15 (FBXL15)	5.40
BF712177	Ssc.7772.1.A1_at	BED-type-containing 5 mRNA	5.40
CF790688	Ssc.22211.1.A1_at	α_2-HS glycoprotein	4.54
CO947301	Ssc.30916.1.A1_at	Adhesion molecule with Ig-like domain 2 (AMIGO2)	4.49
CO941352	Ssc.22551.1.A1_a_at	Protoporphyrinogen oxidase (PPOX)	4.43
BI400382	Ssc.10263.1.A1_at	Kelch repeat and BTB (POZ) domain-containing 7 (KTBBD7)	4.42
CO941201	Ssc.29588.1.A1_at	Dystonin (DST) transcript variant 1	4.41
CF362307	Ssc.19431.1.S1_at	Ectonucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3)	3.69
BF190189	Ssc.20017.2.S1_at	MHC10	3.46
CK463721	Ssc.27429.1.A1_at	Cytoplasmic polyadenylation element-binding protein	3.34
BI817204	Ssc.4368.1.S1_at	F-box protein 32 (FBXO 32)	3.30
CN155052	Ssc.5853.1.S1_at	Nucleoside diphosphate linked moiety X (Nudix)-type motif 16-like 1 (NUDT16L1)	3.27
CK450823	Ssc.5735.1.A1_at	Stabilin 1 (STAB1)	3.19
CN159691	Ssc.2749.1.S1_at	Septin 8 (SEPT8)	3.17
NM_214021.1	Ssc.94.1.A1_at	Myosin 6	3.07
AW308314	Ssc.27794.2.S1_at	Tubulin tyrosine ligase-like family 3 (TTLL3) (putative)	2.96
BX914324	Ssc.15332.1.S1_at	Sequence similarity 46 member A	2.88
BX673920	Ssc.19586.1.S1_at	C1orf210	2.86
BF708392	Ssc.8843.1.A1_at	Fibronectin gene ED-B region	2.77
NM_213868.1	Ssc.137.1.S1_at	Ficolin	2.75

Table S3 (Contd.)

Gene function	Primary sequence name	Our assignment	Fold change
BE232264	Ssc.3383.1.S1_at	LIM domain-binding 2 (LDB2)	2.67
CF789935	Ssc.22287.1.S1_at	Family with sequence similarity 43 member A (FAM46A)	2.65
CO988922	Ssc.30245.1.A1_at	ADAM metallopeptidase with thrombospondin type 1 motif 6 (ADAMTS6) variant 2	2.63
CN163150	Ssc.27170.1.S1_at	Proteinase 3 (PRTN3)	2.63
BQ597894	Ssc.10756.1.A1_at	Hypothetical protein LOC100134123	2.61
BX923048	Ssc.25819.1.S1_at	Hypothetical protein LOC284023	2.60
CF363122	Ssc.18770.1.A1_at	Tigger transposon putative	2.58
CO940939	Ssc.31183.1.S1_at	Acid phosphatase-like 2 (ACPL2)	2.54
CN161021	Ssc.17582.1.A1_a_at	Rieske (Fe-S) domain containing (RFESD)	2.53
CN162687	Ssc.26893.1.A1_at	*Homo sapiens* hemicentin 1 (HMCN1)	2.53
CF367968	Ssc.18103.1.A1_at	LOC515496 hypothetical protein LOC515496	2.51
CF360048	Ssc.28915.1.S1_at	C1orf78	2.51
BG834406	Ssc.4010.1.S1_at	F-box protein 17 (FBXO17)	2.46
BX923373	Ssc.25846.1.S1_at	Phosphofurin acidic cluster sorting protein 2 (PACS2)	2.46
NM_213860.1	Ssc.646.1.S1_at	Stefin A8	2.46
BF702347	Ssc.4597.1.A1_at	Mannan-binding lectin serine peptidase 2 (MASP2)	2.42
AW668731	Ssc.28032.1.S1_at	Ubiquitin-specific peptidase 27	2.39
CN069824	Ssc.26476.1.A1_at	Procollagen-proline 2-oxoglutarate 4-dioxygenase	2.37
CO945178	Ssc.29731.1.A1_at	Procollagen type VII α1	2.35
CF795865	Ssc.21337.1.A1_at	TBC1 domain family 12 (TBC1D12)	2.31
BX665827	Ssc.21780.1.A1_at	Myosin IE transcript variant 5	2.27
CF363086	Ssc.19442.1.A1_at	Fragile histidine triad gene (FHIT) on chromosome 3	2.26
BQ605090	Ssc.2288.1.A1_at	WD repeat domain 6 (WDR6)	2.24
CF368531	Ssc.19052.1.S1_at	Putative transmembrane protein 9 (TMEM9)	2.23
BX925008	Ssc.4177.2.S1_at	WAS protein family 3 (WASF3)	2.22
CF176298	Ssc.11403.1.S1_at	β-Spectrin	2.20
AW414584	Ssc.19323.2.S1_at	Family with sequence similarity 114	2.20
BX674789	Ssc.9560.2.S1_a_at	Actin-binding LIM protein 1 (ABLIM1)	2.20
CO986761	Ssc.30768.1.S1_at	Yippee-like 2 (YPEL2)	2.19
BX926190	Ssc.21745.1.S1_at	Ankyrin 1	2.19
CD572294	Ssc.17766.1.A1_at	Enabled homologue	2.16
BE232076	Ssc.21302.2.S1_at	Ectonucleotide pyrophosphatase/phosphodiesterase 6 (ENPP6)	2.15
BG609680	Ssc.21254.2.S1_at	α_1-Antiproteinase	2.14
CK463668	Ssc.22341.1.A1_at	UV radiation resistance associated gene (UVRAG)	2.14
CF360675	Ssc.18682.1.S1_at	Shroom2	2.13
CO937074	Ssc.29832.1.A1_at	O-sialoglycoprotein endopeptidase (OSGEP)	2.10
BX666791	Ssc.2884.1.S1_at	Villin	2.10
CN159832	Ssc.23465.1.S1_at	Tight junction protein 2 (TJP2)	2.10
BF191966	Ssc.27581.2.S1_at	Trypsin-domain-containing 1 (TYSND1)	2.10
BF711365	Ssc.3966.1.S1_at	KIAA1147	2.08
BX921471	Ssc.24707.1.S1_at	O-linked N-acetylglucosamine (GlcNAc) transferase	2.08
NM_213862.1	Ssc.16228.1.S1_at	Platelet basic protein	2.08
CN166368	Ssc.27556.1.S1_at	Family with sequence similarity 46C (FAM46C)	2.07
BG383739	Ssc.19172.2.S1_at	Polynucleotide kinase 3′-phosphatase (PNKP)	2.07
BX671222	Ssc.21838.1.S1_at	Dynein cytoplasmic 2	2.06
BM659360	Ssc.16609.1.S1_at	Sarcoglycan β	2.04
CO941974	Ssc.24339.1.A1_at	Utrophin transcript variant 3 (UTRN)	2.04
Unknown			
BG608757	Ssc.2774.1.S1_at	Unknown	11.27
AJ236935.1	Ssc.489.1.A1_at	Hypothetical protein	8.29

Table S3 (Contd.)

Gene function	Primary sequence name	Our assignment	Fold change
CO944541	Ssc.29744.1.A1_at	Unknown	7.80
BE235737	Ssc.3344.2.S1_at	Unknown	6.06
BX670653	Ssc.21856.1.S1_at	Unknown	5.18
BQ602909	Ssc.12996.1.A1_at	Unknown	4.73
BI403316	Ssc.12697.1.A1_at	Chromosomal regions	4.42
CN156200	Ssc.24808.1.A1_at	Chromosomal regions	4.09
CN158430	Ssc.7890.1.S1_at	Chromosomal regions	3.89
CN025861	Ssc.28280.1.A1_at	Unknown	3.84
BQ597414	Ssc.10620.1.A1_at	Chromosomal regions	3.78
BG382216	Ssc.3608.1.S1_at	Unknown	3.52
CF175953	Ssc.17899.1.A1_at	Chromosomal regions	3.45
BQ599723	Ssc.7898.1.A1_at	Chromosomal regions	3.37
CF787324	Ssc.21623.1.S1_at	Hypothetical protein	3.22
CN069704	Ssc.26467.1.A1_at	Unknown	3.21
CF792078	Ssc.28416.1.A1_at	Unknown	3.15
BF702977	Ssc.8030.1.A1_at	Hypothetical protein LOC533126	2.97
CO993952	Ssc.30568.1.A1_at	Unknown	2.94
CK465707	Ssc.18024.1.A1_at	Unknown	2.93
BP173153	Ssc.20419.2.S1_at	Chromosomal regions	2.90
CN160660	Ssc.8782.1.S1_a_at	Chromosomal regions	2.89
CO952005	Ssc.29212.1.A1_at	Unknown	2.85
CK463021	Ssc.24683.1.A1_at	Unknown	2.83
CO947798	Ssc.30008.1.A1_at	Unknown	2.82
CO945834	Ssc.29796.1.A1_at	Chromosomal regions	2.80
AJ660919	Ssc.10650.1.A1_at	Hypothetical protein LOC735557	2.73
BI181884	Ssc.11342.1.A1_at	Unknown	2.68
BQ603962	Ssc.11875.1.A1_at	Hypothetical protein LOC606495	2.63
CO943502	Ssc.29640.1.A1_at	Chromosomal regions	2.59
BE233223	Ssc.16538.1.S1_at	*Homo sapiens* chromosome clone	2.50
CF180231	Ssc.18136.1.A1_at	*Homo sapiens* chromosome clone	2.47
CF362574	Ssc.19290.2.A1_at	*Homo sapiens* chromosome clone	2.45
AJ653579	Ssc.28545.1.A1_at	Unknown	2.44
BI404670	Ssc.4464.1.S1_at	*Homo sapiens* cDNA clone IMAGE:5263177	2.41
BI402948	Ssc.12595.1.A1_at	Unknown	2.39
BQ599735	Ssc.6595.1.A1_at	Unknown	2.32
BI181000	Ssc.31159.1.A1_at	MKIAA4095	2.29
BI405073	Ssc.13358.1.A1_at	Unknown	2.25
CK464611	Ssc.24753.1.S1_at	Unknown	2.23
CO990628	Ssc.30351.1.A1_at	Unknown	2.23
CK450820	Ssc.1808.1.S1_at	Unknown	2.22
BI398682	Ssc.9723.1.A1_at	C1orf34	2.21
BI399335	Ssc.9768.1.A1_at	Unknown	2.19
CO945086	Ssc.29728.1.A1_at	Unknown	2.17
BX917576	Ssc.20419.1.S1_at	Unknown	2.16
BX671820	Ssc.21790.1.A1_at	Hypothetical protein LOC509263	2.16
AW480546	Ssc.24671.1.S1_at	Unknown	2.14
BQ601671	Ssc.14277.1.A1_at	Unknown	2.14
CN166761	Ssc.27108.1.A1_at	Unknown	2.10
CA779594	Ssc.16572.1.S1_at	Unknown	2.10
AJ682522	Ssc.23240.2.S1_at	Unknown	2.09
AJ654528	Ssc.28555.2.S1_a_at	Unknown	2.08
CO937361	Ssc.29841.1.A1_at	Unknown	2.05

Table S3 (Contd.)

Gene function	Primary sequence name	Our assignment	Fold change
BG609584	Ssc.3957.1.A1_at	Unknown	2.05
CF362278	Ssc.19535.1.A1_at	Unknown	2.01
Cell cycle and signalling			
CK457102	Ssc.24172.1.A1_at	Natriuretic peptide receptor C (NRP3)	7.66
CN163379	Ssc.19481.1.S1_at	WW domain-binding protein 5 (WBP5)	4.98
AF324155.1	Ssc.3831.1.S1_at	Corticosteroid-binding globulin	4.55
CK454440	Ssc.1791.3.S1_a_at	Secernin 2 (SCRN2)	4.50
CK460180	Ssc.25059.1.A1_at	Neuronal guanine nucleotide exchange factor (NGEF)	4.45
BI183564	Ssc.7039.1.A1_at	Nerve growth factor receptor (TNFRSF16)-associated protein 1	4.20
AW785890	Ssc.2354.1.S1_at	G-protein-coupled receptor 160 (GRP160)	3.86
CN163300	Ssc.848.1.S1_at	Similar to thyroid hormone receptor interactor 13 (TRIP13)	3.55
BI359661	Ssc.6483.2.S1_at	Ataxin 2-related protein	3.36
NM_214088.1	Ssc.15749.1.S1_at	Cyclin D2 (CCND2)	3.32
AJ665385	Ssc.22903.2.A1_at	Gene for mitogen-activated protein kinase kinase kinase kinase 3 (MAP4K3)	3.28
CO954035	Ssc.29337.2.S1_at	Poly(ADP-ribose) polymerase family member 10 (PARP10)	3.08
CO945303	Ssc.29713.1.A1_at	Sfi1 homologue isoform b	2.69
CA781104	Ssc.15291.1.A1_at	Natriuretic peptide receptor 3 (NPR3)	2.62
CN159329	Ssc.16346.1.S1_at	Fibroblast growth factor receptor 2 (FGFR2)	2.60
CA778421	Ssc.21536.2.S1_at	Phosphodiesterase 5A (PDE5A)	2.57
BI186417	Ssc.5836.1.S1_at	Ephrin-B1 (EFNB1)	2.54
CN158780	Ssc.21653.1.S1_at	Leucocyte receptor cluster (LRC) member 8 (LENG8)	2.54
CN163406	Ssc.26881.1.S1_at	Nuclear factor κB (NF-κB) inhibitor	2.49
CF180763	Ssc.18230.1.S1_at	Rac/Cdc42 guanine nucleotide-exchange factor (ARHGEF6)	2.43
BX669202	Ssc.16470.2.A1_at	PAP1B	2.38
CO947208	Ssc.29711.1.S1_at	Neuron navigator 2 (NAV2)	2.36
CO941085	Ssc.31108.1.A1_at	G_1-to-S phase transition 2 (GSPT2)	2.34
BF711393	Ssc.10530.1.A1_at	Paternally expressed 3 (PEG3)	2.34
CK455958	Ssc.24084.1.A1_at	A receptor, type IIB (ACVR2B)	2.33
CK465614	Ssc.20438.1.S1_at	Prostaglandin F receptor (FP) (PTGFR)	2.32
BQ604774	Ssc.13203.1.A1_at	Putative regulator of G-protein signaling 7 (RGS7)	2.27
BQ602486	Ssc.3649.1.A1_at	RCAN family 3 (RCAN3)	2.24
CO956260	Ssc.978.1.S1_at	Von Willebrand factor (VWF)	2.24
BQ603286	Ssc.24314.1.S1_at	Pellino homologue 2 (*Drosophila*) (PELI2)	2.21
CN162732	Ssc.14386.1.A1_at	Cyclin G2 (CCNG2)	2.21
BI402713	Ssc.12529.1.A1_at	Serine/threonine kinase 32B (STK32B)	2.20
CF361753	Ssc.9108.2.S1_at	WAP four-disulfide core domain 1 (WFDC1)	2.19
CK464193	Ssc.22598.2.A1_a_at	A kinase (PRKA) anchor protein 8-like (AKAP8L)	2.15
CF793335	Ssc.3327.1.A1_at	Dapper, antagonist of β-catenin	2.07
NM_214114.1	Ssc.328.1.S1_at	Janus kinase 1 (JAK)	2.05
BI404830	Ssc.13292.1.A1_at	Disabled homologue 2 (DAB2)	2.05
CK466050	Ssc.20716.1.S1_at	Tumor suppressor candidate region gene 2 (GLTSCR2)	2.04
CO955588	Ssc.6156.1.A1_at	Neuroblastoma, suppression of tumorigenicity 1 (NBL1)	2.03
CF794921	Ssc.22082.1.A1_at	Disabled homologue 1 (DAB1)	2.02
Markers of fetal hepatocytes and paternally expressed genes			
CN162607	Ssc.13476.1.A1_at	Paternally expressed 10 (PEG10)	7.04
CK458546	Ssc.25001.1.A1_at	Dual-specificity phosphatase 9 (DUSP9)	7.04
CK456429	Ssc.2397.1.A1_at	Neighbour of Punc E 11 (NOPE)	5.54
CN165575	Ssc.3014.1.S1_at	Melanoma antigen family D4 (MAGED4)	3.48
NM_001001860.1	Ssc.22086.1.A1_at	Melanoma antigen family D1 (MAGED1)	3.44
CK453813	Ssc.22623.2.S1_at	Paternally expressed 3(PEG3)	2.99
NM_214317.1	Ssc.7131.1.A1_at	α Fetoprotein (AFP)	2.98
BF711624	Ssc.7669.1.A1_at	Orosomucoid2 agp-2	2.47
BQ604762	Ssc.2112.1.S1_at	Putative dual-specificity phosphatase 4 (DUSP4)	2.29

Table S4 Similar fold changes are seen in expression of key hepatic genes when determined by gene array or qRT-PCR between piglets born by CS or VD at 7 days post-partum

All values are means; qRT-PCR results were normalized to the expression of RPLP0 (acidic ribosomal phosphoprotein P0).

		Fold change		
Direction of change	Gene name	qRT-PCR	*P* value	Array
Increased by CS	Calbindin D-9k	105.66	0.014	100
	Corticosteroid-binding globulin (SERPINA6)	8.02	0.027	4.55
	Aldehyde dehydrogenase 3 family B1 (ALDH3B1)	2.12	0.624	3.96
	Alcohol dehydrogenase 5 (class III) (ADH5)	1.38	0.462	1.24
	Acyl-CoA synthetase long-chain family 4 (ACSL4)	1.10	0.086	3.98
Increased by VD	Angiopoietin-like 4 (ANGPTL4)	−3.95	0.050	−2.48
	Phosphotyrosine interaction-domain-containing 1 (PID1)	−1.10	1.000	−8.01
	Cytochrome C (P450)	−11.64	0.050	−8.24
	Galanin-like peptide (GALP)	−24.85	0.050	−27.46

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