The mechanism behind the pronounced effect of estrogen on CRP is most likely an effect on gene expression in the liver. Strong support for this assumption that this effect is mediated by estrogen itself and not by a factor stimulated by estrogen comes from the observations that the effect is dose-dependent, is seen in both women and men, and occurs only after oral treatment. It is not clear whether the effect of estrogen on gene expression is mediated by transcriptional activation or RNA stabilization. No hormone response elements have been discovered in the promoter of the CRP gene, but one study has shown that estrogen can stimulate the transcription factor C/EBP-β, which is involved in CRP transcription (9).

Our data contradict findings in mice transgenic for human CRP, in which testosterone but not estrogen was essential for expression of both unstimulated and acute-phase CRP (10). This discrepancy could be explained by the fact that the transgenic mice had the human CRP gene integrated into another chromosomal context. Because of study design, we cannot exclude that testosterone has an effect on expression of the CRP gene.

The findings of the present study might be of major relevance because CRP is associated with an increased risk of CHD, and hormone replacement therapy has been shown to increase the risk of CHD (11). In addition, in the present study, patients who underwent estrogen therapy had more cardiovascular events (6).

One limitation of the present study is that participants suffered from prostate cancer, which could possibly, through some unknown mechanism, trigger CRP expression independently of IL-6. However, the randomized design of the study, which produced treatment groups that were balanced regarding severity of disease, should minimize this risk. In this context, it cannot be excluded that estrogen might have pro-inflammatory effects on the prostate itself. However, the correction for IL-6 stimulation of CRP is likely to exclude this reason for the difference in circulating CRP concentrations between the two groups. Another limitation is that the analyses performed on blood samples that had been frozen for more than 20 years. Such long-term storage might lead to concentration of samples as a result of freeze-drying. Speaking against such an effect is the fact that serum CRP concentrations were within the reference interval. Furthermore, freeze-drying effects would most likely affect both treatment groups similarly.

In conclusion, we have shown that estrogen treatment in middle-aged and elderly men is associated with increased circulating CRP concentrations, indicating a role for estrogen in the regulation of unstimulated CRP. The results clearly emphasize the need for further molecular studies of hormonal effects on the regulation of CRP expression.

References

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N-Terminal Pro-B-Type Natriuretic Peptide Concentrations Are Markedly Higher in the Umbilical Cord Blood of Newborns than in Their Mothers, Angelika Hammerer-Lercher,1* Johannes Mair,2 Geront Tesw,3 Bernd Puschendorf,1 and Rudolf Sommer1

Natriuretic peptides are well-established markers in adult heart failure patients (1) and may also be useful for identifying neonates or children with cardiac diseases. Recent studies demonstrated high N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations in healthy neonates with a subsequent rapid decrease within several days (2, 3). However, in the transition from fetal to neonatal life, the physiologic role of natriuretic peptides is not fully understood. Furthermore, it is currently not known whether natriuretic peptides in the fetal circulation derive from the fetus itself or whether there is a placental exchange of maternal natriuretic peptides. The aim of this study, therefore, was to determine the NT-proBNP concentrations in healthy neonates and to compare their concentrations with the values for their respective mothers to indirectly demonstrate a possible placental NT-proBNP exchange.

From 100 neonates delivered consecutively between
November 2003 and February 2004 (Gynaecologic and Paediatric Hospital Linz), we compared the NT-proBNP concentrations of all healthy vaginally delivered term-born neonates (n = 42) with the NT-proBNP concentrations for their respective healthy mothers. The population characteristics are shown in Table 1. None of the neonates suffered from asphyxia or respiratory distress syndrome. The study is consistent with the Declaration of Helsinki. Venous umbilical cord blood and peripheral venous blood from the mothers had been drawn for routine blood analyses such as blood grouping. NT-proBNP concentrations in the sample remnants were measured by a sandwich electrochemiluminescence immunoassay (Elecsys 1010; Roche) as described previously (4). The Wilcoxon signed-rank test was used to assess differences in fetal and maternal NT-proBNP concentrations, the Mann–Whitney test was used for gender comparison, and Spearman rank correlation coefficients were calculated. A P value <0.05 was considered statistically significant.

Median (25th–75th percentiles) NT-proBNP concentrations [553.4 (413.5–832.9) ng/L] were on average 11.6-fold higher (6.9- to 32-fold) in the cord blood than in the blood samples from the respective mothers [45.5 (22.9–76.8) ng/L; P ≤0.0001; Fig. 1]. The NT-proBNP concentrations for the respective mothers and newborns did not correlate (r = −0.11; P = 0.49). There was also no significant difference (P = 0.76) in NT-proBNP concentrations between sexes in the neonates.

In this study, we, for the first time, compared NT-proBNP concentrations in a significant number of mothers and their newborn infants immediately after delivery, using a commercially available automated assay. To date, only one small study has compared fetal [mean (SE), 1052.0 (181.5) ng/L] and maternal NT-proBNP concentrations [569.3 (74.0) ng/L] by a RIA (5); the results indicated that NT-proBNP concentrations in healthy fetuses in the 21st week of gestation are twice as high as in maternal blood. The use of different nonstandardized immunoassays, which detect different epitopes and fragments of NT-proBNP, may partly explain the differences between our present study and the previous study. Different gestational ages and the stress of delivery may also influence the secretion patterns of NT-proBNP. Furthermore, the mothers in the study of Walther et al. (5) were not well characterized. In the present study, all mothers were healthy and consequently had low NT-proBNP concentrations.

Umbilical venous cord blood NT-proBNP concentrations of neonates are representative of NT-proBNP concentrations in newborns; Mir et al. (2) reported similar NT-proBNP concentrations in the umbilical venous cord blood and peripheral venous blood on the day of delivery. Arterial and venous umbilical cord blood was shown to have similar NT-proBNP concentrations as well (6). Therefore, the high concentrations of natriuretic peptides immediately after delivery may be explained in part by the perinatal circulatory changes from fetus to neonate. After delivery, three important circulatory pathways, the ductus venosus, the foramen ovale, and the ductus arteriosus, are closed, which leads to an increase in pulmonary blood flow in response to lung expansion. Consequently, right ventricular volume and pressure load are increased at birth as well, which may contribute to the peaking of natriuretic peptide concentrations immediately after delivery. Because there was no significant correlation between cord blood and maternal NT-proBNP concentrations, we suggest that there is no placental exchange of NT-proBNP and that increased concentrations in neonates derive from the neonates themselves.

Table 1. Population characteristics (n = 42).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median</th>
<th>25th–75th percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, years</td>
<td>29.4</td>
<td>(19.1–40.1)</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>122.5</td>
<td>(113.8–128.3)</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>39 (39–40)</td>
<td></td>
</tr>
<tr>
<td>Female neonates, %</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3590</td>
<td>(3233–3835)</td>
</tr>
<tr>
<td>Apgar score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 1 min</td>
<td>9 (9–10)</td>
<td></td>
</tr>
<tr>
<td>At 5 min</td>
<td>10 (10–10)</td>
<td></td>
</tr>
<tr>
<td>At 10 min</td>
<td>10 (10–10)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>7.29</td>
<td>(7.25–7.34)</td>
</tr>
<tr>
<td>Venous</td>
<td>7.37</td>
<td>(7.32–7.41)</td>
</tr>
</tbody>
</table>

![Fig. 1. NT-proBNP concentrations in newborns and their respective mothers.](image-url)

Box-plots represent the 25th and 75th percentiles (boxes) and minima and maxima (error bars) for each group. *: outliers and extreme values; N, number of cases.

The NT-proBNP tests were provided by Roche Diagnostics (Austria) free of charge. The company had no influence on study design, data analysis or interpretation, and the content of the manuscript.
Preanalytical Influences on DPC IMMULITE 2000 Intact PTH Assays of Plasma and Serum from Dialysis Patients, Daniel T. Holmes,1 Adeera Levin,2 Barry Forer,3 and Frances Rosenberg1

1 Department of Pathology and Laboratory Medicine and 2 Department of Medicine, Division of Nephrology, University of British Columbia, St. Paul’s Hospital, Vancouver, Canada; 3 Measurement, Evaluation, and Research Methodology Program, University of British Columbia, Vancouver, Canada; * address correspondence to this author at: Department of Pathology and Laboratory Medicine, St. Paul’s Hospital, 1081 Burrard St., Vancouver, BC, V6Z 1Y6, Canada; fax 604-806-8815, e-mail dtholmes@interchange.ubc.ca

Measurement of intact parathyroid hormone (PTH) is essential to the diagnosis and management of metabolic bone disease (1), hypercalcemia, hypocalcemia (2), and renal osteodystrophy (3). The effects of specimen type, collection temperature, and storage temperature on the in vitro stability of PTH differ by method and platform (4–10). Characterization of preanalytical effects unique to each method, platform, and patient population is important to prevent potential clinical misclassification.

The Diagnostics Product Corporation (DPC) IMMULITE 2000 intact PTH assay is a solid-phase two-site chemiluminescent immunoassay with a monoclonal mouse capture antibody and a polyclonal goat signal antibody conjugated to alkaline phosphatase. The effects of preanalytical factors on this assay have been partially investigated. Underfilling of EDTA-plasma tubes decreased measured PTH in samples from both healthy persons and those with chronic kidney disease (CKD) (11). Storage of SST serum samples for 3 days at room temperature decreased measured PTH in SST serum samples, an effect not seen with EDTA plasma (6). Results from EDTA plasma paradoxically increased after storage at 4 °C (12). Despite evidence that PTH is more stable in EDTA-anticoagulated specimens (5–7, 9, 10), the manufacturer describes an instability associated with EDTA-plasma samples and therefore recommends cold (2–8 °C) collection, centrifugation, and storage until analysis (13).

At our institution, PTH is most frequently measured for management of CKD and helps direct therapies, including calcium, vitamin D, bisphosphonates, and parathyroidectomy. Preanalytical influences on PTH assays may be atypical in CKD because samples contain increased concentrations of N-terminal truncated PTH fragments that cross-react with intact PTH assays (14). Accordingly, we have investigated preanalytical variables in a dialysis-dependent CKD population. The study was approved by the St. Paul’s Hospital ethics committee.

Five predialysis samples were obtained from 31 CKD patients between 0800 and 0930 h. Two specimens were drawn into Becton Dickinson (BD) 6-mL dipotassium EDTA Vacutainer™ plastic tubes: the first was immediately placed on ice and then centrifuged at 4 °C (EDTA_cold), whereas the second was collected and centrifuged at room temperature (EDTA_RT). The three remaining samples were drawn into 5-mL BD Vacutainer™ SST® plastic tubes containing gel and clot activator. The first (SST_cold) and second (SST_RT) serum specimens were collected in the same manner as their EDTA counterparts. A third specimen (SST_spuncold) was collected in accordance with the manufacturer’s indication that SST specimens can be collected at room temperature but require cold centrifugation and subsequent refrigeration (15). All tubes were filled completely. Each of the five tubes was separated into four aliquots. Aliquots from the EDTA_cold, SST_cold, and SST_spuncold tubes were maintained at 4 °C until analysis, whereas aliquots from the EDTA_RT and SST_RT tubes remained at room temperature. Each aliquot was analyzed at baseline (within 3 h) and after 24, 48, and 72 h, according to the manufacturer’s protocols (reagent lot 121).

Using SPSS (Ver. 13.0), we performed two separate 2 × 2 × 4 within-subject repeated-measures ANOVA analyses: the first was (EDTA/SST) × (room temperature/cold) × (time), and the second was (EDTA/SST) × (room temperature/spuncold) × (time). We explored the interactions between sample type and temperature and sample type and time and found them to be significant; we therefore considered the effects of temperature and time separately. Bonferroni corrections for multiple comparisons were applied.

The mean PTH concentrations for each sample type are displayed in Table 1 and Fig. 1. Results for specimens collected in EDTA were higher than those for specimens collected in SST tubes irrespective of temperature (P <0.001). Furthermore, mean PTH values were significantly higher in EDTA_RT specimens than EDTA_cold (P <0.001). Time had no significant effect on PTH measurements in EDTA_cold specimens (P = 0.172), whereas for