Placental release of taurine to both the maternal and fetal circulations in human term pregnancies

Maia Blomhoff Holm[1,2], Oddrun Kristiansen[1,2], Ane Moe Holme[1,2], Nasser Ezzatkhah Bastani[3], Hildegunn Horne[1,2], Rune Blomhoff[3,4], Guttorm Haugen [2,5], Tore Henriksen[1,2] and Trond Melbye Michelsen[1,6]

[1]Department of Obstetrics, Division of Obstetrics and Gynecology, Oslo University Hospital, PO BOKS 4950, 0424 Oslo, Norway

[2]Institute of Clinical Medicine, University of Oslo, PO BOKS 1171 Blindern, 0316 Oslo, Norway

[3]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, PO BOKS 1046 Blindern, 0316 Oslo, Norway

[4]Department of Clinical Service, Division of Cancer Medicine, Oslo University Hospital, PO BOKS 4950, 0424 Oslo, Norway

[5]Department of Fetal Medicine, Division of Obstetrics and Gynecology, Oslo University Hospital, PO BOKS 4950, 0424 Oslo, Norway

[6]Norwegian Advisory Unit on Women's Health, Oslo University Hospital, PO BOKS 4950, 0424 Oslo, Norway

Corresponding author:

Maia Blomhoff Holm (maia.blomhoff.holm@outlook.com/ +47 977 40 364)

Abstract

Taurine is regarded as an essential amino acid in utero, and fetal taurine supply is believed to rely solely on placental transfer from maternal plasma. Despite its potential role in intrauterine growth restriction and other developmental disturbances, human *in vivo* studies of taurine transfer between the maternal, placental and fetal compartments are scarce. We studied placental transfer of taurine in uncomplicated human term pregnancies *in vivo* in a cross-sectional study of 179 mother-fetus pairs. During cesarean section, we obtained placental tissue and plasma from incoming and outgoing vessels on the maternal and fetal sides of the placenta. Taurine was measured by liquid chromatography–tandem mass spectrometry. We calculated paired arteriovenous differences, and measured placental expression of the taurine biosynthetic enzyme cysteine sulfinic acid decarboxylase (CSAD) with Quantitative Real-time polymerase chain reaction and western blot. We observed a fetal uptake ($p<0.001$), a uteroplacental release ($p<0.001$) and a negative placental consumption of taurine ($p=0.001$), demonstrating a bilateral placental release to the maternal and fetal compartments. Increasing umbilical vein concentrations and fetal uptake was associated with the uteroplacental release to the maternal circulation ($r_s=-0.19$, $p=0.01$/$r_s=-0.24$, $p=0.003$), but not with taurine concentrations in placental tissue. CSAD-mRNA was expressed in placental tissue, suggesting a potential for placental taurine synthesis. Our observations show that the placenta has the capacity to a bilateral taurine release, indicating a fundamental role of taurine in the human placental homeostasis beyond the supply to the fetus.

Key words

Taurine, placenta, fetus, transfer, arteriovenous differences, CSAD

Introduction

Taurine is a vital amino acid for the developing fetus. It is involved in numerous cellular processes such as regulation of cell volume, proliferation, apoptosis, and cytoprotection (Ripps and Shen 2012; Ditchfield et al. 2015). Reduced fetal plasma levels of taurine are associated with intrauterine growth restriction, retinal degradation, disrupted skeletal and myocardial muscle development, and dysfunction of the nervous system and the pancreatic islets (Ripps and Shen 2012; Cetin et al. 1990).

In adult humans, taurine is a non-essential amino acid synthesized primarily from methionine and cysteine via cysteine sulfinic acid and hypotaurine (Hayes and Sturman 1981; Stipanuk et al. 2006). Due to reports of a lack of synthesizing enzymes in fetal tissues (Sturman et al. 1970; Gaull et al. 1972), the prevailing opinion has been that the human fetus and placenta have limited or no ability to synthesize taurine, and that the fetus thus solely relies on transfer from maternal plasma to meet its demand (Ditchfield et al. 2015; Tappaz 2004). *In vitro* studies of perfused placental tissue, cell cultures and isolated membrane vesicles have demonstrated that taurine is transported actively across the placental barrier by a sodium dependent taurine transporter primarily located in the microvillous membrane of the syncytiotrophoblast, and that the activity of these transporters is reduced in intrauterine growth restriction (Hibbard et al. 1990; Karl and Fisher 1990; Miyamoto et al. 1988; Roos et al. 2004; Norberg et al. 1998). Studies in animal models have shown that perinatal maternal

taurine supplementation may ameliorate detrimental effects of intrauterine growth restriction. For instance, supplementation of taurine to pregnant rats fed a low-protein diet has been shown to improve both neuronal and pancreatic islet development in the offspring (Liu et al. 2011; Boujendar et al. 2002). These studies imply that maternal taurine concentrations and placental uptake from the maternal circulation are important determinants of concentrations and uptake of taurine in the fetus. However, studies in pregnant sheep receiving infusion of other amino acids have shown that an increase in maternal amino acid concentrations not necessarily leads to an increased uptake in the fetus (Jozwik et al. 1999).

Despite the potential role of taurine deficiency in pregnancy complications like fetal growth restriction and other developmental disturbances, *in vivo* studies of taurine transfer between the maternal, placental and fetal compartments in the human are scarce. We therefore aimed to study placental transfer of taurine in non-complicated term pregnancies in the human *in vivo*. We hypothesized that increased umbilical plasma concentrations and uptake of taurine by the fetus are associated with increased maternal arterial plasma concentrations, uteroplacental uptake from the maternal circulation and placental concentrations of taurine. Unexpectedly, we observed a net placental *release* of taurine to both the maternal and fetal circulations. We therefore proceeded to challenge the prevailing view that placental tissue lacks the ability to synthesize taurine by studying the human placental expression of cysteine sulfinic acid decarboxylase (CSAD), one of the main enzymes in the taurine biosynthetic pathways.

Methods

Design and study population

We performed a cross-sectional *in vivo* study of 179 women scheduled for planned cesarean section and their infants. We invited healthy, non-smoking women with uncomplicated singleton pregnancies to participate. We excluded women with significant pre-existing comorbidity, medication, pregnancy complications and onset of labor prior to scheduled cesarean section.

Data collection

We have previously described the procedure for flow measurements, and sampling of plasma and placental tissue in detail (Holme et al. 2017). Using ultrasound including Doppler we measured the internal vessel diameter (D) and time-averaged maximum velocity (TAMX) in the uterine artery and the intra-abdominal umbilical vein in the morning before the scheduled cesarean section. The women were fasting (median [Q1, Q3] duration: 10 [9, 11] hours). We collected maternal blood samples from the radial artery, the antecubital vein and the uterine vein on the anterolateral surface of the uterus during planned cesarean section in spinal anesthesia (bupivacaine 10 mg, fentanyl 20 µg). We collected fetal blood samples from the umbilical artery and vein immediately after delivery of the infant, before delivery of the placenta. The blood was centrifuged and stored at -80 °C until analysis.

The placenta was placed on an ice chilled tray immediately after delivery. We removed the decidua and collected chorionic villous tissue from 4-5 sampling sites randomly located in the center and each quadrant of the placenta. Placental tissue for taurine analysis was washed in ice cold PBS, snap frozen in liquid nitrogen and stored at -80 °C until

homogenization. The tissue samples were weighed and homogenized cold for 90 seconds in radioimmunoprecipitation assay buffer (25 mM Tris pH 7.4, 150 mM sodium chloride, 1% nonyl phenoxypolyethoxylethanol -40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate) containing 1:100 protease (Sigma P8340) and phosphatase (Sigma P5726 and Sigma P0044) inhibitors, using 0.7 mm zirconia beads in a mini beat beater (BioSpec 607EUR). The homogenates were centrifuged at 4°C, 14 000 g for 15 minutes, and the supernatant was removed, aliquoted and snap frozen in liquid nitrogen. Placental tissue for CSAD expression analysis was washed in ice cold 10 mM HEPES-tris saline. We stored one piece of placental tissue in RNA stabilizing solution (Sigma-Aldrich RNAlater Storage Solution) at -80 °C until Quantitative Real-time polymerase chain reaction (qRT-PCR) analysis. The remaining placental tissue was homogenized directly in buffer D (250 mM sucrose and 1mM EDTA in 10 mM HEPES-tris, pH 6.95) containing 1:1000 protease and phosphatase inhibitors in an ice cold blender for 1-2 minutes. We added additional protease and phosphatase inhibitors to the homogenate to a final dilution of 1:100, aliquoted and snap froze in liquid nitrogen. We stored the homogenates at -80 °C until western blot analysis.

Analyses

Taurine analysis

25 µL of placental homogenate or plasma sample, calibration standards, quality control samples and blank matrix (water) were added to a 0.3 mL 96 well extraction plate. We added 10 µL internal standard mix and 100µl methanol to all wells and mixed on a plate shaker. The plate was centrifuged at 4000 rpm for 15 minutes, and 20 µL of the supernatant from the extraction plate was spiked into 80 µL Mobile Phase A in the analysis plate. We analyzed the extracted amino acid with an Applied Biosystems 4000 Q TRAP linear MS/MS spectrometer by electro spray ionization in positive mode (Foster City, CA, USA). The chromatographic separation was performed on a Phenomenex Kinetex Core Shell C18 (100 x 4.6 mm, 2.6 µm) HPLC column with a temperature of 30°C. The mobile phases were (A) Water + 0.5% formic acid + 0.3% heptafluorobutyric acid and (B) acetonitrile + 0.5% formic acid + 0.3% heptafluorobutyric acid at a flow rate of 0.8 mL/min. The separation was achieved with a linear gradient from 98% (A) for 2 min, 40 % (A) from 0 to 3.5 min followed by a linear gradient back to 98 % (A) over 5 min. The whole run was 8.5 min and the injection volume was 15 µL. We determined the concentration of taurine from the ratio of analyte peak area/internal standard (D4-Taurine) peak area against a linear multiple point calibration curve. The intra-day and inter-day accuracy and precision of the assay were determined by analyzing two different quality control samples and calculating the mean value and standard deviation. The coefficient of variation was 7.17. We normalized the measured concentrations against the mean value of the quality control to adjust for day-to-day variation in the analyses.

RNA Isolation and qRT-PCR for CSAD

The placental RNA expression of CSAD was analyzed by qRT-PCR. Approximately 25 mg tissue was extracted from each sample (n=6), rinsed in RNAse free water and homogenized in 700 µl lysis buffer containing β-mercaptoethanol using a pellet pestle (Kontes). We isolated total RNA using the E.Z.N.A. Total RNA kit 1 (R6834-02, Omega Bio-

Tek) according to the manufacturer's instructions. DNA was removed using DNase-1 treatment (18068015, Invitrogen). We measured RNA quantity using the ND1000 Spectrophotometer (Saveen Werner) and determined RNA quality using the Agilent 6000 nano-kit. RNA-integrity (RIN)-values varied from 1.8 to 7.8 amongst the samples. A quantity of 225 ng RNA was reversely transcribed in a total volume of 20 µL using the High capacity RNA-to-cDNA kit (4387406, Applied Biosystems). Quantitative Real-time PCR (qRT-PCR) was performed on a CFX96 Real-Time System c1000 Touch (BIO-RAD) using Taq Man Gene Expression Master Mix (4369016, Applied Biosystems) and predesigned TaqMan Gene Expression Assay targeting CSAD (Hs00971306_m1, Applied Biosystems). We used beta-actin (ACTB) as endogenous control (Hs99999903_m1). The Ct cutoff value was set to 40 cycles, and the cycle threshold (Ct) values for CSAD was normalized against the Ct values of ACTB (= ΔCt) for each sample.

Western blot analyses for CSAD

The placental CSAD protein expression was analyzed using western blotting. We used both a commercial mouse anti-human CSAD antibody (0.21 mg/mL Abcam, cat no. ab82613) and a custom made sheep anti-mouse CSAD antibody (0.1 mg/mL). The custom antibody was a generous gift from Professor Jan Haavik at the Department of Biomedicine at the University of Bergen. It was generated against full-length mouse CSAD, but has been shown to react against human CSAD protein (Winge et al. 2015).

Frozen placental homogenates were thawed on ice. We determined the protein concentration using BCA Protein Assay Kit (Thermo Scientific, cat no. 23225). The placental homogenate samples were diluted in buffer D (250 mM sucrose in 10 mM HEPES-tris, pH 6.95) and pretreated with 3X DTT sample buffer (8 M urea, 170 mM SDS, 0.04 units of bromophenol blue, and 450 mM DTT in 50 mM Tris-HCl; pH 6.8) before they were heated at 95 ^{o}C for 5 min. Proteins (30 µg/well) were separated on Mini-Protean TGX Precast gels, 12 and 10 % (BioRad, cat. no. 4561041 and 4561036) and transferred to Immuno-Blot PVDF Membrane (BioRad #1620177). Amido Black total protein staining (Sigma Aldrich, Cat no. A8181) was used as a protein loading control. Prior to primary antibody exposure, the membrane was blocked for 1 hour at room temperature in 5 % milk in TBST (50 mM Tris, 150 mM NaCl, 0.1 % Tween, pH 7.6). We incubated the membrane with the CSAD antibody (1:1000 for the commercial and 1:2000 for the custom antibody analyses in 5 % BSA in TBST) over night at 4 ^{o}C. As positive controls we used mouse liver homogenates (30 µg for the commercial and 15 µg protein for the custom antibody analyses) and recombinant CSAD protein. For the commercial antibody analyses we used human CSAD protein (Abcam, cat no. ab162535, 1.0 µg), and for the custom antibody analyses we used recombinant mouse CSAD (Signalway Antibody, cat no: AP70194, 0.05 µg). As a negative control we included peptide competition, where the respective recombinant CSAD proteins was incubated with the CSAD antibody (25:1 for the commercial and 20:1 for the custom antibody analyses) over night at 4 ^{o}C prior to membrane incubation at 4^{o}C over 24h for the commercial and 48 hours for the custom antibody analyses. Antigens were detected using horse anti-mouse IgG, HRP-linked antibody (Cell Signaling, cat no. 7076S, 1/3000 in 5% Bovine Serum Albumin and TBST) for the commercial and rabbit anti-sheep IgG (H+L)-HRP Conjugate (BioRad, cat no.1721017,1/5000 in 5 % milk and TBST) for the custom antibody analyses, Finally, we

used enhanced chemiluminescence detection with PierceTM ECL substrate (Thermo Scientific, Cat no. 32109) and the ChemiDocTM Imaging System (BioRad) to detect the signal.

Calculations

We assumed similar blood composition in the maternal radial and uterine artery. The uteroplacental arteriovenous (A-V) concentration difference (µmol/L) was thus calculated as the difference in taurine concentration between the radial artery and the uterine vein. A higher concentration in the radial artery than in the uterine vein was interpreted as a uteroplacental uptake (µmol/L) from the blood passing in the maternal circulation. A higher concentration in the uterine vein compared to the radial artery was interpreted as a uteroplacental release (µmol/L) to the maternal circulation. The umbilical venoarterial (v-a) difference (µmol/L) was calculated as the difference in taurine concentration between the umbilical vein and the umbilical artery. A higher concentration in the umbilical vein compared to the umbilical artery was interpreted as a fetal uptake (µmol/L) from the blood passing in the umbilical circulation. A higher concentration in the umbilical artery than in the umbilical vein was interpreted as a fetal release (µmol/L) to the umbilical circulation. By definition, a fetal uptake corresponds to a placental release to the umbilical circulation, while a fetal release corresponds to a placental uptake from the umbilical circulation.

In a subgroup of our mother-fetus pairs, we calculated blood flow (Q) in the uterine arteries and the umbilical vein as

$$Q = h \times \left[\frac{D}{2}\right]^2 \times \pi \times TAMX$$

where D is the vessel diameter, TAMX is the time averaged maximum velocity and h is the coefficient for the spatial blood velocity profile. We used 0.5 as the coefficient for the umbilical vein and 0.6 for the uterine artery (Haugen et al. 2004; Rigano et al. 2010). Based on Fick's principle we calculated the uteroplacental and fetal mass uptake or release in µmol/min by multiplying the uterine and umbilical blood flow with the uteroplacental A-V and umbilical v-a concentration difference, respectively. The positive or negative placental consumption of taurine was calculated as the difference between the uteroplacental and the fetal uptake or release.

Statistics

Clinical data are reported as mean values with standard deviations or numbers with percentages. Due to skewed distributions, BMI and taurine concentrations are reported as medians and two quartiles; the lower quartile, Q1, and the upper quartile, Q3. We performed paired comparisons between taurine concentrations in the different vessels, the placenta and between the uteroplacental and fetal uptake or release with Wilcoxon sign rank tests. Correlation analyses were performed with Spearman's rank correlation coefficient. A two-sided p-value <0.05 was considered significant. We performed all analyses using Statistical Package for the Social Sciences, Version 21.0 (SPSS Inc., Hong Kong).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the

1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the data protection officials at Oslo University Hospital and the Regional Committee for Medical and Health Research Ethics, Southern Norway 2419/2011. Informed consent was obtained from all individual participants included in the study.

Results

Clinical characteristics

Table 1 shows the clinical characteristics of the participating women and infants. The women had a mean age of 35.4 years, and 24.6% were nulliparous. They were generally lean, but the range in BMI and gestational weight gain was quite large, reflecting the population we intended to study. The infants were born at term with a wide range of birth weights and placental weights.

Table 1 Clinical characteristics of the participating women and infants

	n	%	Mean (SD)	Range
Women	179			
Age (years)			35.4 (3.8)	23-44
Para≥1	135	75.4		
BMI before pregnancy (kg/m^2)[a]			22.3 [20.8, 25.4]	17.0-47.6
Gestational weight gain (kg)			15.0 (4.8)	-1.2-31.3
Higher education (>15 years)	155	86.6		
Smoking during pregnancy[b]	0	0		
Gestational diabetes[c]	4	2.2		
Infants	179			
Gestational age (weeks)			39.3 (0.6)	37.1-42.0
Birthweight (g)			3546 (443)	2297-4955
Placenta weight (g)[d]			617.2 (133.4)	310-1115
Sex (boys)	99	55.3		
Apgar score < 7 after 5 min	0	0		

[a]Value presented as median and the lower and upper quartile [Q1, Q3] due to skewed distribution. [b]Seven women stopped smoking when pregnancy was confirmed in first trimester. [c]Not insulin treated. Defined based on WHO criteria; fasting plasma glucose of 5.1-6.9 mmol/L or plasma glucose of 8.5-11.0 mmol/L 2 hours after an oral glucose tolerance test of 75 g glucose. [d]Untrimmed, without blood clots.

Plasma and placental taurine concentrations

Table 2 shows the median taurine concentrations and paired concentration differences with quartiles in five vessels. There were large individual differences. The taurine concentrations were significantly higher in the uterine vein compared to the radial artery

(p<0.001), i.e., a uteroplacental release. There was no significant concentration difference between the radial artery and the antecubital vein (p=0.58). The taurine concentrations in the umbilical vein were significantly higher compared to the umbilical artery (p<0.001), i.e, a fetal uptake.

Table 2 Median and the lower and upper quartile [Q1, Q3] of the taurine concentrations and the paired arteriovenous differences

Vessel	**Median Taurine µmol/L**
Radial artery (n 168)	20.1 [15.6, 28.3]
Uterine vein (n 177)	25.0 [18.8, 36.7]
Antecubital vein (n 164)	21.5 [15.7, 27.0]
Umbilical vein (n 177)	111.0 [87.4, 147.5]
Umbilical artery (n 162)	83.3 [70.1, 108.0]
Paired differences	
Uteroplacental A-V (n 166)	-3.8*[-13.3, 3.7]
Antebrachial a-v (n 158)	0.5^{NS} [-6.7, 8.8]
Umbilical v-a (n 160)	20.7* [-5.4, 45.4]

*p<0.001 with Wilcoxon sign rank test. NS: not significant

We observed a median taurine concentration in placental tissue of 67.8 [57.3, 76.0] µmol/mg protein. Normalized for placental wet weight the concentration was 2.87 [2.52, 3.36] µmol/g placenta. Assuming that 1 g of placental tissue equals 1 ml blood, the median paired ratio between the taurine concentrations in the placenta and the umbilical vein was 27:1, and the median paired ratio between the taurine concentrations in the placenta and the radial artery was 139:1.

Mass uptake and release of taurine in the fetal-placental unit

In a subgroup of the included mother-fetus pairs we measured a median [Q1, Q3] total uterine artery flow of 500.5 [362.2, 615.0] mL/min (n=70) and a median [Q1, Q3] umbilical venous flow of 196.4 [159.1, 238.6] mL/min (n=115). In this group we calculated a median [Q1, Q3] uteroplacental taurine release of 0.7 [-2.1, 6.2] µmol/min) to the maternal circulation (p=0.04), and a median [Q1, Q3] fetal uptake from (placental release to) the umbilical circulation (p<0.001) of 4.0 [-1.1, 8.9] µmol/min. We observed a negative median [Q1, Q3] placental consumption of taurine: -8.9 [-12.8, 3.6] µmol/min (p= 0.001).

Correlations between plasma and placental taurine concentrations

The taurine concentration in the fetal umbilical vein and the umbilical v-a difference were not significantly correlated with the taurine concentration in the maternal radial artery (r_s=-0.04, p=0.63 and r_s=-0.02, p=0.83 respectively), but were negatively correlated with the maternal uteroplacental A-V difference (r_s=-0.19, p=0.02 and r_s=-0.25, p=0.003) (Fig 1a and b). The fetal umbilical v-a difference was positively correlated with the taurine concentration in the maternal uterine vein (r_s=0.17, p=0.03) (Fig 1c). There was no significant correlation between the concentration in the umbilical artery and the maternal uteroplacental A-V difference (r_s=0.07, p=0.37). The taurine concentration in the umbilical circulation and the umbilical v-a difference were not correlated with the taurine concentration in placental tissue

(Table 3). There were no correlations between the taurine concentration in placental tissue and the concentrations in the maternal circulation or the uteroplacental A-V difference.

Fig 1 Correlations between a) the fetal umbilical vein and the maternal uteroplacental A-V difference b) the fetal umbilical v-a difference and the maternal uteroplacental A-V difference c) the fetal umbilical v-a difference and the maternal uterine vein

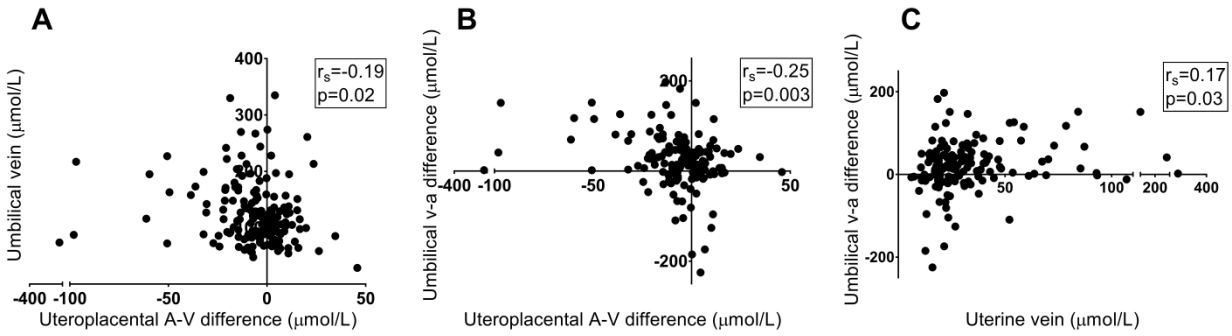


Table 3 Spearman correlations between placental taurine concentrations and plasma concentrations and concentration differences.

	Taurine µmol/L				**Paired differences µmol/L**	
	Radial artery (n 166)	Uterine vein (n 175)	Umbilical vein (n 175)	Umbilical artery (n 160)	Uteroplacental A-V (n 164)	Umbilical v-a (n 158)
Taurine µmol/mg placental protein	r_s= -0.03, p= 0.70 NS	r_s= -0.08, p= 0.29 NS	r_s= -0.07, p= 0.36 NS	r_s= 0.05, p= 0.54 NS	r_s= 0.08, p= 0.34 NS	r_s= 0.01, p= 0.93 NS

NS: not significant with Wilcoxon sign rank test

CSAD expression analysis

qRT-PCR

There was detectable expression of CSAD-mRNA in human placenta homogenate (n=6), with a mean Ct value of 31.3, range: 30.5 – 32.3. The expression was significantly lower than the endogenous control ($p<0.001$), beta actin, with a mean Ct value of 27.8, range: 26.3 – 29.0. The sample quality, analyzed by the RNA integrity number (RIN,) varied with a mean value of 5.3, range: 1.8 – 7.8. RIN values range from 0 – 10, where 10 is completely intact and 0 is totally degraded RNA. Random hexamers in TaqMan gene expression assays allows analysis of sample with varying quality.

Western blot

In the present study, we used both a commercial antibody against human CSAD and a custom made antibody against mouse CSAD that previously has been shown to detect CSAD in human brain tissue (Winge et al. 2015). In a series of experiments we observed variable staining in the western blots of human placental tissue. In the blots using the commercial antibody, we observed staining of a band at 55 kDa (example of blot shown in Online Resource 1), but in the blots using the custom antibody there was no reproducible staining of

CSAD (example of blot shown in Online Resource 2). CSAD protein was detected in both of the positive controls (i.e. mouse liver homogenate and the recombinant CSAD). Peptide competition reduced the expression of CSAD protein significantly for the bands observed in the human placental homogenates, as well as in mouse liver and recombinant protein.

Discussion

In the present study of placental taurine transfer in non-complicated human term pregnancies, we observed a significant median fetal uptake of taurine from the umbilical circulation, a significant median uteroplacental release of taurine to the maternal circulation and a significant negative median placental taurine consumption. These findings demonstrate an unexpected bilateral placental release to both the maternal and fetal compartments. In contrast to our hypothesis, increasing venous umbilical concentrations and fetal uptake (μmol/L) of taurine was associated with an increasing uteroplacental release (μmol/L) of taurine to the maternal circulation, rather than a uteroplacental uptake. A higher fetal uptake of taurine was also associated with higher concentrations in the maternal uterine vein, i.e., the blood drained from the placenta into the maternal circulation, rather than the concentrations in the radial artery and the blood entering the placenta. Further, the taurine concentrations in the umbilical vein and the fetal uptake were not correlated with the concentration of taurine in placental tissue.

To our knowledge, this is the first study to explore maternal uteroplacental A-V differences, fetal umbilical v-a differences and placental concentrations of taurine in the same human mother-fetus pairs simultaneously. Further, no previous study has, as far as we know, quantified the mass uptake or release of taurine across the placenta in the maternal and fetal circulations in the human. A few human *in vivo* studies have previously measured maternal uteroplacental A-V or fetal umbilical v-a concentration differences of taurine (Tsuchiya et al. 2009; Hayashi et al. 1978; Velazquez et al. 1976; Cetin et al. 1988; Cetin et al. 2005; Steingrimsdottir et al. 1993; Prenton and Young 1969). Hayashi et al observed a significant fetal uptake from the umbilical circulation, but none of the other studies were able to show statistically significant concentration differences. This is possibly due to large variabilities in maternal and fetal taurine concentrations and a limited number of study subjects (n=4-31). Several reports, including studies by our group, have described a positive correlation between maternal and fetal plasma concentrations for most of the proteogenic amino acids (Cetin et al. 1996; Holm et al. 2017; Cetin et al. 1988). Cetin et al (Cetin et al. 1996) have further reported a positive correlation between taurine concentrations in arterialized maternal plasma and the umbilical vein, supporting the notion that the maternal taurine level is the major determinant of fetal taurine supply. We did not observe a similar relationship between taurine concentrations in the radial artery and umbilical vein in the present study. This discrepancy may be due to smaller sampling size, the heterogenic study population or different analysis methods.

We observed large individual variations in the flux across both the maternal facing microvillous and fetal facing basal membrane of the syncytiotrophoblast, indicating that placental taurine transfer in healthy term pregnancies is a dynamic process which fluctuates between uptake and release. The negative correlations between the umbilical vein taurine concentration and v-a difference on one hand, and the uteroplacental A-V difference in the

maternal circulation on the other, show that increased placental release (µmol/L) of taurine to the fetus was accompanied by a greater release of taurine to the maternal circulation. Less placental release to, or even uptake from, the umbilical circulation was associated with reduced release to or uptake from the maternal circulation. This observation suggests that the placenta itself may play a dominating part in the government of the dynamic taurine transfer between the maternal and fetal circulations. Taurine is the most abundant free amino acid in the placental syncytiotrophoblast, and placental taurine concentrations observed in the current work are in line with previous findings in non-complicated pregnancies (Philipps et al. 1978). A study of women with hypertensive pregnancies, however, found much lower values (Velazquez et al. 1976). In our cohort the concentration of taurine in the placenta was 27 times higher compared to the fetal plasma, facilitating efflux to the fetus. Compared to maternal plasma, the placental taurine concentration was 139 times higher. Perfusion studies have shown that the human placenta is able to achieve and maintain such a high gradient through active, energy requiring transport of taurine across the microvillous membrane (Miyamoto et al. 1988; Hibbard et al. 1990). However, the steep placental-maternal gradient could facilitate a release of taurine from the placenta to the maternal circulation as observed in the present study.

The bilateral placental release of taurine observed in the present study indicates that the placenta is an immediate source of taurine in healthy term pregnancies. Interestingly, there was no association between taurine concentrations in placental tissue and umbilical concentrations or fetal uptake. This observation could imply that the placental concentrations of taurine in healthy term pregnancies far exceed the amount required to facilitate sufficient transfer to the fetus, and that under such circumstances there is no dose-response relationship between placental taurine concentrations and fetal supply. This notion is in line with a sophisticated, governed release of taurine in the fetal placental-unit at term, rather than a placental release due to overflow. The concomitant bilateral placental release of taurine could be a placental response to the prolonged fasting, the anesthesia, or to other circumstances regarding the cesarean section in our study. It could also be a part of osmoregulation in the placenta, since taurine is a vital osmolyte in the syncytiotrophoblast, and a study of isolated membrane vesicles have demonstrated that taurine may be released across the microvillous membrane via chloride channels as a response to a hypo-osmotic insult (Vallejos and Riquelme 2007). Further, cell culture studies have shown that taurine is likely to play a critical role not only in fetal development, but also in the development and functioning of the placenta by facilitating syncytiotrophoblast renewal and cell survival (Desforges et al. 2013). Intracellular taurine depletion in the placenta impaired the syncytiotrophoblast regeneration and lead to an increased susceptibility to oxidative stress, resulting in nuclear and mitochondrial DNA damage (Desforges et al. 2015). It is possible that the portion of the placental taurine pool that exceeds what is necessary to secure fetal supply plays an important role in placental homeostasis and that the fetal-placenta taurine transfer also is primarily governed by placental needs.

The observed bilateral placental taurine release could be a result of accumulative placental uptake of taurine across gestation or in the postprandial state. Alternatively, it could be due to de novo taurine synthesis in the placenta. It has been the prevailing opinion that the fetus and the placenta have limited or no ability to synthesize taurine (Ditchfield et al. 2015;

Tappaz 2004). This assumption has been based on reports of lacking synthesizing enzymes in placental and fetal tissues (Gaull et al. 1972; Sturman et al. 1970). However, these studies have measured enzymes in the transsulfuration pathway converting the essential amino acid methionine to cysteine, several steps earlier in the synthesis pathway for taurine. Until recently, no study had, to our knowledge, explored the presence of cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD) in the human placenta. CDO and CSAD are the enzymes responsible for converting cysteine to cysteine sulfinic acid and hypotaurine for further oxidation to taurine, reactions which are considered to be major steps in the biosynthesis of taurine in mammals (Tappaz 2004; Park et al. 2017). Interestingly, a recent study by Korneeva and coworkers has provided evidence of both CDO mRNA and protein expression in the human placenta (Korneeva et al. 2016). CDO converts cysteine to cysteine sulfinic acid, which can either be further transaminated by aspartate amino transferase and converted to sulfur and pyruvate, or decarboxylized to hypotaurine for taurine synthesis by CSAD. CSAD activity can thus regulate the partitioning of cysteine sulfinic acid between decarboxylation and transamination, and potentially govern taurine production (Stipanuk et al. 2006). Despite its vital role in the biosynthesis of taurine, CSAD expression in the human placenta has not been established. In the present study, we observed expression of CSAD-mRNA in human placental tissue, findings that agreed with data presented by Korneeva and coworkers (Korneeva et al. 2016). The expression of CSAD protein in the human placenta, however, has not been studied previously. We observed placental expression of the CSAD protein in the western blots using a commercial antibody, but no reproducible CSAD expression in the blots using a custom made antibody. The potential discrepancy between protein and mRNA data could be due to variable or lacking translation of CSAD in the syncytiotrophoblast in healthy term pregnancies, or possibly false negative results in most of our western blots. Although the expression data is not conclusive as yet, our data on placental CSAD, together with the recent findings by Korneeva and coworkers, challenge the prevailing view that there is no expression of taurine synthesizing enzymes in the human placenta, and suggest that there is a potential for a CSAD mediated placental taurine synthesis at term. Notably, it is also a possibility that taurine synthesis through other pathways, for instance via coenzyme A and cysteamine (Stipanuk et al. 2006) may contribute to the observed bilateral placental release to the maternal and fetal circulations.

Major strengths of the current work are the *in vivo* sampling method with paired plasma and placental samples and the LC-MS/MS methodology which is considered to be the gold standard for amino acid analyses (Alterman and Hunziker 2011). Another important strength is the substantial number of participants, since the taurine concentrations and arteriovenous differences show large individual variations. A limitation of the present study is the cross-sectional design which does not provide any opportunity to evaluate longitudinal taurine concentration changes or taurine transport and metabolism at earlier gestational ages. We were only able to obtain flow measurements in a subset of our mother-fetus pairs due to the logistically challenging sampling procedure. Our estimates of uteroplacental release of taurine to the maternal circulation are based on the concentration differences between the radial artery and the uterine vein. As the uterine vein drains the uterus in addition to the placenta, a release or uptake of taurine by the uterine tissue could influence our observations. As a comparison, we did not observe a significant difference between taurine concentrations

in the radial artery and the antecubital vein, representing the arteriovenous concentration difference across the capillary bed of the forearm. A net transfer of water between the maternal, placental and fetal compartments could affect the calculated arteriovenous concentration differences. However, an adjustment based on arteriovenous hemoglobin concentration differences according to a previously described method (Holm et al. 2017) did not affect our overall results.

In conclusion, our *in vivo* study of taurine transfer in non-complicated human term pregnancies showed an unexpected bilateral placental release to the maternal and fetal compartments. Contrary to our hypothesis, increased umbilical plasma concentrations and uptake of taurine by the fetus were not associated with increased maternal arterial plasma concentrations, uteroplacental uptake from the maternal circulation or taurine concentrations in placental tissue, but with the concomitant uteroplacental taurine release. We observed evidence of CSAD expression in human placental tissue, suggesting a potential for taurine synthesis in the human term placenta. Our observations show that the human placenta has the capacity to a bilateral taurine release, indicating a fundamental role of this amino acid in the human placental homeostasis beyond the supply to the fetus.

Acknowledgements

First we would like to thank the participating women for contributing to our study. We would also like to acknowledge the anesthesiologists, specialist nurses, obstetricians, and midwives at Oslo University Hospital for offering their time and skills to help us with the sampling procedure. We further thank Trine M. Reine and Anne Randi Enget at the Department of Nutrition at the University of Oslo who performed the qRT-PCR analysis, and Jan Haavik at the Department of Biomedicine at the University of Bergen who generously provided the CSAD antibody for western blot analysis.

Funding

This study was funded by grants from the South-Eastern Norway Regional Health Authority, the Throne Holst foundation, the Norwegian National Advisory Unit on Women's Health, Oslo University Hospital, and the Department of Obstetrics, Oslo University Hospital.

References

Alterman MA, Hunziker P (2011) Amino Acid Analysis: Methods and Protocols. Humana Press,

Boujendar S, Reusens B, Merezak S, Ahn MT, Arany E, Hill D, Remacle C (2002) Taurine supplementation to a low protein diet during foetal and early postnatal life restores a normal proliferation and apoptosis of rat pancreatic islets. Diabetologia 45 (6):856-866. doi:10.1007/s00125-002-0833-6

Cetin I, Corbetta C, Sereni LP, Marconi AM, Bozzetti P, Pardi G, Battaglia FC (1990) Umbilical amino acid concentrations in normal and growth-retarded fetuses sampled in utero by cordocentesis. Am J Obstet Gynecol 162 (1):253-261

Cetin I, de Santis MS, Taricco E, Radaelli T, Teng C, Ronzoni S, Spada E, Milani S, Pardi G (2005) Maternal and fetal amino acid concentrations in normal pregnancies and in pregnancies with gestational diabetes mellitus. Am J Obstet Gynecol 192 (2):610-617. doi:10.1016/j.ajog.2004.08.011

Cetin I, Marconi AM, Bozzetti P, Sereni LP, Corbetta C, Pardi G, Battaglia FC (1988) Umbilical amino acid concentrations in appropriate and small for gestational age infants: a biochemical difference present in utero. Am J Obstet Gynecol 158 (1):120-126

Cetin I, Ronzoni S, Marconi AM, Perugino G, Corbetta C, Battaglia FC, Pardi G (1996) Maternal concentrations and fetal-maternal concentration differences of plasma amino acids in normal and intrauterine growth-restricted pregnancies. Am J Obstet Gynecol 174 (5):1575-1583

Desforges M, Parsons L, Westwood M, Sibley CP, Greenwood SL (2013) Taurine transport in human placental trophoblast is important for regulation of cell differentiation and survival. Cell Death Dis 4:e559. doi:10.1038/cddis.2013.81

Desforges M, Whittaker H, Farmer E, Sibley CP, Greenwood SL (2015) Effects of taurine depletion on human placental syncytiotrophoblast renewal and susceptibility to oxidative stress. Adv Exp Med Biol 803:63-73. doi:10.1007/978-3-319-15126-7_6

Ditchfield AM, Desforges M, Mills TA, Glazier JD, Wareing M, Mynett K, Sibley CP, Greenwood SL (2015) Maternal obesity is associated with a reduction in placental taurine transporter activity. Int J Obes (Lond) 39 (4):557-564. doi:10.1038/ijo.2014.212

Gaull G, Sturman JA, Raiha NC (1972) Development of mammalian sulfur metabolism: absence of cystathionase in human fetal tissues. Pediatr Res 6 (6):538-547. doi:10.1203/00006450-197206000-00002

Haugen G, Kiserud T, Godfrey K, Crozier S, Hanson M (2004) Portal and umbilical venous blood supply to the liver in the human fetus near term. Ultrasound Obstet Gynecol 24 (6):599-605. doi:10.1002/uog.1744

Hayashi S, Sanada K, Sagawa N, Yamada N, Kido K (1978) Umbilical vein-artery differences of plasma amino acids in the last trimester of human pregnancy. Biol Neonate 34 (1-2):11-18

Hayes KC, Sturman JA (1981) Taurine in metabolism. Annu Rev Nutr 1:401-425. doi:10.1146/annurev.nu.01.070181.002153

Hibbard JU, Pridjian G, Whitington PF, Moawad AH (1990) Taurine transport in the in vitro perfused human placenta. Pediatr Res 27 (1):80-84. doi:10.1203/00006450-199001000-00021

Holm MB, Bastani NE, Holme AM, Zucknick M, Jansson T, Refsum H, Morkrid L, Blomhoff R, Henriksen T, Michelsen TM (2017) Uptake and release of amino acids in

the fetal-placental unit in human pregnancies. PLoS One 12 (10):e0185760. doi:10.1371/journal.pone.0185760
Holme AM, Holm MB, Roland MCP, Horne H, Michelsen TM, Haugen G, Henriksen T (2017) The 4-vessel Sampling Approach to Integrative Studies of Human Placental Physiology In Vivo. Journal of visualized experiments : JoVE (126). doi:10.3791/55847
Hultman K, Alexanderson C, Manneras L, Sandberg M, Holmang A, Jansson T (2007) Maternal taurine supplementation in the late pregnant rat stimulates postnatal growth and induces obesity and insulin resistance in adult offspring. J Physiol 579 (Pt 3):823-833. doi:10.1113/jphysiol.2006.124610
Jozwik M, Teng C, Battaglia FC, Meschia G (1999) Fetal supply of amino acids and amino nitrogen after maternal infusion of amino acids in pregnant sheep. Am J Obstet Gynecol 180 (2 Pt 1):447-453
Karl PI, Fisher SE (1990) Taurine transport by microvillous membrane vesicles and the perfused cotyledon of the human placenta. Am J Physiol 258 (3 Pt 1):C443-451
Korneeva KL, Rodriguez RR, Ralchenko SV, Martunovska OV, Frolova AO, Martsenyuk OP, Manzhula LV, Melnyk VT, Shkoropad OY, Obolenska MY (2016) Expression of genes, encoding the enzymes of cysteine metabolism in human placenta in the first and third trimesters of uncomplicated pregnancy. Ukrainian biochemical journal 88 (1):88-98. doi:10.15407/ubj88.01.088
Liu J, Liu L, Chen H (2011) Antenatal taurine supplementation for improving brain ultrastructure in fetal rats with intrauterine growth restriction. Neuroscience 181:265-270. doi:10.1016/j.neuroscience.2011.02.056
Miyamoto Y, Balkovetz DF, Leibach FH, Mahesh VB, Ganapathy V (1988) Na+ + Cl- - gradient-driven, high-affinity, uphill transport of taurine in human placental brush-border membrane vesicles. FEBS Lett 231 (1):263-267
Norberg S, Powell TL, Jansson T (1998) Intrauterine growth restriction is associated with a reduced activity of placental taurine transporters. Pediatr Res 44. doi:10.1203/00006450-199808000-00016
Park E, Park SY, Cho IS, Kim BS, Schuller-Levis G (2017) A Novel Cysteine Sulfinic Acid Decarboxylase Knock-Out Mouse: Taurine Distribution in Various Tissues With and Without Taurine Supplementation. Adv Exp Med Biol 975:461-474. doi:10.1007/978-94-024-1079-2_37
Philipps AF, Holzman IR, Teng C, Battaglia FC (1978) Tissue concentrations of free amino acids in term human placentas. Am J Obstet Gynecol 131 (8):881-887
Prenton MA, Young M (1969) Umbilical vein-artery and uterine arterio-venous plasma amino acid differences (in the human subject). J Obstet Gynaecol Br Commonw 76 (5):404-411
Rigano S, Ferrazzi E, Boito S, Pennati G, Padoan A, Galan H (2010) Blood flow volume of uterine arteries in human pregnancies determined using 3D and bi-dimensional imaging, angio-Doppler, and fluid-dynamic modeling. Placenta 31 (1):37-43. doi:10.1016/j.placenta.2009.10.010
Ripps H, Shen W (2012) Review: taurine: a "very essential" amino acid. Mol Vis 18:2673-2686
Roos S, Powell TL, Jansson T (2004) Human placental taurine transporter in uncomplicated and IUGR pregnancies: cellular localization, protein expression, and regulation. Am J Physiol Regul Integr Comp Physiol 287 (4):R886-893. doi:10.1152/ajpregu.00232.2004
Steingrimsdottir T, Ronquist G, Ulmsten U (1993) Balance of amino acids in the pregnant human uterus at term. Eur J Obstet Gynecol Reprod Biol 50 (3):197-202

Stipanuk MH, Dominy JE, Jr., Lee JI, Coloso RM (2006) Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. J Nutr 136 (6 Suppl):1652s-1659s

Sturman JA, Rassin DK, Gaull GE (1970) Distribution of transsulphuration enzymes in various organs and species. Int J Biochem 1 (2):251-253. doi:http://dx.doi.org/10.1016/0020-711X(70)90102-3

Tappaz ML (2004) Taurine biosynthetic enzymes and taurine transporter: molecular identification and regulations. Neurochem Res 29 (1):83-96

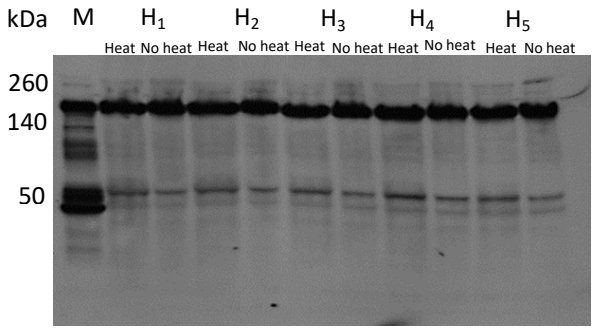
Tsuchiya H, Matsui K, Muramatsu T, Ando T, Endo F (2009) Differences between the amino acid concentrations of umbilical venous and arterial blood. Arch Dis Child Fetal Neonatal Ed 94 (2):F155-156. doi:10.1136/adc.2008.147256

Vallejos C, Riquelme G (2007) The maxi-chloride channel in human syncytiotrophoblast: a pathway for taurine efflux in placental volume regulation? Placenta 28 (11-12):1182-1191. doi:10.1016/j.placenta.2007.06.005

Velazquez A, Rosado A, Bernal A, Noriega L, Arevalo N (1976) Amino acid pools in the feto-maternal system. Biol Neonate 29 (1-2):28-40

Winge I, Teigen K, Fossbakk A, Mahootchi E, Kleppe R, Sköldberg F, Kämpe O, Haavik J (2015) Mammalian CSAD and GADL1 have distinct biochemical properties and patterns of brain expression. Neurochem Int 90:173-184. doi:http://dx.doi.org/10.1016/j.neuint.2015.08.013

Online Resource 1 Western Blot, CSAD with commercial antibodies. Placental homogenates (H_n) and mouse liver control (M). We found detectable bands at 55 kDa in all human placenta homogenate samples. We also found a distinct band around 180 kDa. Peptide competition removed the staining in both the placental homogenates and mouse liver control (data not shown).



Online Resource 2 Western Blot, CSAD with custom made antibodies. a) No detectable bands at 55 kDa in human placental homogenate samples (H_n). Protein loading control is illustrated by amido black. CSAD was, as expected, detected at 55 kDa for mouse liver homogenate (M) and at 70 kDa for recombinant mouse CSAD (RP). Peptide competition reduced the expression of CSAD protein significantly for both mouse liver and recombinant protein ($p<0.001$), with a mean (SD) reduction of 98 (0.02) % and 84 (0.06) % respectively. b) The fold difference level of CSAD protein in positive relative to negative control (peptide competition) is illustrated by the bar chart. CSAD protein level in mouse liver is shown as relative to recombinant protein. *** indicates ($p<0.001$).