C-Type Natriuretic Peptide Forms in the Ovine Fetal and Maternal Circulations: Evidence for Independent Regulation and Reciprocal Response to Undernutrition

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C-type natriuretic peptide (CNP) has a crucial role in postnatal endochondral bone growth and is rapidly responsive to changes in nutrition. Although CNP is expressed in the placenta, little is known about the regulation and role of CNP in fetal-maternal health. We hypothesized that CNP may be similarly responsive to undernutrition in the growing fetus, in which maternal nutrition is crucial to normal growth and development. We therefore studied maternal and fetal CNP and theaminoterminal (bioinactive) fragment of proCNP (NTproCNP) in 39 chronically catheterized pregnant sheep before and after a 3-d maternal fast from 121 d gestation. Maternal CNP and NTproCNP levels were higher than in the fetus (CNP 12-fold, NTproCNP 1.5-fold, both P < 0.001). The ratio of NT-proCNP to CNP was higher in the fetus than the mother (53 ± 3 vs. 8.7 ± 0.6, P < 0.001), suggesting enhanced synthesis and/or degradation of CNP in the fetus. As in postnatal lambs, fetal plasma CNP forms fell promptly during maternal fasting. In contrast, maternal levels exhibited reciprocal and contemporaneous increase, which was reversed by refeeding. Utero-placental production of CNP was suggested by a high venoarterial concentration gradient across the gravid uterus, and a correlation between maternal NTproCNP levels and placental weight (r² = 0.26, P = 0.01). These studies provide the first evidence that CNP is regulated independently in the fetus. Reciprocal increases in maternal CNP forms may reflect the response of the uteroplacental unit to substrate deficiency. CNP may have a role in maintaining fetal welfare and provides a possible marker of uteroplacental nutrient supply. (Endocrinology 148: 4015–4022, 2007)

Unlike the circulating cardiac hormones, atrial natriuretic peptide and brain natriuretic peptide, C-type natriuretic peptide (CNP) is synthesized within a broad range of tissues including the vascular endothelium, brain, and reproductive and skeletal tissues (1–3), in which the hormone serves to regulate cell proliferation and hypertrophy (3, 4). In keeping with its putative paracrine action, transorgan CNP gradients (in contrast to atrial natriuretic peptide and brain natriuretic peptide) are low (5) and the circulating concentrations of the bioactive forms, chiefly CNP-22, are barely detectable in health (6). Presumably the low circulating levels of CNP result from rapid catabolism at sites of synthesis and/or rapid elimination from plasma. However, the recent discovery and measurement of the aminoterminal fragment of proCNP (NTproCNP) (7), which circulates at levels 10–50-fold higher than those of CNP, have opened up new approaches to the study of CNP synthesis and regulation in vivo. Plasma NTproCNP has been found to be strongly correlated with skeletal growth and markers of bone formation in children and lambs, consistent with the activity of the CNP signaling pathway in growth plate tissues (8) and the crucial role of the hormone in endochondral bone growth (9, 10).

In recent studies in rapidly growing lambs, we have shown that reduced nutrition, long known to be important in skeletal growth, strongly impacts on CNP synthesis and is similar to the effects of glucocorticoid administration (11). Acute changes in energy balance induced by caloric restriction (25% of normal food intake) in 4-wk-old lambs rapidly and reversibly reduces plasma NTproCNP and CNP. From these findings we hypothesized that CNP synthesis may be similarly responsive to undernutrition in the growing fetus in which maternal nutrition is crucial to normal growth and development (12).

Little is known of the part played by CNP in fetal-maternal health. However, in vitro studies show that CNP is strongly expressed in placenta (13) and uterine tissues (14). In the mouse embryo, CNP expression is also evident in brain and spinal cord (13) and is reported to increase in embryo truncal tissues during the course of gestation (15). In the human, the extremely high levels of NTproCNP in fetal plasma (16) and the evidence of a small fetal-maternal CNP gradient (16, 17) suggest that the fetal and maternal production of CNP may be independent. Whether CNP synthesis in pregnancy is subject to acute regulation and separately regulated in the fetus remains unknown.

Here we report on the CNP forms in the ovine maternal and fetal circulation and show that the uteroplacental tissues are a major contributor to maternal levels. Furthermore, re-
circacl changes in fetal and maternal circulating CNP levels in response to acute maternal undernutrition provide the first evidence that CNP synthesis is regulated independently in the mother and fetus in late gestation.

**Materials and Methods**

**Animal studies**

Five-year-old Romney ewes were acclimatized for a week to indoor conditions and feeding on a concentrate diet. A fortnight before mating, the estrous cycles of ewes were synchronized with intravaginal devices containing progesterone (18). Ewes were scanned 42–56 d after mating, and dry ewes and those with triplets were excluded. Pregnant ewes were delivered to the laboratory on d 105 gestation (term = 145 d), and instrumented on d 112. Under general anesthesia, polyvinyl catheters were inserted into the tarsal vein and artery of each fetal hind limb and in six singleton bearing ewes into the uteroovarian vein draining the pregnant uterine horn. All catheters were then exteriorized through the maternal flank. Catheters for blood sampling were also placed in a maternal femoral artery and vein. Antibiotics were given to ewes before surgery (5 ml Streptopen; Pitman-Moore, Wellington, New Zealand) and the amniotic fluid before closure of the membranes and uterus (80 mg gentamicin; Roussel, Auckland, New Zealand). All vascular catheters were flushed with heparinized saline at least every second day until experiments commenced.

Ewes were fasted for 3 d, beginning after the morning blood sample on d 121 gestation, and refed after sampling on d 124. Paired arterial blood samples were drawn for measurement of CNP forms, glucose, urea, insulin, and IGF-I from both mother and fetus between (0800 and 0900 h before (d 114, 117, and 121), during (d 122–124), and after fasting (d 125, 126, 128, and 131). To examine possible uteroventral contributions to maternal circulating levels, paired blood samples were also drawn for measurement of CNP forms from the maternal arterial and uteroovarian venous catheters before (d 114 and 121), at the end of (d 124), and after fasting (d 125, 126, and 131). The ewes were killed with an overdose of pentobarbitone on d 132 and placental and fetal weights measured.

Experiments were approved by the Animal Ethics Committee of The University of Auckland.

**Plasma assays**

Blood samples were drawn into chilled tubes containing EDTA (CNP assay, 7.5 mg/ml EDTA, Vacutainer; Becton and Dickinson, Lincoln Park, NJ) or lithium heparin (NTproCNP assay and other analytes), centrifuged at 4°C, and plasma stored at −80°C until analysis. Plasma glucose and urea concentrations were measured on a Hitachi 902 autoanalyzer (Hitachi Australia, North Ryde, New South Wales, Australia) by enzymatic colorimetric assay (Roche, Basel, Switzerland) and kinetic UV assay (Roche), respectively. Plasma hormone concentrations were measured by specific RIA established and validated for maternal and fetal sheep plasma. Plasma insulin was measured according to previously published methods (19) except that ovine insulin was used as the standard (Sigma Chemical, St. Louis, MO; batch I9254). The standard curve displaced in parallel with ovine plasma samples and cross-reactivity with IGF-I or IGF-II was 0.01%. The minimal detectable concentration was 40 pg/ml plasma and the inter- and intraassay coefficients were 11 and 11%, respectively. Plasma IGF-I was measured using an IGF binding protein-blocked RIA (20, 21). The detection limit was 0.7 ng/ml and the inter- and intraassay coefficients of variation were 20 and 10%, respectively.

NTproCNP was assayed as previously described (7, 16) using the primary rabbit antiserum (J39) raised against NTproCNP (1–15) (100 µl, 1:6000 diluted antiserum per assay tube). Peptide standards were made from synthetic human proCNP (1–19) taking into account the purity data supplied (Chiron Technologies Pty. Ltd., Melbourne, Australia). Within- and between-assay coefficients of variation were 4.9 and 6.4%, respectively, at 22 pmol/liter.

Plasma CNP was assayed as previously described (22) using a commercial antiserum (Phoenix Pharmaceuticals, Inc., Belmont, CA; catalog no. RAB-014-03). The rabbit antiserum raised against proCNP(82–103) shows 100% cross-reactivity with CNP-22 and hCNP-53 (Phoenix Pharmaceutilicals data sheet). Within- and between-assay coefficients of variation were 3.6 and 8.3%, respectively, at 7.5 pmol/liter.

**HPLC analysis of fetal and maternal immunoreactive CNP**

Representative fetal or maternal plasma (20 ml) was separately extracted using Sep-Pak C18 cartridges (Waters Corp., Milford, MA) as described previously (23). Extracts were resuspended in either 0.1% trifluoroacetic acid or 20% (vol/vol) acetonitrile in 0.1% trifluoroacetic acid before reverse-phase (C18) or size exclusion (G2000, Toyo Soda, Tokyo, Japan) HPLC, respectively.

**Statistical methods**

Data are presented as mean ± sem. Student’s t test was used to analyze differences in analyte levels between mother and fetus. Repeated-measures ANOVA with Bonferroni post hoc analysis was used to assess changes in levels over time, with group (single vs. twin) included as a covariate where appropriate. Relationships between variables were explored using simple linear regression. Statistical significance was assumed when P < 0.05.

**Results**

The experimental flock comprised 39 ewes, 21 with singleton and 18 with twin pregnancies. Not all samples were available from all animals due to a combination of catheter failure and some fetal losses, but complete data to the end of the experiment were obtained from 13 singleton and 10 twin-bearing ewes.

Mean fetal weight at d 132 was 4514 ± 125 g for singletons and 3687 ± 100 g for twins (P < 0.01). Mean total placental weight was 582 ± 29 g for singleton-bearing and 808 ± 32 g for twin-bearing ewes (P < 0.01).

**Fetal and maternal plasma CNP and NTproCNP**

Maternal plasma concentrations of NTproCNP (273 ± 10 pmol/liter, n = 39, d 114) and CNP (40 ± 4 pmol/liter, n = 39) were higher than fetal levels (NTproCNP, 221 ± 7 pmol/liter, n = 56; CNP, 3.8 ± 0.2 pmol/liter, n = 43) before fasting (both P < 0.001), with the mean maternal to fetal ratio 1.3 ± 0.1 for NTproCNP and 11.8 ± 1.0 for CNP. In fetal plasma, the ratio of NTproCNP to CNP was also much higher than in maternal plasma (57 ± 3 ± 7.8; 8.8 ± 0.6, P < 0.001), consistent with enhanced degradation of CNP in fetal tissues or plasma. Over the course of the 18-d study, fetal levels of NTproCNP declined significantly (F = 8.0, P < 0.001, Fig. 1).

**Response to fasting**

Within 24 h of food withdrawal, there was a rapid fall of fetal plasma NTproCNP levels (207 ± 5 to 179 ± 5 pmol/liter, P < 0.001). Levels remained low for the duration of the fasting period and then increased within 24 h of refeeding (Fig. 1A). Similar proportional and directional changes (P < 0.001) were observed in fetal plasma CNP levels (Fig. 1B).

Coincident with these changes in the fetus were reciprocal changes in maternal levels. Maternal plasma NTproCNP levels increased from 253 ± 10 to 282 ± 11 pmol/liter within 24 h of food withdrawal (P < 0.05), rising to a peak of 295 ± 14 pmol/liter (P < 0.001) at the end of the fast (d 124). Levels then fell progressively after refeeding to attain basal levels by d 128. Changes in maternal CNP levels were similar in di-
TABLE 1. Fetal and maternal plasma glucose, urea, insulin, and IGF-I levels before, during, and after maternal fasting

<table>
<thead>
<tr>
<th></th>
<th>Day 114</th>
<th>Day 121*</th>
<th>Day 124*</th>
<th>Day 131</th>
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<tbody>
<tr>
<td>Glucose (mmol/liter)</td>
<td></td>
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<tr>
<td>Maternal</td>
<td>3.34 ± 0.08</td>
<td>3.61 ± 0.05</td>
<td>1.76 ± 0.07</td>
<td>3.49 ± 0.07</td>
</tr>
<tr>
<td>Fetal</td>
<td>0.86 ± 0.03</td>
<td>0.96 ± 0.02</td>
<td>0.49 ± 0.02*</td>
<td>0.90 ± 0.03</td>
</tr>
<tr>
<td>Urea (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal</td>
<td>5.00 ± 0.16</td>
<td>4.48 ± 0.19</td>
<td>7.69 ± 0.32</td>
<td>4.39 ± 0.29</td>
</tr>
<tr>
<td>Fetal</td>
<td>5.73 ± 0.14</td>
<td>5.24 ± 0.16</td>
<td>9.04 ± 0.26*</td>
<td>4.87 ± 0.23</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td></td>
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<tr>
<td>Maternal</td>
<td>0.19 ± 0.03</td>
<td>0.30 ± 0.04</td>
<td>0.03 ± 0.00*</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Fetal</td>
<td>0.13 ± 0.01</td>
<td>0.20 ± 0.02</td>
<td>0.07 ± 0.01*</td>
<td>0.15 ± 0.01</td>
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<tr>
<td>IGF-I (ng/ml)</td>
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<tr>
<td>Maternal</td>
<td>49.8 ± 4.5</td>
<td>67.7 ± 6.3</td>
<td>57.2 ± 4.6</td>
<td>82.8 ± 8.0</td>
</tr>
<tr>
<td>Fetal</td>
<td>62.4 ± 2.8</td>
<td>73.8 ± 3.6</td>
<td>52.8 ± 5.2*</td>
<td>76.2 ± 5.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 50 fetuses and 35 ewes.

*a Beginning of fasting period.

*b End of fasting period.

*c P < 0.01 for changes over time.

*d P < 0.05 for changes over time.

*P < 0.01 for post hoc comparison of concentrations at d 121 and 124.

**P < 0.05 for post hoc comparison of concentrations at d 121 and 124.

Fig. 1. Circulating maternal (closed circles, n = 39) and fetal (open circles, n = 57) concentrations of NTproCNP (A) and CNP (B) during gestation. Ewes were fasted during d 121–124. Results are expressed as means ± SEM. Significant differences from baseline values (d 121) are indicated by asterisks. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Maternal and fetal plasma levels of glucose, insulin and IGF-I all decreased with fasting, whereas urea levels increased (Table 1). All levels returned toward baseline after refeeding.

Effect of twinning

Maternal plasma levels of both NTproCNP and CNP were higher in twin than singleton pregnancies (F = 11.7, P = 0.001 and F = 18.4, P < 0.001, respectively, Table 2). The reverse was true in the fetus, in which mean NTproCNP and CNP levels were lower in twins than singletons, although this difference did not reach statistical significance for NTproCNP (F = 2.6, P = 0.1 and F = 5.1, P = 0.027, NTproCNP and CNP, respectively). Maternal and fetal plasma levels of glucose and insulin were higher in singleton than twin pregnancies throughout the experimental period (Table 2).

Identity and source of CNP forms in maternal plasma

When subjected to size exclusion HPLC/RIA, a major peak of immunoreactive proCNP (1-15) eluted in a position consistent with the 5-kDa aminoterminal fragment (proCNP 1–50) in both maternal and fetal plasma extracts. Smaller (unidentified) later eluting peaks were also present (Fig. 2). Two distinct immunoreactive CNP peaks, consistent with CNP-53 and CNP-22 were identified in maternal plasma extracts (Fig. 3). These peaks were just detectable in fetal extracts, consistent with their much lower levels as measured in fetal plasma. No proCNP (1–103) was detected in maternal or fetal plasma extracts.

Mean plasma levels of CNP in the uteroovarian vein were at least 3- to 4-fold higher than the corresponding maternal arterial levels (F = 63, P < 0.001, Fig. 4). During the 18-d study period, plasma levels of CNP in the uteroovarian vein declined significantly (F = 4.0, P < 0.003).

Mean plasma levels of NTproCNP were also slightly higher in the uteroovarian vein than in the maternal artery, but the differences were not statistically significant, presumably reflecting the longer half-life of the amino terminal (bioinactive) fragment in the circulation.

There were significant correlations between placental weight and maternal NTproCNP levels on d 131 (r² = 0.26,
TABLE 2. Fetal and maternal plasma CNP forms, glucose, insulin, and IGF-I levels in singleton and twin pregnancies

<table>
<thead>
<tr>
<th></th>
<th>Single</th>
<th>Twin</th>
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<tr>
<td></td>
<td>Day 114</td>
<td>Day 131</td>
</tr>
<tr>
<td>CNP (pmol/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal</td>
<td>26.4 ± 2.3</td>
<td>25.2 ± 3.5</td>
</tr>
<tr>
<td>Fetal</td>
<td>4.22 ± 0.39</td>
<td>4.22 ± 0.48</td>
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<tr>
<td>NTproCNP (pmol/liter)</td>
<td></td>
<td></td>
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<tr>
<td>Maternal</td>
<td>245 ± 10</td>
<td>238 ± 14</td>
</tr>
<tr>
<td>Fetal</td>
<td>233 ± 16</td>
<td>178 ± 10</td>
</tr>
<tr>
<td>Glucose (mmol/liter)</td>
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</tr>
<tr>
<td>Maternal</td>
<td>3.58 ± 0.05</td>
<td>3.64 ± 0.06</td>
</tr>
<tr>
<td>Fetal</td>
<td>1.02 ± 0.03</td>
<td>1.02 ± 0.05</td>
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<tr>
<td>Insulin (ng/ml)</td>
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<tr>
<td>Maternal</td>
<td>0.26 ± 0.03</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>Fetal</td>
<td>0.18 ± 0.02</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal</td>
<td>47.8 ± 6.0</td>
<td>75.8 ± 10.3</td>
</tr>
<tr>
<td>Fetal</td>
<td>68.8 ± 4.4</td>
<td>82.7 ± 8.2</td>
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</table>

Values are mean ± SEM for 20 singleton fetuses and their mothers and 30 twin fetuses and their 15 mothers.

a P < 0.001 for singles vs. twins.
b P < 0.01 for singles vs. twins.
c P < 0.05 for singles vs. twins.
d P < 0.05 for group by time interaction.

P = 0.01 (Fig. 5) and between placental weight and maternal CNP (r² = 0.18, P < 0.05). Fetal CNP and NTproCNP levels were not related to fetal or placental weight.

Discussion

These studies in healthy chronically cannulated pregnant ewes reveal a number of new findings pertaining to the circulating forms and regulation of CNP in ovine pregnancy. First, significantly higher concentrations of both CNP (11.8-fold) and NTproCNP (1.3-fold) in the maternal plasma than in fetal plasma constitute strong evidence that synthesis and/or metabolism in mother and fetus are independent. Second, the striking and reciprocal responses in CNP levels in fetal plasma, consistent with high rates of CNP degradation in the maternal transfer of bioactive forms to the fetus is minimal, consistent with the very limited transplacental passage of other peptide hormones in ruminants (24). However, the plasma NTproCNP levels were high in the fetus (200–230 pmol/liter) and similar to those observed in the human fetus at term (16). Furthermore, the ratio of NTproCNP to CNP was some 6-fold higher in the fetal than maternal circulation. Assuming that the clearance rate of NTproCNP does not differ in the two circulations, these findings suggest that both synthesis and degradation of CNP are enhanced in the ovine fetus. A similar (5-fold) difference in the ratio of NTproCNP to CNP has been reported in human mothers and fetuses (16) and might suggest that limiting circulating bioactive CNP concentration in the fetus may carry advantages, i.e. in preventing skeletal tissue overgrowth before birth in mammals. Clearly further work is required to localize sources of CNP synthesis and degradation in the fetus. For example, comparative studies of CNP degradation rates in maternal and fetal circulations and the contribution of the clearance receptor (natriuretic peptide receptor-C, NPR-C) and other degradative enzymes need to be assessed. Hydrolysis by nephrilysin (EC 3.4.24.11, neutral endopeptidase), the main CNP-catabolizing enzyme (25) warrants particular attention in future studies. The 2-fold higher concentration of nephrilysin activity in human fetal than maternal plasma (26), widespread peripheral expression of nephrilysin in rodent embryo (including skeletal) tissues (27), and raised nephrilysin immunoreactivity in bone tissues of the newborn when compared with the adult (28) could be relevant to our findings on fetal CNP, which is an excellent substrate for nephrilysin (29).

Additional evidence that fetal and maternal CNP are reg-
Changes in plasma glucose, insulin, and IGF-I levels were similar in direction and time course to the changes in fetal plasma CNP levels during fasting and refeeding. However, insulin suppresses CNP expression in vitro (30), and large increases in plasma IGF-I do not affect CNP levels in growing lambs (11), making it unlikely that these hormones have any direct role in the fetal CNP response to maternal fasting.

In contrast to the fall in fetal plasma levels of NTproCNP and CNP during maternal fasting, maternal levels rose, with a time course and magnitude of peak response that reciprocated those observed in the fetus. However, the fall in maternal plasma glucose, insulin, and IGF-I levels were broadly similar to those seen in the fetus. These unexpected findings, although confirming the independence of CNP synthesis within fetal and maternal circulations, strongly suggest separate regulatory systems, and presumably separate functions, for CNP in mother and fetus.

The mechanisms underlying these differential responses were not explored in this study, but the close temporal relationships raise the possibility that the maternal rise could be in response to the abrupt fall in fetal CNP synthesis or action. Previous findings of raised plasma CNP forms in the presence of loss-of-function mutations of the CNP receptor

![Fig. 2. Immunoreactive (ir) NTproCNP size exclusion HPLC profiles of a maternal (A) and fetal (B) plasma extract. Column void volume ($V_v$) and elution positions of molecular markers are shown by arrows.](image)

![Fig. 3. Immunoreactive (ir) CNP size exclusion HPLC profiles of (A) a maternal and fetal (B) plasma extract. Column void volume ($V_v$) and elution positions of molecular markers are shown by arrows.](image)
(natriuretic peptide receptor-B, NPR-B) suggest the possibility of feedback inhibition in this system (31). However, the fact that fetal plasma NTproCNP levels fell over the time course of our study without any apparent effect on maternal levels makes this explanation unlikely. Reduced renal clearance of the hormone is another possible explanation. Plasma NTproCNP but not CNP levels increase as renal function declines in adult humans and dogs (Prickett, T. C. R., unpublished observations). However, there is no evidence that fasting for 3 d would induce acute but reversible renal impairment in the ewes in our study. The small transient rise in plasma urea concentrations observed with fasting is consistent with the expected increase in protein oxidation over this period and was not of sufficient magnitude to suggest impaired renal function. Moreover, the proportionate increase in both maternal CNP and NTproCNP concentration makes it unlikely that changes in renal function underlie the maternal response.

Another intriguing possibility is that an increase in maternal CNP synthesis represents a homeostatic (compensatory) response of the uteroplacental unit to nutrient deprivation. Both the nitric oxide (NO) and CNP signaling pathways within the uterine vascular wall play an important part in maintaining the greatly increased uterine blood flow during late gestation (32). Fasting may lead to a fall in maternal plasma arginine and citrulline concentrations (Harding, J. E., unpublished observations), leading to reduced NO production (33) and a compensatory increase in CNP synthesis (34) and activity (35). Furthermore, vascular endothelial growth factor (VEGF) is important in maintaining NO production (36, 37), and placental VEGF expression is reduced during short-term (5 d) fasting in sheep in midgestation (12). Because VEGF inhibits CNP synthesis at concentrations within the physiological range (38), fasting-induced reduction in VEGF production (12) or activity (39–41) in placental tissues would also lead to an increase in maternal CNP concentrations. This is consistent with our previous report that in women with preeclampsia, in which nutrient supply to the fetus is also threatened, plasma NTproCNP levels were increased 35% in maternal plasma and reduced 17.5% in the fetus when compared with values observed in healthy normotensives at term (16). Further studies are required to determine the role of CNP in utero placental physiology and evaluate its potential as a possible marker of nutrient supply to the fetoplacental unit.

Our finding of consistently higher CNP levels in plasma from the uteroovarian vein than from the simultaneously sampled maternal artery suggests that the uteroplacental tissues make a major contribution to systemic plasma levels of CNP in pregnant sheep. Plasma NTproCNP levels were also higher in the uteroovarian vein than the maternal artery, but the difference was smaller and not statistically significant, in keeping with the much longer half-life of this bioinactive form when compared with CNP. Our study was not designed to determine which tissues contributed to the higher levels in the uteroovarian vein. These may include placental tissues (13), myometrium (42), and the wall of the uterine arteries themselves (32). However, our findings that maternal plasma levels of both CNP and NTproCNP were higher in ewes carrying twins than in those carrying single-

![Graph A](image1.png)

**Fig. 4.** Plasma concentrations of CNP (A) and NTproCNP (B) drawn simultaneously from the maternal artery (closed circles) and uteroovarian vein (open circles) from six pregnant ewes with singleton pregnancies. (Note that the lower arterial CNP concentrations in this figure, compared with those of ewes in Fig. 1, reflect the lower CNP levels in singleton pregnancies.) Results are expressed as means ± SEM. Significant differences between arterial and venous values are indicated by asterisks. *P < 0.05; **P < 0.01; ***P < 0.001.

![Graph B](image2.png)

**Fig. 5.** Relationship between placental weight and maternal NTproCNP levels on d 131. Singletons (closed squares) and twins (open squares). $r^2 = 0.26; P = 0.01$. 

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**TABLE 1**

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<tr>
<th>Placental weight (g)</th>
<th>NTproCNP (pmol/L)</th>
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<tbody>
<tr>
<td>400</td>
<td>100</td>
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<tr>
<td>600</td>
<td>200</td>
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<td>800</td>
<td>300</td>
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<tr>
<td>1000</td>
<td>400</td>
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<td>1200</td>
<td>500</td>
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tons and the correlation between maternal NTproCNP levels and placental weight both suggest that at least some is likely to be of placental origin. This study shows that circulating CNP levels in pregnant ewes are more than 10-fold higher than those of healthy pregnant women (mean 1.8 ± 0.2 pmol/liter) at term. Anatomical differences in placentation (modified epitheliochorial in sheep, hemochorial in humans) may perhaps contribute to this difference, although the additional intervening tissue layers would reduce the access of any CNP produced by the fetal cotyledonal tissues to maternal circulation in the sheep, potentially resulting in lower rather than higher maternal plasma levels in this species. Another possible explanation is the marked difference in maternal plasma VEGF concentration (an inhibitor of CNP synthesis), which increases progressively during pregnancy in the human but not ovine pregnancy (43). However, mediated, sustained plasma CNP concentrations ranging from 20 to 60 pmol/liter may have important hemodynamic effects (44) over and above the paracrine vasodilator actions within the uterine vasculature and now need to be reevaluated.

Whether circulating levels of CNP forms in the fetus reflect skeletal growth rates, as shown in postnatal lambs and children (8), has not yet been studied. Transcripts for components of the CNP signaling pathway have been identified in fetal long bones of rodents, and the fetal mouse tibia is highly responsive to exogenous CNP ex vivo (3). However, genetic manipulations (9, 45, 46) and spontaneous mutations (10) may have important hemodynamic effects (44) over and above the paracrine vasodilator actions within the uterine vasculature and now need to be reevaluated.

In summary, this is the first report of circulating CNP levels in normal pregnant ewes and their fetuses in late gestation. We have shown that plasma NTproCNP and CNP levels are acutely and independently regulated by nutrient supply in both mother and fetus. Our data suggest that rates of CNP synthesis and degradation are high in the fetus, whereas the uteroplacental tissues are a major source of CNP in the mother. CNP may have a role in maintaining fetal welfare and provides a possible marker of uteroplacental nutrient supply.

Acknowledgments

We gratefully acknowledge the expert technical assistance of Eric Thorstensen and Jo Whittlow.

Received March 1, 2007. Accepted May 9, 2007.

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This work was supported by grants from the Health Research Council of New Zealand, the National Research Centre for Growth and Development, the Auckland Medical Research Foundation, and the Canterbury Medical Research Foundation.

Disclosure Statement: The authors have nothing to disclose.

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