British Journal of Nutrition (2009), **102**, 1445–1452
doi:10.1017/S0007114509990389

Maternal diets deficient in folic acid and related methyl donors modify mechanisms associated with lipid metabolism in the fetal liver of the rat

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(Received 11 November 2008 – Revised 6 April 2009 – Accepted 30 April 2009 – First published online 1 July 2009)

Previously we have examined the effects of diets deficient in folic acid (−F) or folate deficient with low methionine and choline (−F LM LC) on the relative abundance of soluble proteins in the liver of the pregnant rat. In the present study we report the corresponding changes in the fetal liver at day 21 of gestation. The abundance of eighteen proteins increased when dams were fed the −F diet. When dams were fed the −F LM LC diet, thirty-three proteins increased and eight decreased. Many of the differentially abundant proteins in the fetal liver could be classified into the same functional groups as those previously identified in the maternal liver, namely protein synthesis, metabolism, lipid metabolism and proteins associated with the cytoskeleton and endoplasmic reticulum. The pattern was consistent with reduced cell proliferation in the −F LM LC group but not in the −F group. Metabolic enzymes associated with lipid metabolism changed in both the −F and −F LM LC groups. The mRNA for carnitine palmitoyl transferase were up-regulated and CD36 (fatty acid translocase) down-regulated in the −F group, suggesting increased mitochondrial oxidation of fatty acids as an indirect response to altered maternal lipid metabolism. In the −F LM LC group the mRNA for acetyl CoA carboxylase was down-regulated, suggesting reduced fatty acid synthesis. The mRNA for transcriptional regulators including PPARα and sterol response element-binding protein-1c were unchanged. These results suggest that an adequate supply of folic acid and the related methyl donors may benefit fetal development directly by improving lipid metabolism in fetal as well as maternal tissues.

Pregnancy: Methionine: Choline: Folic acid: Endoplasmic reticulum

It is well established that folic acid supplements reduce the risk of neural tube defects[1] and improve the growth of the human fetus[2]. Although the detailed mechanism has not been elucidated, folate supplements are believed to improve the flow of methyl groups through the methionine cycle[3,4]. Studies of laboratory animals also suggest that altered one-carbon metabolism has long-term effects on cardiovascular and metabolic health of the offspring[5]. In rats, the adverse effects of protein restriction can be reversed by the addition of a supplement of either folic acid, or the related amino acid glycine[6–8]. Folate-deficient (−F) diets also produce changes in the insulin axis, with a transient increase in the insulin content of the fetal pancreas and a tendency for glucose-stimulated insulin release to be increased in the offspring of rats fed a −F diet[9].

Previously we have shown that when pregnant rats are fed diets low in folic acid (−F) there are extensive changes in the metabolism of lipids and amino acids[10], and when the diet is additionally low in methionine and choline (−F LM LC) lipids accumulate in the maternal liver[11]. Differentially abundant proteins in the soluble fraction of the maternal liver could be allocated into nine functional groups, five involved in metabolic processes, namely the folate–methionine cycle, tyrosine metabolism, protein metabolism, energy metabolism and lipid metabolism and three in cellular processes, namely endoplasmic reticulum function, bile production and antioxidant defence. There were also changes in the levels of mRNA coding for the rate-determining enzymes of fatty acid synthesis and oxidation as well as the transcriptional regulators that control them (PPAR and sterol response element-binding protein-1c). These transcriptional activators are also involved in the development of non-alcoholic fatty liver disease[12]. The present experiments were conducted to establish whether there are corresponding changes in the fetal livers of the animals used in our previous studies[10,11]. Fetal development may be affected directly by the lack of folate, but since −F diets produce such large changes in maternal metabolism there is also the possibility of indirect effects caused by changes in the nutrient supply derived from the maternal circulation.

Methods

Experimental diets

The experimental diets were based on the American Institute of Nutrition (AIN)-76 formula[13] and contained 90 g casein/kg supplemented with a mixture of synthetic amino acids

Abbreviations: −F, folate-deficient; −F LM LC, folate-deficient with low methionine and choline; gadd153, growth arrest and DNA damage protein 153.

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equivalent to those found in a further 90 g casein/kg as described previously[10]. Folic acid was omitted from the −F and −F LM LC diets. No additional methionine was added to the −F LM LC diets (total methionine concentration 2·3 mg methionine/kg diet compared with 5·6 mg methionine/kg diet in the control) and choline chloride was reduced to 0·1 % (w/w) (compared with 0·2 % (w/w) in the control).

Animals

All experimental procedures were approved by the ethical review committee of the Rowett Research Institute and conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986. Female rats of the Rowett hooded strain bred in the Institute were allocated to three groups of eight animals (mean body weight 208 g), housed on sawdust bedding and fed control, −F or −F LM LC diets *ad libitum*. The animals were offered the experimental diets for a 2-week adaptation period and then were mated with males of the same strain. The day on which a vaginal plug was detected was denoted day 0. The female rats were maintained on their corresponding diets until day 21 of gestation, when they were anaesthetised by halothane inhalation and killed by exsanguination. The fetuses were rapidly removed, weighed and killed by decapitation. The fetal livers were rapidly dissected; samples were frozen in liquid N_2 and subsequently stored at −80°C until required.

Proteomics

Samples from a second portion of the same fetal liver used for the RNA preparation were homogenised in buffer containing protease inhibitors and centrifuged for 30 min at 100 000 ***g*** at 4°C (Beckman TL-100 centrifuge; Beckman Coulter Ltd, High Wycombe, Bucks, UK) as described previously[11]. Proteins in the supernatant fraction (300 μg from each extract) were separated in the first dimension on BioRad immobilised pH gradient strips (pI 3–10; BioRad, Hemel Hempstead, Herts, UK). Following equilibration with the second dimension buffer, which contained 135 mM-iodoacetamide, proteins were separated on an 18 × 18 cm SDS polyacrylamide gel at 200 V for 9·5 h. Molecular weights were determined with a Bio-Rad Precision Plus Protein Mr standard. Gels were stained with colloidal Coomassie brilliant blue, analysed using PDQuest software (v. 7; BioRad) and spots of interest excised with a robotic spot cutter (BioRad). Proteins were digested with trypsin and the identities were determined by matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) MS (Voyager-DE PRO; Applied Biosystems, Warrington, Cheshire, UK) or by LC/MS/MS (Q-trap; Applied Biosystems). The peptide mass list profiles from both systems were analysed using the Matrix Science 'Mascot' web tool (Matrix Science Ltd, London, UK; http://www.matrixscience.com) using the MSDB database (maintained by the Proteomics Department, Hammersmith Campus, Imperial College London, UK). The Mascot database search criteria allowed one missed cleavage, carbamidomethyl modification of cysteine, partial oxidation of methionine and a charged state of MH^+. A Mascot score of at least 50 with 20 % matched peptides covering at least 10 % of the protein sequence was required for a positive identification when the probability value was $P<0·05$.

Real-time polymerase chain reaction

Total RNA was extracted from a portion of fetal liver from one female fetus chosen randomly from the litter, using Trizol reagent (Sigma, Poole, Dorset, UK) as described previously[14]. Samples of 200 ng total RNA were reverse transcribed using the TaqMan Reverse Transcription Reagents Kit (Applied Biosystems) primed with random hexamers. The levels of cDNA relative to the 18S ribosomal RNA were measured using the SYBR Green real-time PCR kit (Applied Biosystems). The identity of the products was confirmed by sequencing and a calibration curve showed that the response was linear over the range measured. Relative target quantity was calculated from the standard curve and the results expressed as the ratio of the product relative to the product from the 18S rRNA.

Statistical analysis

Data are presented as mean values with their standard errors. The gene expression data (Table 1) were analysed by ANOVA followed by Fischer's multiple comparison test (Genstat 7 statistical package; Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Herts, UK). Images of the proteomic gels (six animals per treatment) were normalised using the PDQuest software (BioRad) to determine the mean corrected spot density, followed by pair-wise comparisons (Student's *t* test) of each spot on the control gels with either the −F or the −F LM LC gels. The spot densities of proteins identified in this preliminary screen were subsequently analysed by ANOVA followed by Fischer's multiple comparison test (Genstat).

Results

Fetal growth

The growth and metabolic changes in the animals have been described in detail previously[9–11]. Briefly, the fetuses of dams fed the −F diet were approximately 18 % heavier (4·84 (SEM 0·07) g) than the controls (4·10 (SEM 0·03) g), while those from dams fed the −F LM LC diet were approximately 10 % smaller than the controls (3·71 (SEM 0·07) g; $P=0·004$; ANOVA). The fetal liver in the dams fed the −F LM LC diets weighed less (0·192 (SEM 0·024) g) compared with those in the control (0·259 (SEM 0·023) g) and −F groups (0·268 (SEM 0·026) g; $P=0·003$; ANOVA). Folate deficiency increased the concentrations of homocysteine, glycine, serine and threonine in the fetal serum but the changes were not as pronounced as in the maternal serum. The −F diets reduced the folate content of the fetal liver to approximately 30 % of the control[10].

Proteomic analysis of soluble proteins

Approximately 800 proteins were resolved on the two-dimensional electrophoresis gels of the soluble protein fraction of the fetal liver at day 21 of gestation. Comparisons of the

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Table 1. Proteins associated with methionine metabolism, protein synthesis and proteolysis*

(Mean values with their standard errors for six animals per group)

Treatment...		Mean pixel density							
		Control		− F		− F LM LC			
Spot no.	Protein name	Mean	SEM	Mean	SEM	Mean	SEM	*P*	NCBI accession
Methyl metabolism									
4403	S-adenosylhomocysteine hydrolase chain A	641[a]	53	761[a]	74	1025[b]	122	0·016	1B3RB
4510	Adenosylhomocysteinase	91[a]	21	86[a]	19	194[b]	40	0·023	P10760
4107	Protein-L-isoaspartate (D-aspartate) *O*-methyltransferase	80[a]	12	81[a]	18	132[b]	14	0·036	P22062
4007	PCD/DCoH	286[a]	87	235[a]	54	514[b]	74	0·045	EDL93037
0028	Uroc1 (urocanase-containing domain)	80[a]	17	57[a]	7	176[b]	39	0·009	AAH22133
Protein synthesis									
3512	Phenylalanine-tRNA synthetase-like, *Mus musculus* (mouse)	1931[a]	257	2148[a,b]	177	2734[b]	204	0·054	AAH79364
2502	Translation initiation factor eIF-4A1	1700[a]	218	1521[a]	106	851[b]	189	0·012	BC063812
Proteolysis									
5002	Proteasome endopeptidase complex chain C9	9388[a]	1195	13 501[b]	1100	8243[a]	465	0·006	X53304
5108	Proteasome endopeptidase complex β chain C7-I	249[a]	29	351[a,b]	67	484[b]	73	0·031	S38725
5602	Lap3	1301[a]	113	1591[b]	101	1225[a]	53	0·042	AAH79381
6812	Proteasome protein p45/SUG (fragment)	46[a]	8	44·5[a]	8	73[b]	6	0·029	BAA22935
1306	Rat protein 2B28 (mediator of ubiquitin pathway)	144[a]	25	177[a]	43	276[b]	96	0·017	AAH06701
1105	Cathepsin B precursor	383[a]	85	498[a,b]	149	801[b]	100	0·048	AAA40993
3002	Bendless protein (Ube2n)	134[a]	24	146[a]	23	241[b]	36	0·034	Q9EQX9

− F, folate-deficient diet; − F LM LC, folate-deficient diet with low methionine and choline; NCBI, National Center for Biotechnology Information; PCD, pterin-4a-carbinolamine dehydratase; DCoH, dimerisation cofactor of hepatic nuclear factor-1; eIF-4A1, eukaryotic initiation factor-4A1; Lap3, leucine aminopeptidase 3; SUG, sugarbabe; Ube2n, ubiquitin-conjugating enzyme E2.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

* Data were analysed by one-way ANOVA followed by Fischer's unprotected test.

relative abundance of proteins from the liver of animals fed the control diet with those fed the −F diet showed that eighteen proteins were up-regulated ($P<0·05$; Student's *t* test). There were no proteins down-regulated in the fetal livers of dams fed the −F diet. Comparisons of the patterns from animals fed the −F LM LC diet with the control showed that thirty-three proteins were up-regulated and eight proteins were down-regulated ($P<0·05$; Student's *t* test). All of the proteins identified in the initial analysis were sequenced by MS and allocated to different functional groups. Table 1 shows the proteins associated with methionine metabolism, protein synthesis and proteolysis. Table 2 shows the proteins associated with energy metabolism, fat metabolism and cell signalling. Table 3 shows the proteins associated with endoplasmic reticulum function and Fe metabolism. Table 3 also includes a small number of proteins which could not be allocated to these groups. A number of proteins produced more than one spot. This may be the result of a number of factors which are either technical factors such as proteolysis during sample preparation and biological factors including differences in post-translational processing.

Gene expression

The levels of mRNA coding for enzymes associated with lipid metabolism and the transcriptional regulators that control them are shown in Table 4. The mRNA for acetyl CoA carboxylase, associated with fatty acid synthesis, was decreased by approximately 23 % in the livers of fetuses fed the −F LM LC diet when compared with the control. The mRNA for fatty acid synthase also tended to be approximately 40 % lower in the −F LM LC group. The −F diet had no effect. In contrast, the levels of the mRNA for liver type carnitine palmitoyl transferase-1 increased 273 % in the −F group but was unchanged in the −F LM LC group when compared with the control. The mRNA for acyl CoA oxidase was unchanged in all groups. There was a 42 % decrease in the mRNA coding for the fatty acid transporter CD36 (fatty acid translocase) in the −F group whereas the levels were unchanged in the −F LM LC group.

There were no changes in the mRNA coding for the main transcriptional regulators of hepatic lipid metabolism (PPARα, liver X receptor-α, sterol response element-binding protein-1c, and the CCAAT/enhancer-binding proteins C/EBPα and C/EBPβ) in either the −F or −F LM LC groups. The levels of the mRNA coding for growth arrest and DNA damage protein 153 (gadd153) were not significantly different when the uncorrected data were analysed by ANOVA ($P=0·240$). However, litter size was found to be a strong covariate ($P=0·007$). This correction reduced the overall variance and when it was included in the analysis, the levels of the mRNA for gadd153 were unchanged in the −F group but increased by 33 % in the −F LM LC group compared with the control.

Discussion

The relative abundance of proteins in the soluble fraction of the liver is determined by a number of factors, including rates of production, sequestration into other fractions and changes in post-translational processing, giving a unique insight into the impact of maternal diet on numerous processes occurring within a tissue. Many of the differentially abundant proteins in the fetal liver can be classified into the same functional groups as those previously identified in the maternal liver[11], namely protein synthesis, metabolism, lipid metabolism and proteins associated with the cytoskeleton and endoplasmic reticulum. Similar classes of proteins have also been identified as being differentially abundant in the livers of adult animals fed −F rations[15] or following drug-induced steatosis[16]. Two additional groups of proteins were differentially abundant in the fetal liver: a group of proteins associated with cell signalling and one associated with Fe metabolism. Additionally a group of proteins associated with oxidative stress, which were found to be elevated in the adult liver, do not appear to be differentially abundant in the fetal liver. Only one of the proteins up-regulated in the fetal liver was found to be common to both the −F and −F LM LC groups, although the levels of a further seven proteins in the −F group were intermediate between the control and −F LM LC groups, suggesting that there may have been small change in these proteins as a result of the folate deficiency alone. There were no common proteins down-regulated in the −F and −F LM LC groups although there was one protein with levels intermediate between the control and −F LM LC groups.

Our previous studies have suggested that the reduction in fetal growth observed in the animals fed the −F LM LC diet is due to the restricted availability of sulfur amino acids[9]. The changes in the abundance of proteins associated with protein synthesis, proteolysis and metabolism in the fetal livers of this group are consistent with this proposition. For example, the eukaryotic initiation factor-4A1 (eIF4-A1) is unchanged in the −F group whereas it is down-regulated in the −F LM LC group. At the same time, DCoH (dimerisation cofactor of hepatic nuclear factor-1), associated with the transcriptional response to amino acid deficiency[17] and p45/SUG, part of the proteosome which plays a critical role in supplying amino acids for sustained protein synthesis in response to amino acid deficiency[18], are up-regulated in the −F LM LC group. The increase in the levels of the mRNA for gadd153, which increases in response to amino acid deficiency in cell cultures[19], also suggests that there is a limitation in the amino acid supply in the −F LM LC group but not in the −F group.

The reduced growth of the −F LM LC group may also underlie some of the changes in the abundance of proteins associated with cell signalling. The up-regulation of growth factor receptor bound protein-2 (Grb-2), phosphohistidine phosphatase-1 (Phpt-1) and Rho-GDP dissociation inhibitor (Rho-GDI) suggests that there are important changes in the signalling from activated cell surface receptors, affecting processes such as epithelial morphogenesis, angiogenesis and vasculogenesis[20–22]. Similar changes in cell–cell interactions and signalling via mitogen-activated protein (MAP) kinase and extracellular signal-regulated kinase (ERK) signalling pathways are observed in microarray studies of the fetal liver of dams fed low-protein diets[23], suggesting that there may be changes in the three-dimensional structure of the developing liver. However, the abundance of these signalling proteins is not altered in the −F group, suggesting that folate deficiency alone does not have the same effect on signalling as the combined deficiency of all three methyl donors. In the −F

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Table 2. Proteins associated with energy metabolism, fat metabolism and cell signalling*

(Mean values with their standard errors for six animals per group)

Treatment...		Mean pixel density							
		Control		−F		−F LM LC			
Spot no.	Protein name	Mean	SEM	Mean	SEM	Mean	SEM	*P*	NCBI accession
Energy metabolism									
6411	Malate dehydrogenase, cytoplasmic	324[a]	19	588[b]	33	416[a,b]	64	0·063	
3202	Cytosolic malate dehydrogenase	330[a]	97	384[a]	76	858[b]	154	0·008	AAH59124
4505	Isocitrate dehydrogenase (NADP)	6992[a]	488	8940[b]	722	5804[a]	426	0·004	A54756
3307	UDP-glucose 4-epimerase	161[a]	37	361[b]	80	375[b]	75	0·051	P18645
2716	α-Enolase	169[a]	38	92[a]	36	397[b]	118	0·025	P04764
2009	α-Enolase	61[a]	36	49[a]	49	209[b]	16	0·036	AAH63174
4808	Aldehyde dehydrogenase 1 family, member B1 (mouse)	18·5[a]	3	11·3[a]	1	29·7[b]	5	0·007	Q9CZS1
4613	Aldehyde dehydrogenase 1 family, member B1	672[a]	56	964[b]	109	607[a]	25	0·007	AAH81884
6702	Aldehyde dehydrogenase 2, mitochondrial	88[a]	25	243[b]	64	224[b]	25	0·028	AAS75815
Fat metabolism									
2104	Phosphatase 2A inhibitor	506[a]	83	640[a,b]	197	962[b]	136	0·093	Q63945
2302	Farnesyl-pyrophosphate synthetase (cholesterol synthesis)	476[a,b]	55	626[a]	54	349[b]	74	0·023	AAH59125
3206	PEBP	2734[a]	162	3715[b]	322	2372[a]	123	0·002	P31044
3501	4-Trimethylaminobutyraldehyde dehydrogenase	342[a]	44	493[b]	70	314[a]	26	0·05	Q9JLJ3
5203	Dienoyl-CoA isomerase	1010[a]	67	1458[b]	204	1069[a,b]	136	0·081	1DCIA
6109	Fatty acid-binding protein 5	128[a]	17	138[a]	138	266[b]	266	0·016	S83247
Cell signalling									
3106	Grb2 (*Rattus norvegicus*) (Ash-m)	358[a]	88	371[a]	101	758[b]	134	0·03	BAA08645
8101	Rho-GDIα	238[a]	68	450[a,b]	105	574[b]	67	0·027	AAH04732
1016	Similar to phosphohistidine phosphatase 1	82[a]	22	79[a]	17	209[b]	29	0·002	BAB24222
3402	Ran-GTPase activating protein 1 (predicted)	797[a,b]	92	1068[b]	111	762[a]	64	0·067	AAH82056
2004	Rab-GDP dissociation inhibitor-β	706[a]	56	1121[b]	142	792[a]	116	0·034	P50399

− F, folate-deficient diet; − F LM LC, folate-deficient diet with low methionine and choline; NCBI, National Center for Biotechnology Information; PEBP, phosphatidylethanolamine-binding protein; Grb2, growth factor receptor-bound protein 2; Rho-GDIα, Rho-GDP dissociation inhibitor-α.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

* Data were analysed by one-way ANOVA followed by Fischer's unprotected test.

Table 3. Proteins associated with endoplasmic reticulum (ER) function and iron metabolism, and other proteins*
(Mean values with their standard errors for six animals per group)

Treatment...		Mean pixel density							
		Control		−F		−F LM LC			
Spot no.	Protein name	Mean	SEM	Mean	SEM	Mean	SEM	*P*	NCBI accession
ER and cytoskeleton									
1812	Transitional endoplasmic reticulum ATPase	339[a]	83	344[a]	123	711[b]	69	0·02	A55190
2806	Transitional endoplasmic reticulum ATPase	640[a]	66	525[a]	93	943[b]	91	0·009	A55190
1210	Annexin A4 (36 kDa)	75[a]	11	94·6[a]	24	157·2[b]	15	0·009	NP_077069
2109	Apolipoprotein A-I	79[a]	11	74[a]	19	170[b]	24	0·003	NP_036870
2003	S100 Ca-binding protein A8 (calgranulin A)	282[a]	39	272[a]	72	572[b]	87	0·009	P50115
4209	Regucalcin	170[a,b]	29	251[b]	51	128[a]	22	0·080	Q03336
3510	Tubb5	1023[a]	66	1276[b]	83	923[a]	83	0·016	AAH60540
4718	Moesin (membrane-organising extension spike protein)	186[a]	55	167[a]	37	384[b]	77	0·037	O35763
1602	ER-60 protease	7878[a]	620	6345[a]	670	10 995[b]	1338	0·009	D63378
5105	DnaK-type molecular chaperone Hsp70·3	115·8[a]	10	158[a,b]	19	171·1[b]	15	0·032	P55063
5403	DnaK-type molecular chaperone Hsp72-ps1	3920[a,b]	350	5020[b]	672	3389	127	0·056	AAH98914
2503	DnaK-type molecular chaperone Grp75 precursor	665[a,b]	84	482[a]	62	977[b]	170	0·024	I56581
4002	Hip (co-chaperone in the Hsc70/Hsp40 reaction cycle)	984[a]	124	1700[b]	117	1008[a]	111	<0·001	CAA57546
4405	Dnajb11, DnaJ (Hsp40) homologue, subfamily B, member 11	126[a]	21	188[b]	23	98[a]	9	0·015	AY387070
4504	Chaperonin containing TCP1	1044[a,b]	155	1392	110	968	58	0·06	AAH83650
Fe metabolism									
4001	Ferritin light chain	187[a]	58	170[a]	170	521[b]	521	0·004	AAH61525
4407	Liver regeneration-related protein LRRG03 (transferrin)	288[a]	55	301[a]	72	195[b]	46	0·033	AAQ91040
Other									
2721	Rat α 1-fetoprotein	745[a]	51	520[a,b]	135	417[b]	92	0·064	V01254
3603	Protein disulfide isomerase-associated protein 3	843[a]	85	821[a]	142	432[b]	36	0·016	P11598
3608	Epoxide hydrolase 2, cytoplasmic	684[a,b]	76	829[a]	102	545[b]	36	0·065	AAH85732
3609	Serum albumin precursor	3192[a]	160	3142[a]	335	2420[b]	156	0·052	P020770
4605	T-kininogen I precursor	500[a]	66	1013[b]	168	594[a,b]	184	0·049	AAA41489
5204	Ribonuclease A family 4 (Rnase4)	842[a]	62	1361[b]	193	804[a]	47	0·006	AAH05569
7207	Alloantigen F	72[a]	85	150[b]	66	191[b]	181	0·041	AAA40740

− F, folate-deficient diet; − F LM LC, folate-deficient diet with low methionine and choline; NCBI, National Center for Biotechnology Information; Hsp, heat shock protein; Grp75, glucose-regulated protein 75; TCP1, t-complex 1.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Data were analysed by one-way ANOVA followed by Fischer's unprotected test.

Table 4. Gene expression in the fetal liver (relative expression in arbitrary units)*

(Mean values with their standard errors)

Treatment...	Control (*n* 5)		−F (*n* 5)		−F LM LC (*n* 7)		
	Mean	SEM	Mean	SEM	Mean	SEM	*P*
ACC-1	26·4[a]	1·5	25·5[a]	0·8	19·5[b]	2·2	0·028
FAS	1·5[a]	0·1	1·2[a]	0·2	0·9[b]	0·1	0·053
L-CPT-1	11·8[a]	2·6	32·3[b]	8·7	14·3[a]	2·6	0·029
ACO	1·4	0·12	1·8	0·2	1·5	0·3	0·607
CD36	4·5[a]	0·41	2·7[b]	0·5	3·9[a]	0·5	0·049
PPARα	2·0	0·1	1·9	0·1	2·0	0·1	0·755
SREBP-1c	1·2	0·1	1·2	0·2	1·1	0·1	0·651
LXRα	1·5	0·1	1·4	0·1	1·6	0·2	0·572
C/EBPα	32·0	1·3	30·3	3·1	28·1	3·4	0·652
C/EBPβ	4·0	0·5	5·3	1·0	6·6	1·4	0·222
gadd153†	33·9[a]	4·6	32·9[a]	3·4	45·2[b]	4·4	0·019

− F, folate-deficient diet; −F LM LC, folate-deficient diet with low methionine and choline; ACC-1, acetyl CoA carboxylase; FAS, fatty acid synthase; L-CPT-1, liver type carnitine palmitoyl transferase; ACO, acyl CoA oxidase; CD36, fatty acid translocase; SREBP-1c, sterol response element-binding protein; LXRα, liver X receptor nuclear receptor subfamily 1, group H, member 3; C/EBPα, CCAAT/enhancer-binding protein α; C/EBPβ, CCAAT/ enhancer-binding protein β; gadd153, growth arrest and DNA damage protein 153.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

* Data were analysed by ANOVA followed by Fischer's unprotected test. The primer sequences have been described previously[11,29–31] except for CD36; forward 5′-GGA AAG TTA TTG CGA CAT GAT TAA TG-3′ and reverse 5′-GGA AAG AAC CTC AGT GTT TGA GAC TTC-3′.

† Corrected for litter size as a covariate: control = 15·0 (SEM 0·6), −F = 14·0 (SEM 13·1), −F LM LC = 13·1 (SEM 0·9); regression coefficient = 4·90 (SEM 1·54) ($P=0·007$).

group the Ran-GTPase-binding protein and the Rab-GDP dissociation inhibitor are both increased. These proteins are associated with the regulation of several cellular processes including nucleo-cytoplasmic transport, cell-cycle progression and post-mitotic nuclear assembly[24]; however, the functional significance of these changes is not clear.

Proteins associated with the cytoskeleton and the endoplasmic reticulum are differentially abundant in the soluble fraction of both the fetal and maternal livers. Amongst the proteins in this group, the endoplasmic reticulum ATPase, apoA1 and Ca-binding proteins have all been associated with packaging of VLDL[25]. Another differentially abundant protein, the endoplasmic reticulum-localised protein ER-60, which has both proteolytic and chaperone activities, has been shown to associate with newly synthesised apoB[26]. Changes in the abundance of annexin A4, which belongs to a class of Ca^{2+}-binding proteins that bind acidic phospholipids[27], suggest that there may also be changes in exocytosis. Overall the changes in this group of proteins suggest that the maternal diet deficient in folic acid and low in methionine and choline (−F LM LC) is modifying the function of the endoplasmic reticulum in the fetal liver in a similar way to that seen in the maternal liver. There is no evidence that these processes are affected in the −F group, suggesting that the methyl supply is sufficient to support these processes.

There are also changes in groups of proteins associated with metabolism and especially the metabolism of lipids in both the −F and −F LM LC groups. Proteins in these groups, including cytosolic malate dehydrogenase, enolase and fatty acid-binding proteins, undergo similar changes in abundance in the maternal liver. These changes suggest that both −F diets and diets deficient in all three methyl donors (−F LM LC) are altering lipid metabolism in the fetal liver. However, analysis of mRNA associated with the regulation of lipid metabolism in adults suggests that there are important differences. The present study shows that the mRNA for the sterol response element-binding protein-1c and acetyl CoA carboxylase (fatty acid synthesis), both of which are down-regulated in the maternal liver, are unchanged in the fetal liver. There is also no change in the PPARα which is up-regulated in the maternal liver in the −F LM LC group[11]. There is also no change in the levels of other transcriptional activators associated with lipid metabolism, such as liver X receptor and the CCAAT/enhancer-binding proteins in the fetal liver. The up-regulation of the mRNA for carnitine palmitoyl transferase in the fetal livers of the −F group suggests an increase in mitochondrial fatty acid oxidation which does not occur in the −F LM LC group. The mRNA for acetyl CoA carboxylase and fatty acid synthase is reduced by approximately 20 % in the −F LM LC group and is consistent with a down-regulation of fatty acid production. There are also changes in the abundance of proteins specifically associated with fat metabolism and these are consistent with the changes in mRNA levels. For example, the changes in the cytoplasmic malate dehydrogenase (malate aspartate shuttle), α-enolase and mitochondrial aldehyde dehydrogenase in the −F LM LC group all suggest a reduction in fatty acid synthesis. These data suggest that there are some similarities in the effects of the deficient diets, with a decrease in fatty acid synthesis in both the maternal and fetal livers of animals fed the −F LM LC diet. However, the data suggest that there are also some important differences, especially in animals fed the −F diet, which increase fatty acid oxidation in the fetal liver but have no effect in the maternal liver.

These results suggest that an adequate supply of folic acid and the related methyl donors may benefit fetal development directly by improving methionine cycle activity and hence lipid metabolism in fetal as well as maternal tissues. The data suggest that in the case of folate deficiency (−F) the main effects are on lipid metabolism in the maternal system with only compensatory changes in fatty acid oxidation in the fetal liver, whereas when folate deficiency is combined with a shortage of the related methyl donors then there is a more widespread change in fat metabolism and effects on the endoplasmic reticulum in both maternal and fetal livers. The effect of folate supplements on lipid accumulation, especially in humans, is unknown; however, it is increasingly clear that the formation of lipid deposits within organs is a risk factor for many diseases[28]. The potential for folate deficiency to reduce this accumulation suggests several potential links to abnormal fetal development and subsequent disease. Folic acid and related methyl donors may have a hitherto unknown role in fetal lipid metabolism, which may be an important link between folate status, fetal growth and neural development in human pregnancies.

Acknowledgements

The present study was supported by the Rural and Environment Research and Analysis Directorate of The Scottish

Government as part of the core funds of the Rowett Institute for Health and Nutrition and by the European Union sixth Framework programme EARNEST (CT-2005-007036). We wish to express our thanks to staff from the Bioresources Unit for animal care and to Dr C. Mayer (Biomathematics and Statistics, Scotland) for advice on the statistical analysis.

W. D. R. and C. J. M. designed the research; S. M. H., C. J. M. and W. D. R. performed the research; R. J. G., M. J. R. and G. J. D. carried out the gel electrophoresis and MS; C. J. M., S. M. H. and W. D. R. analysed the data; W. D. R. and C. J. M. wrote the paper.

The authors declare no conflict of interest.

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British Journal of Nutrition