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Placental amino acid transport may be regulated by maternal vitamin D and vitamin D-binding protein: results from the Southampton Women's Survey

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Abstract

Both maternal 25-hydroxyvitamin D [25(OH)D] concentrations during pregnancy and placental amino acid transporter gene expression have been associated with development of the offspring in terms of body composition and bone structure. Several amino acid transporter genes have vitamin D response elements in their promoters suggesting the possible linkage of these two mechanisms. We aimed to establish whether maternal 25(OH)D and vitamin D-binding protein (DBP) levels relate to expression of placental amino acid transporters. RNA was extracted from 102 placental samples collected in the Southampton Women's Survey (SWS) and gene expression was analysed using quantitative real-time PCR. Gene expression data were normalised to the geometric mean of three housekeeping genes and related to maternal factors and childhood body composition. Maternal serum 25(OH)D and DBP levels were measured by radioimmunoassay. Maternal 25(OH)D and DBP levels were positively associated with placental expression of specific genes involved in amino acid transport. Maternal 25(OH)D and DBP concentrations were correlated with the expression of specific placental amino acid transporters and thus may be involved in the regulation of amino acid transfer to the fetus. The positive correlation of DBP levels and placental transporter expression suggests that delivery of vitamin D to the placenta may be important. This exploratory study identifies placental amino acid transporters which may be altered in response to modifiable maternal factors and provides a basis for further studies.

Introduction

Vitamin D insufficiency is common in women of childbearing age and is associated with reduced fetal growth and poor postnatal health [12, 18]. The biologically inactive 25-hydroxyvitamin D [25(OH)D] is used to monitor vitamin D status as this is the major circulating form [14]. In the Southampton Women's Survey (SWS), a prospective longitudinal study of maternal nutrition and lifestyle before and during pregnancy, lower maternal 25(OH)D was associated with morphological changes in the fetal femur [24], lower neonatal fat mass and greater fat mass and lower grip strength in childhood [8, 13]. Reduced 25(OH)D during late pregnancy was also associated with reduced bone mineral content in children at 9 years of age in another Southampton cohort study [19].

The mechanisms underlying these associations are not fully understood, but are likely to involve the placenta; the sole conduit for nutrition from mother to fetus. We previously reported that placental mRNA expression of the vitamin D sensitive calcium transporter *PMCA3* and the imprinted gene *PHLDA2* is associated with offspring bone mass development and composition [21, 25]. Other than calcium transport, a key element for fetal bone development is placental amino acid transport. Placental amino acid transfer is vital for fetal growth [2] and animal studies suggest that decreased amino acid transport precedes fetal growth restriction [17]. Amino acid transfer to the fetus involves amino acid transport across the microvillous (MVM) and basal membranes (BM) of the placental syncytiotrophoblast and potentially metabolic interconversion within the placenta [7, 9]. Placental amino acid transfer is thought to be regulated by maternal nutritional and hormonal factors [10, 16, 29].

There are three classes of amino acid transporter in the human placenta; accumulative transporters, amino acid exchangers [3, 22] and facilitated transporters [6] (Figure 1). The facilitated transporters TAT1, LAT3 and LAT4 are essential for net amino acid transport to the fetus and their gene expression in human placenta is associated with measures of fetal growth [6]. The factors that regulate these changes in gene expression are not understood. However, as these and several other amino acid transporters have vitamin D response elements (VDRE) in their promoter regions they could theoretically be regulated at the transcriptional level by maternal vitamin D. Specifically the biologically active 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] regulates transcription of specific genes by binding the vitamin D receptor (VDR) and interacting with VDRE in their promoter regions.

We therefore investigated whether maternal 25(OH)D and Vitamin D binding protein (DBP) concentrations during pregnancy are related to placental amino acid transporter gene expression in samples collected from a population based cohort: the Southampton Women's Survey.

Methods

The study was conducted according to the guidelines in the Declaration of Helsinki, and the Southampton and South West Hampshire Research Ethics Committee approved all procedures (276/97, 307/97, 089/99, 153/99, 005/03/t, 06/Q1702/104). Written informed consent was obtained from all participating women and by parents or guardians with parental responsibility on behalf of their children.

Maternal measurements

We used data and samples from the SWS, a cohort study of 3,158 pregnancies with information collected from the mothers before conception [15]. Non-pregnant women aged 20-34 years were recruited via their General Practitioners; assessments of lifestyle, diet and anthropometry were performed by trained research nurses at study entry and then in early (11 weeks) and late (34 weeks) gestation among those women who became pregnant. Subscapular skinfold thicknesses were measured to the nearest 0.1 mm in triplicate using Harpenden skinfold callipers [11].

At 34 weeks of gestation, a maternal venous blood sample was obtained and an aliquot of maternal serum was frozen at -80°C. Serum 25(OH)D and DBP concentrations were analyzed by radioimmunoassay (Diasorin, Stillwater, MN). The 25(OH)D assay measures both 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. The assays met the requirements of the UK National Vitamin D External Quality Assurance Scheme, and intra- and inter-assay coefficients of variance were <10%.

Placental samples

Placentas were collected from term pregnancies within 30 minutes of delivery. Placental weight was measured after removing blood clots, cutting the umbilical cord flush with its insertion into the placenta, trimming away surrounding membranes and removing the amnion from the basal plate. To ensure that samples collected were representative of the placentas as a whole, 5 villous tissue samples were selected using a stratified random sampling method and stored at -80°C. For this study, a cohort of 102 placentae was selected from 300 collected in total based on availability of neonatal dual energy X-ray absorptiometry (DXA) data.

RNA extraction and cDNA synthesis

For each placenta 5 snap frozen samples were pooled and powdered in a frozen tissue press. Total RNA was extracted from 30 mg powdered placental tissue using the RNeasy fibrous tissue RNA

isolation mini kit (Qiagen, UK) according to the manufacturer's instructions. The integrity of total RNA was confirmed by agarose gel electrophoresis.

Total RNA (0.2 µg) was reverse transcribed with 0.5 µg random hexamer primer, 200 units (u) M-MLV reverse transcriptase, 25 u recombinant RNasin ribonuclease inhibitor and 0.5 mM each of dATP, dCTP, dGTP and dTTP in a final reaction volume of 25 µl in 1x MMLV reaction buffer (Promega, Wisconsin, USA). All 102 samples were produced in one batch to reduce variation.

Probe and primer design

Intron spanning oligonucleotide probes and primers were designed using the Roche (West Sussex, UK) ProbeFinder version 2.45 for human. Probes were supplied by Roche from the human universal probe library and primers were synthesised by Eurogentec (Seraing, Belgium). Control genes were selected using the geNormTM human Housekeeping Gene Selection Kit (Primer Design Limited, Southampton, UK).

Target genes

The genes measured in this study along with primer and probe details are listed in Table 1. mRNA levels were measured using quantitative real-time PCR using a Roche LightCycler 480. For Roche universal probe library probes the cycle parameters were 95^{o}C for 10 min, followed by 40 cycles of 95^{o}C for 15 s and 60^{o}C for 1 min. For the Primer Design Perfect Probes the cycle parameters were 95^{o}C for 10 min, followed by 40 cycles of 95^{o}C for 10 s and 60^{o}C and 72^{o}C for 15 s. Intra-assay CV's for each gene were 5-8%. Each of the 102 samples was run on the same plate in triplicate. All mRNA levels are presented relative to the geometric mean of the three control genes, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (*YWHAZ*), ubiquitin C (*UBC*) and topoisomerase (*TOP1*) [4].

Postnatal measurements

At birth (n = 102) and 4 years of age (n = 42-46) a whole-body DXA scan was obtained using a Hologic Discovery instrument (Hologic Inc) in pediatric scan mode (Apex 3.1 software), yielding fat mass, lean mass, and bone mineral content. The coefficient of variation for body composition analysis with the DXA instrument was 1.4% to 1.9%.

Statistics

Maternal and placental mRNA data that were not normally distributed were transformed logarithmically. Previous data showed that gene expression of the control genes and many of the

target genes was higher in male than in female placentae [5]. Adjustment was therefore made for sex in the correlation analysis between mRNA and all other variables. Pearson's correlation coefficient (r_p) was used to determine partial correlations adjusted for sex and gestational age between placental mRNA levels, neonatal body composition and maternal factors (IBM SPSS Statistics 20, USA). The partial correlation between placental gene expression and maternal vitamin D measures was also adjusted for potential confounding factors: maternal sum of skinfold thickness, walking speed, parity and smoking during pregnancy. A value of $p \leq 0.05$ was accepted as statistically significant, and, given the observational nature of the study together with the substantial co-linearity among both predictors and outcomes, testing for multiple comparisons was felt to be inappropriate [28].

Results

Characterisation of the subjects from the SWS cohort

The mean age (SD) of the 102 mothers at the birth of their child was 30.9 (3.9) years; 37.9% were primiparous. The median (inter-quartile range) gestational age was 39.6 (38.8–40.7) weeks. The mean (SD) placental/fetal weight ratio was 0.13 (0.02). Of the 102 placentas from SWS pregnancies studied here, 53 of the infants were male and 49 were female.

Maternal plasma vitamin D and placental gene expression

34-week plasma 25(OH)D levels were measured for 91 of the 102 women and DBP levels for 85 of the 102 women. Of the genes investigated mRNA for *EAAT1*, *EAAT4* and *EAAT5* were not detected in human placenta.

In this subset of SWS woman, there was a positive correlation between maternal 34-week plasma 25(OH)D levels and the mRNA expression of *LAT3*, *ASCT1* and y^+*LAT1* and a negative correlation with *SNAT1* (Table 2). Maternal DBP levels correlated positively with mRNA expression of *TAT1*, *LAT3*, *LAT4*, *SNAT1*, *SNAT2*, y^+*LAT2*, *4F2HC*, *EAAT3* and there was a trend with *LAT1* (Table 2).

When the correlation was also adjusted for maternal confounding factors all correlations were still present except for the relationships between 25(OH)D and *ASCT1* and DBP and *TAT1* which were no longer statistical significant at the $p \leq 0.05$ level (Table 2). The adjusted data also showed a positive association between DBP and *LAT1* mRNA (Table 2).

Neonatal body composition

At birth, there were no significant associations between placental amino acid transporter gene expression and neonatal lean mass, fat mass or bone mineral content (data not shown).

At 4 years of age total lean mass was positively associated with y^+*LAT1*, *LAT3* and *TAT1* mRNA expression (Table 3). Bone mineral density was positively associated with *LAT4* mRNA and negatively associated with *ASCT2* and *EAAT3* mRNA expression (Table 3). *EAAT3* mRNA expression levels (n = 42) were also negatively associated with bone mineral content ($r_p = -0.46$, $p = 0.003$) and total bone area (cm^2 without heads; $r_p = -0.43$, $p = 0.01$). *SNAT4* ($r_p = -0.40$, $p = 0.01$) and y^+*LAT2* ($r_p = -0.32$, $p = 0.04$) expression levels were negatively associated with total bone area.

Discussion

Many genes related to placental function may be regulated directly or indirectly by vitamin D. This study aimed to establish whether there are relationships between maternal vitamin D levels and changes in gene expression in placentas from the SWS. Maternal 25(OH)D and DBP levels were positively associated with placental expression of genes involved in amino acid transport. This suggests that maternal vitamin D status may regulate the expression of placental amino acid transporters and potentially influence the transfer of amino acids to the fetus and subsequent fetal growth. The observations that DBP was associated with the expression of twice as many genes as vitamin D suggests that delivery of vitamin D to the placenta may be a crucial determinant of vitamin D activity. The associations seen may however involve a more complex relationship between maternal vitamin D status and maternal body composition.

Vitamin D

Placental amino acid transport is important for fetal growth and development, so understanding how the amino acid transporters are regulated in the placenta will help us understand the mechanisms underlying fetal growth restriction and the associated postnatal phenotype. Maternal vitamin D status has also been shown to associate with both fetal and neonatal growth, and, taken with the fact that it modulates gene transcription; this suggests there may be an interaction between vitamin D and placental amino acid transport. This interaction could be a direct effect of vitamin D and its receptor acting directly on the placental amino acid transporter genes at a VDRE or an indirect effect mediated via vitamin D's activation of another gene. Both the *LAT3* and *ASCT1* genes have been shown to have VDREs in their promoter region [31] which could underlie the association between their mRNA expression and maternal 25(OH)D levels. Vitamin D can also down regulate gene expression via VDR blocking the activity of the cyclic AMP response element (CRE) in the promoter [32]. This may explain the observed negative association between 25(OH)D and *SNAT1* mRNA expression; a gene regulated by cyclic AMP at the CRE [27]. Vitamin D can also directly affect gene transcription by an interaction between VDR and histone acetyltransferases leading to an open/active chromatin state [20]. The amino acid transporter genes could therefore be in a region of DNA affected by vitamin D mediated epigenetic changes or could be regulated indirectly via an effect on another gene in the placenta.

The relationship between vitamin D and placental function may be more complex than VDR mediated changes in placental gene expression and could be very indirect via an effect on maternal physiology or metabolism. It could be that vitamin D levels are influencing aspects of the maternal

environment which in turn regulate placental gene expression. Alternatively, maternal factors could simply be regulating both vitamin D levels and placental amino acid transporter expression in a similar manner. Plasma vitamin D status is known to be related to factors such as maternal smoking, parity and body mass index (BMI) [30]. It could be that maternal body composition is influencing the placenta as a signal reflecting the mother's nutrient reserves and capacity to support the pregnancy. We have previously demonstrated an association between maternal muscle mass and placental amino acid transfer indicating that maternal body composition can affect placental amino acid handling [23].

Vitamin D levels could therefore be a proxy for another aspect of the maternal environment and not a direct mediator of amino acid transporter expression levels. When we corrected our correlation analysis to adjust for maternal factors we did indeed see that the amino acid transporters *ASCT1* and *SNAT1* no longer related to the maternal 25(OH)D levels. These transporters may therefore be regulated by aspects of maternal body composition rather than vitamin D status, or vitamin D levels may be mediating the effects of body composition on the placenta. *LAT3* and y^+*LAT1* did still show strong associations with maternal 25(OH)D levels suggesting it is the vitamin D rather than body composition affecting their regulation. Further studies are needed to establish the mechanisms underlying this association.

Interestingly there were a number of positive associations between DBP and amino acid transporter expression levels. This suggests that the delivery of the vitamin D to the placenta by its binding protein may be an important determinant of vitamin D action, possibly mediated by receptor mediated endocytosis [26]. Further investigation into the uptake of vitamin D and levels of the active $1,25(OH)_2D$ within the placenta are needed. This will help us understand and improve the effects of 25(OH)D supplementation during pregnancy, which may also require the DBP to be upregulated.

Postnatal outcome

We previously reported that placental *TAT1* and *LAT3* mRNA expression levels in this cohort are positively related to measures of fetal growth, with *TAT1* mRNA being associated with fetal growth in terms of lean mass [6]. Consistent with these observations we found that y^+*LAT1*, *TAT1* and *LAT3* mRNA expression in placentas are positively related to 4 year old lean mass. As lean mass contains a high proportion of muscle, a protein rich tissue, its growth will require a substantial amino acid supply so may rely on appropriate amino acid supply in early development.

Limitations

This study has the advantage of using a well characterised population representative of the general population, with detailed phenotyping of mother-offspring pairs. However, the exploratory nature of this study, small sample size and the possibility of chance findings need to be acknowledged. Compared to adults, DXA assessment of body composition in children is more problematic due to their smaller size and tendency to move. These DXA measures have however been validated previously in piglets using biochemical assessment of carcass nitrogen content and lipid extraction to determine lean and fat mass, respectively [(1)]. In this study specific paediatric software was used and movement artefacts were minimal. It is not possible in this observational study to determine whether the observed associations are causal. Nevertheless, the patterns of observations are indicative of a role for vitamin D in the regulation of placental amino acid transporter expression and form the basis for future studies.

Conclusion

In conclusion this study demonstrates relationships between maternal vitamin D levels and in particular vitamin D binding protein and placental gene expression. As there are associations between vitamin D and body composition these observations provide a possible mechanism by which maternal factors influence placental function. Further work needs to be undertaken to investigate the association between maternal vitamin D binding protein and placental gene expression and whether these are direct or indirect effects.

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Conflict of Interest: None.

Authorship: JC RL CS and NH formulated the specific research question and designing the study. HI KG MH CC NH and the SWS Study Group designed the cohort (SWS) study. The experiments were carried out by: PD JC RL and PM. JC and SB analysed the data. The article was written by JC PD RL and NH with input from all other authors.

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Figure Legends:

Figure 1: Transport of amino acids across the placental syncytiotrophoblast. Amino acids are transported across the microvillous membrane (MVM) into the placental syncytiotrophoblast by active accumulative transporters (**Ac;** e.g. SNATs) and exchangers (**X;** e.g. ASCTs). Amino acids transported by accumulative transporters (aa_A) are then exchanged back for those only transported by exchangers (aa_B). Amino acids are transported out of the placenta across the basal membrane (BM) by facilitated transporters (TAT1, LAT3 & 4) and exchangers (**X**). The facilitated transporters transport specific amino acids (aa_1) down their concentration gradient to the fetus. In order to transport other amino acids (aa_2) to the fetus, aa_1 must be exchanged for aa_2 via exchangers (**X**).

Figure 2: LAT3 mRNA expression is associated with postnatal body composition. LAT3 relative mRNA expression in human placenta is positively correlated with maternal 25(OH)D (**A**; $r_p = 31$, $p = 0.003$, $n = 102$) and lean mass at 4 years of age (**B**; $r_p = 38$, $p = 0.01$, $n = 46$).

Table 1: Information on genes, primers and probes

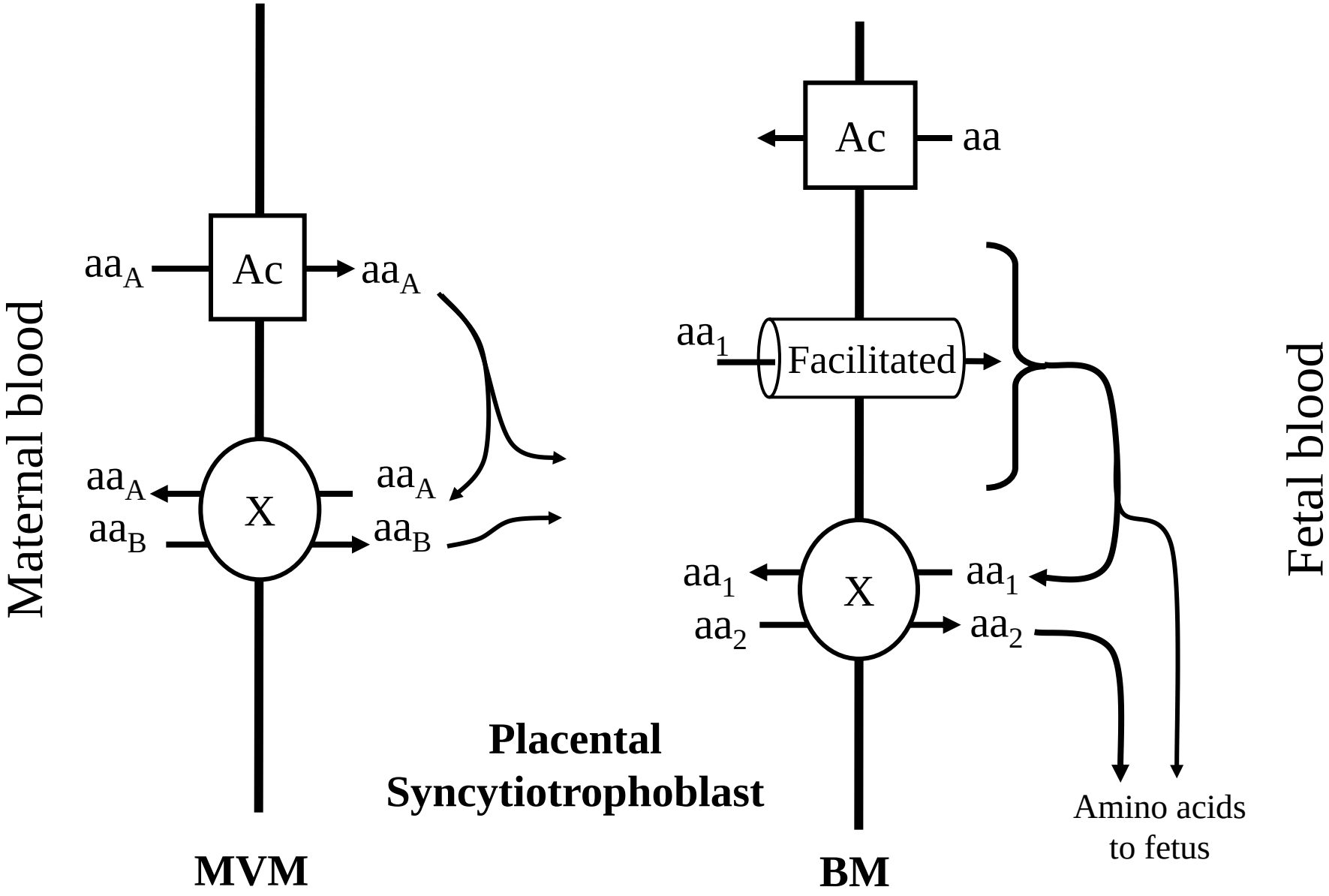
Transporter	Gene	Gene ID	Genebank accession #	Primers	Roche universal probe library #
ASCT1	SLC1A4	6509	NM_003038.2	F-5′-tttgcgacagcatttgctac-3’	78
				R-5′-gcacttcatcatagagggaagg-3’	
ASCT2	SLC1A5	6510	NM_005628.2	F-5′-gaggaatatcaccggaacca-3’	43
			NM_001145144.1	R-5′-aggatgttcatcccctcca-3’	
EAAT1	SLC1A3	6507	NM_004172.4	F-5′-ttgaactgaacttcggacaaatta-3’	76
				R-5′-attccagctgccccaatact-3’	
EAAT2	SLC1A2	6506	NM_004171.3	F-5′-aaaatgctcattctccctctaatc-3’	78
				R-5′-gccactagccttagcatcca-3’	
EAAT3	SLC1A1	6505	NM_004170.4	F-5′-agttgaatgacctggacttgg-3’	9
				R-5′-gcagatgtggccgtgatac-3’	
EAAT4	SLC1A6	6511	NM_005071.1	F-5′-tgcagatgctggtgttacct-3’	19
				R-5′-gttgtccagggatgccata-3’	
EAAT5	SLC1A7	6512	NM_006671.4	F-5′-cgcccaggtcaacaactac-3’	9
				R-5′-gctgcagtggctgtgatact-5’	
LAT1	SLC7A5	8140	NM_003486.5	F-5′-gtggaaaaacaagcccaagt-3’	25
				R-5′-gcatgagcttctgacacagg-3’	
LAT2	SLC7A8	23428	NM_182728.1	F-5′-ttgccaatgtcgcttatgtc-3’	17
			NM_012244.2	R-5′-ggagcttctctccaaaagtcac-3’	
LAT3	SLC43A1	8501	NM_003627.5	F-5′-gccctcatgattggctctta-3’	29
			NM_001198810.1	R-5′-ccggcatcgtagatcagc-3’	
LAT4	SLC42A2	124935	NM_001284498.1	F-5′-acaagtatggcccgaggaa-3’	3
			NM_152346.2	R-5′-gcaatcagcaagcaggaaa-3’	
SNAT1	SLC38A1	81539	NM_030674.3	F-5′-attttgggactcgcctttg-3’	47
			NM_001077484.1	R-5′-agcaatgtcactgaagtcaaaagt-3’	
SNAT2	SLC38A2	54407	NM_018976.3	F-5′-cctatgaaatctgtacaaaagattgg-3’	9
				F-5′-ttgtgtacccaatccaaaacaa-3’	
SNAT4	SLC38A4	55089	NM_018018.4	F-5′-tgttctggtcatccttgtgc-3’	29
			NM_001143824.1	R-5′-aaaactgctggaagaataaaaatcag-3’	
TAT1	SLC16A10	117247	NM_018593.4	F-5′-ggtgtgaagaaggtttatctacagg-3′	6
				R-5′-agggccccaaagatgcta-3′	
y^+LAT1	SLC7A7	9056	NM_001126105.1	F-5′-acactgccgtgagaacctg-3’	72
			NM_001126106.1	R-5′-aggagaggaaacccttcacc-3’	
y^+LAT2	SLC7A6	9057	NM_001076785.1	F-5′-gctgtgatcccccatacct-3’	66
			NM_003983.4	R-5′-ggcacagttcacaaatgtcag-3’	
4F2HC	SLC3A2	6520	NM_001012661.1	F-5′-tggttctccactcaggttga-3’	49
				R-5′-cagccaaaactccagagcat-3’	

Table 2: The associations between placental amino acid transporter mRNA expression and maternal serum 25-hydroxyvitamin D and vitamin D binding protein levels.

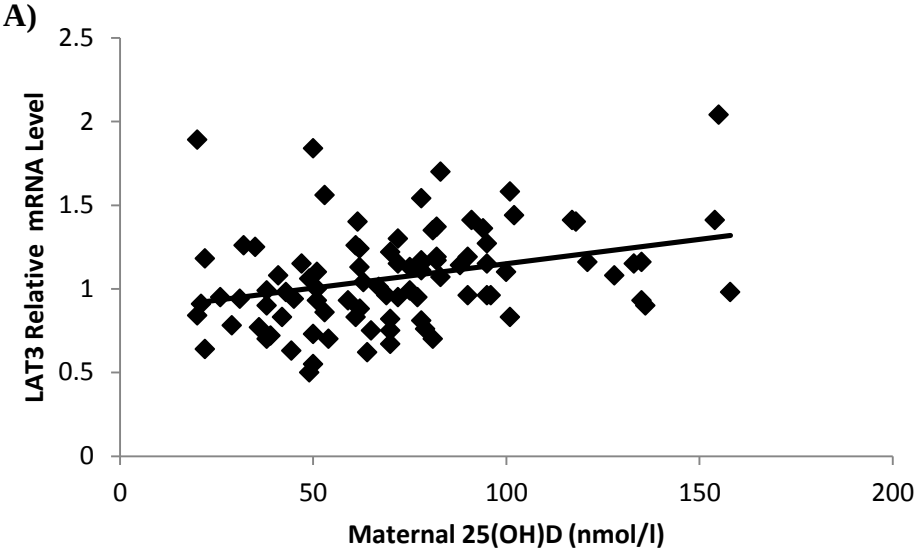
	34-week Vitamin D (nmol/l)		*Vitamin D binding protein (mg/dl)*		*34-week Vitamin D (nmol/l)*		*Vitamin D binding protein(mg/dl)*	
	r	p	r	p	r	p	r	p
TAT1	0.07	0.50	**0.23**	**0.03**	0.14	0.21	**0.12**	**0.10**
LAT 3	**0.31**	**0.003**	**0.22**	**0.04**	**0.37**	**0.003**	**0.22**	**0.05**
LAT 4	-0.12	0.25	**0.28**	**0.01**	-0.13	0.26	**0.28**	**0.01**
SNAT1	**-0.23**	**0.03**	**0.25**	**0.02**	**-0.20**	**0.07**	**0.23**	**0.05**
SNAT2	0.01	0.96	**0.23**	**0.03**	0.04	0.70	**0.23**	**0.04**
SNAT4	0.14	0.19	0.08	0.45	0.12	0.30	0.12	0.29
ASC1	**0.23**	**0.03**	0.06	0.62	**0.20**	**0.07**	0.11	0.33
ASC2	0.04	0.74	0.18	0.10	0.05	0.63	0.17	0.14
y+LAT1	**0.31**	**0.003**	0.03	0.81	**0.36**	**0.001**	0.02	0.99
y+LAT2	0.04	0.73	**0.26**	**0.02**	-0.08	0.94	**0.33**	**0.003**
EAAT2	0.12	0.26	-0.07	0.53	0.06	0.56	-0.01	0.91
EAAT3	0.09	0.39	**0.30**	**0.01**	0.12	0.24	**0.29**	**0.009**
LAT1	-0.14	0.19	**0.21**	**0.06**	-0.17	0.12	**0.23**	**0.04**
LAT2	-0.08	0.44	0.18	0.10	-0.07	0.54	0.17	0.14
4F2HC	-0.12	0.26	**0.25**	**0.02**	-0.08	0.48	**0.23**	**0.04**
	Adjusted for sex and dGA				Adjusted for sex, dGA and- maternal confounding factors			

Table 3: The associations between placental amino acid transporter mRNA expression and 4 year old DXA measurements of body composition.

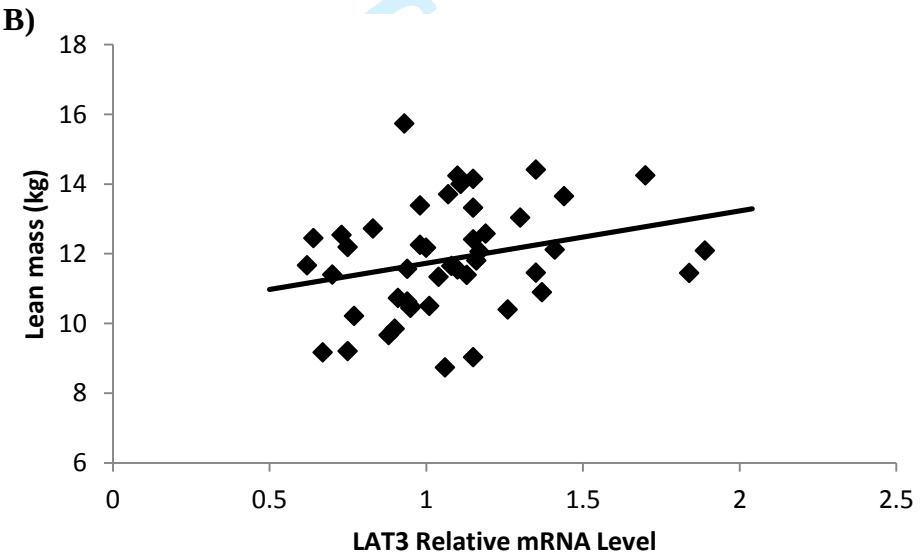
4 year DXA	*Total lean (kg) (n = 46)*		*Total Prentice BMD (g), without heads (n = 42)*	
	r	**p**	**r**	**p**
TAT 1	**0.33**	**0.03**	-0.17	0.28
LAT 3	**0.38**	**0.01**	-0.15	0.33
LAT 4	-0.09	0.57	**0.41**	**0.01**
SNAT1	-0.12	0.45	0.06	0.72
SNAT2	0.06	0.68	-0.11	0.49
SNAT 4	0.08	0.62	-0.18	0.26
ASCT1	0.23	0.13	-0.27	0.09
ASCT2	0.24	0.11	**-0.42**	**0.01**
y+LAT1	**0.31**	**0.04**	-0.25	0.11
y+LAT2	0.20	0.18	-0.27	0.09
EAAT2	0.28	0.07	0.04	0.82
EAAT3	-0.04	0.80	**-0.59**	**0.00005**
LAT1	-0.21	0.16	0.09	0.59
LAT2	-0.04	0.82	-0.02	0.90
4F2HC	0.05	0.73	0.14	0.38


Maternal blood
aa$_A$
Ac
aa$_A$
aa$_A$
X
aa$_A$
aa$_B$
aa$_B$
MVM
Placental
Syncytiotrophoblast
Ac
aa
aa$_1$
Facilitated
aa$_1$
X
aa$_1$
aa$_2$
aa$_2$
BM
Amino acids
to fetus
Fetal blood

A)


LAT3 Relative mRNA Level
2.5
2
1.5
1
0.5
0
0
50
100
150
200
Maternal 25(OH)D (nmol/l)

B)


Lean mass (kg)
18
16
14
12
10
8
6
0
0.5
1
1.5
2
2.5
LAT3 Relative mRNA Level