Maternal and fetal levels of methionine and homocysteine in early human pregnancy

*†Régine P. M. Steegers-Theunissen Clinical epidemiologist, **Neville C. Wathen Consultant, †Tom K. A. B. Eskes Professor, †Bertie van Raaij-Selten Laboratory technician, **Tim Chard Professor
Departments of *Epidemiology, †Obstetrics and Gynaecology and †Paediatrics, University Hospital Nijmegen, The Netherlands; **Fetal Medicine Unit, Homerton Hospital, London, UK

Objective To investigate methionine metabolism during normal human embryonic development by measuring levels of methionine and total homocysteine in samples of maternal serum, extra-embryonic coelomic fluid, and amniotic fluid.

Design Cross-sectional observational study.

Setting Collaboration between St Bartholomew’s Hospital, London, and the University Hospital of Nijmegen in The Netherlands.

Participants Twenty-three women with uncomplicated pregnancies between 8 and 12 weeks of gestation before surgical termination of an ultrasonographically normal fetus.

Methods Maternal serum samples were collected prior to surgery. Samples of extra-embryonic fluid and amniotic fluids were obtained by transvaginal ultrasound-guided coelocentesis and amniocentesis. Methionine was measured using an aminoacid analyser and total homocysteine by high performance liquid chromatography.

Results Levels of methionine were four times higher in extra-embryonic coelomic fluid and twice as high in amniotic fluid compared with maternal serum. In contrast, the total homocysteine concentrations were much lower in both extra-embryonic coelomic fluid and amniotic fluid than in maternal serum. All differences were significant ($P < 0.01$).

Conclusions The comparatively high concentrations of methionine in extra-embryonic coelomic fluid and amniotic fluid, and the concomitant low levels of total homocysteine in these fluids, suggest a role for methionine metabolism during early human pregnancy.

INTRODUCTION
The mechanisms involved in the nutrition of the embryo and fetus are poorly understood. The dynamic physiology of the transplacental transport of the amino acid methionine, its derivative homocysteine, folate and vitamin $B_{12}$ which are involved in homocysteine remethylation (Fig. 1) has been studied previously by us in the second and third trimester of pregnancy$^{1,2}$, as well as by others$^{3,4}$. Alteration of methionine metabolism in humans due to folate or vitamin $B_{12}$ shortage may play a role in the aetiology of neural-tube defects, recurrent miscarriage, placental infarcts and placental abruption$^{5-9}$. The causes of these pregnancy complications might be found in the first gestational weeks. During the first trimester of pregnancy embryonic nutrition is provided by the transfer of nutrients from the extra-embryonic coelomic and amniotic fluids to the embryo. Campbell et al.$^{10}$ reported that the main route for maternal–fetal exchange of folate and methylcobalamin in early human pregnancy may be via the extra-embryonic coelomic cavity. Knowledge about the composition of the extra-embryonic coelomic and amniotic fluids and the transport mechanisms of methionine and homocysteine during that period of pregnancy is lacking.

Therefore the aim of the present study was to investigate the importance of tissue specific methionine metabolism during normal early human pregnancy by measuring the levels of methionine and total homocysteine in samples of maternal serum, extra-embryonic coelomic fluid and amniotic fluid at 8 to 12 weeks of gestation.

METHODS
Twenty-three pregnant women were studied after informed consent was obtained. The experimental protocol was approved by the Ethics Committee of St
Bartholomew’s Hospital, London. All women were undergoing a therapeutic termination of pregnancy for psycho-social reasons at a gestational age of 8 to 12 weeks. The duration of pregnancy was established by menstrual history and ultrasound measurement of crown–rump length.

Transvaginal ultrasound was performed using a 5 MHz curvilinear vaginal probe (Aloka SSD 620, Aloka Co Ltd, Tokyo, Japan). In each case ultrasonography confirmed a singleton pregnancy with normal development and normal fetal heart activity. The procedure for transvaginal ultrasound, coelocentesis and amniocentesis has been described in detail. Matched samples of amniotic and coelomic fluid were collected in every case.

Maternal blood was collected into glass tubes before induction of anaesthesia; within 30 min, the samples were centrifuged for 10 min at 3000 g and the serum aspirated into dry plastic tubes. The fluid or serum was stored at −20°C until assayed for total homocysteine (free plus protein-bound) and methionine. Total homocysteine concentrations were measured by automated high performance liquid chromatography and fluorometric detection in serum and extra-embryonic coelomic fluid (50 μL) were deproteinised by adding an equal volume of ice-cold sulphosalicylic acid (25% w/v) and were placed on ice. After 10 min the samples were centrifuged for 10 min at 3500 g. The supernatant was filtrated through a 0.45 μm filter. A 140 μL sample was injected on a column. After post-column derivatisation with O-phtaldialdehyde, the eluent was fluorometrically. The detection limit was 15 pmol. Both intra- and inter-assay coefficients of variation were < 5%.

Results have been expressed as median (range). Comparisons between gestational age, blood and fluid concentrations of methionine and homocysteine were performed by the Wilcoxon matched-pairs signed-rank test. Correlations were evaluated by determination of the Spearman coefficient of correlation and careful interpretation of the scatter diagrams. P-values < 0.05 were considered statistically significant.

RESULTS

The concentrations of methionine and total homocysteine in maternal venous blood, extra-embryonic coelomic fluid and amniotic fluid from 23 pregnancies at 8 to 12 weeks of gestation are shown in Table 1.

Table 1. Methionine levels in 23 matched samples of extra-embryonic coelomic fluid, amniotic fluid, and maternal serum at 8 to 12 weeks of gestation. Values are given as median (range).

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Methionine (μmol/L)</th>
<th>Homocysteine (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>11 (2–20)</td>
<td>8-7 (6–13-1)</td>
</tr>
<tr>
<td>Coelomic fluid</td>
<td>46 (32–63)</td>
<td>2-5 (0-8–4-2)</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>26 (18–40)</td>
<td>1-0 (0-5–1-7)</td>
</tr>
</tbody>
</table>

Fig. 1. A simplification of the folate and cobalamin dependent methionine metabolism in humans. CS = cystathionine synthase; MeCbl = methylcobalamin; MS = methionine synthase; MTHFR = 5,10-methylene THF reductase; SAH = S-adenosylhomocysteine; SAM = S-adenosylmethionine; THF = tetrahydrofolate.
DISCUSSION

This is the first report on concentrations of methionine and total homocysteine in human extra-embryonic coelomic and amniotic fluids at 8 and 12 weeks of gestation. The comparatively high levels of methionine in both embryonic fluids may suggest that this nutrient is important during early human pregnancy.

Methionine is essential for cell proliferation and DNA and tRNA methylation. It is converted to S-adenosylmethionine and, after decarboxylation, this methyl donor is the source of the 3-carbon moieties of the polyamines spermidine and spermine. In addition, S-adenosylmethionine is involved in the methylation of DNA. In the rat a shortage of methionine and S-adenosylmethionine in embryo cultures can lead to disturbed morphogenesis, especially the development of neural tube defects. The concomitant low total homocysteine concentrations in both fluids together with the high methionine concentrations suggests that the remethylation pathway is likely to be important as well in the extra-embryonic tissues during this early stage of development. Because folate and vitamin B12 are essential in the remethylation of homocysteine into methionine, this hypothesis is supported by the results of the study of Campbell et al., showing high

levels of folate and vitamin B₁₂ in the extra-embryonic and amniotic cavity. High methionine may suggest that the coelomic cavity may act as a store of concentrated methionine prior to utilisation by the yolk sac and rapidly growing fetus and trophoblast. The gradient of methionine levels suggests active transport of methionine from maternal serum to the coelomic cavity followed by active transport or diffusion into the amniotic cavity. This is supported by the finding of a positive correlation between the methionine levels in the coelomic and amniotic fluids. The positive correlation between total homocysteine in serum and coelomic fluid might also be explained by a passive diffusion process.

The positive correlation between the methionine concentration in serum and the total homocysteine concentration in the coelomic cavity is more difficult to explain. It is possible that if large amounts of methionine are actively transported from maternal blood to coelomic fluid, then the remethylation of homocysteine to methionine by extra-embryonic structures would be decreased leading to relatively high total homocysteine concentrations in the coelomic fluid.

The methionine levels in the serum of pregnant women in the present study were slightly lower than those determined in nonpregnant women, and the serum total homocysteine concentrations were slightly higher than those published previously by Andersson et al. in the first trimester of pregnancy.

The derivative homocysteine of methionine is normally present in blood in low concentrations. Elevated intracellular and extracellular homocysteine levels may be cytotoxic, though whether elevated circulating levels of homocysteine are embryotoxic is unknown. In vitro studies in the rat suggest that the embryotoxic effect of L-homocysteine is due to inhibition of methyl donation by S-adenosylmethionine. Also, toxicity of homocysteine for vascular endothelium interfering with spiral or yolk sac arteries cannot be excluded. The development of neural tube defects might be partially explained by a decreased availability of methionine, folate and cobalamin, and subsequent derangement of methionine metabolism during early human pregnancy resulting in decreased synthesis of DNA and thus disordered cell proliferation. The prevention of neural tube defects by periconceptional folate supplementation might partly be explained by the correction of disturbed methionine metabolism. This concept is supported by the results of the present study.

Although the supply, metabolism and synthesis of nutrients during early human pregnancy is poorly understood, the results of the present study suggest that folate and vitamin B₁₂ dependent methionine metabolism may be important for growth and development of the human embryo.

Acknowledgements

The authors acknowledge the laboratory supervision of Dr H. Blom and the expert technical assistance of Mrs A. De Graaf-Hess of the laboratory of neurology and paediatrics, University Hospital Nijmegen, The Netherlands.
References


Received 2 November 1995
Returned to Authors 1 February 1996
Resubmitted 1 April 1996
Accepted 9 July 1996