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Laboratory reference intervals during pregnancy, delivery and the early postpartum period

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Abstract

Background: Physiological changes during pregnancy may affect laboratory parameters. Reference values based on samples from non-pregnant women are not necessarily useful for clinical decisions during pregnancy. There is a need to establish reference values during pregnancy in order to recognize pathological conditions.
Methods: Eight hundred and one women with expected normal pregnancies were included in the study. Of these, 391 had no complications during pregnancy, delivery, or the early postpartum period. Blood samples were obtained at gestational weeks 13–20, 21–28, 29–34, 35–42, at labor, and 1 and 2 days postpartum. Reference intervals were calculated for 36 tests as recommended by the International Federation of Clinical Chemistry and Laboratory Medicine.
Results: Many tests showed such large variations indicating that gestational age-specific reference intervals were necessary. Other tests had different but stable values when compared to non-pregnant women. A minor decrease in albumin levels was observed. This was not only due to pregnancy-associated hemodilution, since other components with the same or a larger molecular diameter did not show a similar decrease. Many tests exhibited a broad distribution around vaginal delivery and in the early postpartum period.
Conclusions: Only a few parameters were unaffected during uncomplicated pregnancy, delivery, and the early postpartum period suggesting that implementation of gestational age-specific reference intervals is necessary.
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Keywords: clinical chemistry tests; female; human; pregnancy trimesters; reference value.

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Introduction

Physiological changes that occur during pregnancy may affect biochemical parameters. Most laboratory information systems (LIS) report reference values based on samples obtained from non-pregnant women, and these are not necessarily useful for clinical decisions during pregnancy. Some gestational age-specific reference intervals have been reported. However, the studies often used different analytical methods and many were based on a mixture of complicated and uncomplicated pregnancies (1–21). The reported differences in results are difficult to interpret, and most of these studies do not fulfill the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommendation of a minimum sample size of 120 individuals for calculation of reference values (22).

In the present study, we report gestational age-specific reference intervals for 36 analytical quantities in 391 women with uncomplicated pregnancies, vaginal deliveries, and the early postpartum period.

Materials and methods

Study participants and samples

Eight hundred and one healthy Caucasian women >18 years of age with a singleton pregnancy were recruited from among 2147 women attending first trimester screening between June 2006 and October 2007. All the women invited to participate in the study had normal nuchal translucency scans, as well as normal free β-human chorionic gonadotropin and pregnancy-associated plasma protein A. Women with any disease, medicine intake, or previous obstetric complications were excluded. Gestational age was estimated by crown rump length, as determined by an ultrasound scan performed at 11–13 weeks by three Fetal Medicine Foundation certified midwives. The study was approved by the Local Research Ethics Committee (No. KA 05065), and all women gave informed oral and written consent. Each woman was scheduled for collection of blood samples at gestational weeks 13–20, 21–28, 29–34, 35–42, during active labor, and at postpartum days 1 and 2. Blood pressure, urine dipstick results, and other clinical data were obtained from special pregnancy charts and from the medical record. Of the 801 women recruited, 26 were excluded (non-Caucasian, medicine intake, transferred to another hospital, miscarriage/still birth, and one death caused by pulmonary embolism). In addition, 55 women dropped out of the study and 78 women had pregnancy-related complications (group B streptococcal infection, verified urinary infection, vaginal bleeding, preeclampsia, hypertension, pruritus gravidarum, and intrauterine growth retardation). Sixty-seven women had an elective cesarean section and 180 had complicated deliveries (preterm delivery, premature rupture of membranes for more than 24 h, vacuum extraction, acute cesarean section, bleeding more than 500 mL, third

and fourth degree perineal laceration, placental retention/intrauterine palpation, and shoulder dystocia). Four women had postpartum complications (endomyometritis and urinary infection). Thus, 391 women had a complete uncomplicated pregnancy, vaginal delivery, and normal postpartum period.

Laboratory methods

Blood samples were drawn into Vacutainer tubes containing trisodium ethylenediaminetetraacetic acid (EDTA) for hematological analysis, and lithium heparin gel tubes for biochemical analysis (BD Medical Systems, Franklin Lakes, NJ, USA or Greiner Bio-One, Kremsmuenster, Austria). Samples were transported to the laboratory by the phlebotomists and processed according to routine laboratory methods. The samples were registered in the LIS and analyzed upon arrival with all other samples arriving at the laboratory. Samples with pre-analytical processing times longer than the stability of the analyte in blood (as indicated in Table 1) were excluded from the calculations. The analyzed parameters, with abbreviations, traceability, stability, and method characteristics, are listed in Table 1. Analysis was performed using the Advia 2120 Hematology System (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA); Immulite 2500 (Siemens Healthcare Diagnostics, Deerfield, IL, USA); Vitros 950 Chemistry System (Johnson & Johnson, Rochester, NY, USA); and Cobas Integra 400 plus (Roche, Basel, Switzerland). All tests were performed according to the manufacturers' specifications and laboratory standards, according to ISO-15189 certification. At the end of the study, all data were retrieved from the LIS. Laboratory results were not made available to the clinicians during the study.

Statistical methods

Reference ranges (2.5th and 97.5th percentiles) with 90% confidence interval (CI) were calculated for each test and gestational period. The non-parametric bootstrap method with 500 iterations (RefVal version 4.11 software) was used in accordance with IFCC recommendation (22). Outliers were removed using Dixon's algorithm. Box-plots were constructed using SPSS 15.2 for Windows (SPSS Inc., Chicago, IL, USA).

SAS version 9.1. was used for all other calculations (SAS Institute Inc., Cary, NC, USA). A mixed model with repeated measures test was performed separately on data during pregnancy and around delivery after examination of the distribution using histograms and probability graphs and the Kolmogorov-Smirnov statistic. For samples <50 observations, the Shapiro Wilks statistic was used to test for normality. The equality of variances was assessed using the Levene test. To obtain normal distributions with good approximation, it was necessary to transform 23 of the 36 quantities. Holm's test was used to correct for multiple testing. Statistical significance was defined as $p<0.05$.

Results

The women included in the study had a mean age of 32 years and a body mass index (BMI) of 22 kg/m^2. Forty-four percent were nulliparous. The mean gestational age at delivery was 283 days, and mean birth weight was 3601 g. Two newborns had Apgar scores of <7 after 5 min. For comparison, all women delivering at Gentofte Hospital during the same period had a mean age of 33 years, a BMI of 23 kg/m^2, and 43% were nulliparous.

Reference intervals are shown in Table 2, which also includes general reference intervals (23, 24). Only a few tests did not change significantly during pregnancy or around time of delivery (Table 3). However, many of the changes are clinically insignificant. Graphical illustrations of the distribution of some tests are indicated by box plots in Figure 1, with shaded general reference intervals (23, 24). Our suggested reference intervals are listed in Table 4.

Discussion

We report the 2.5th and 97.5th percentiles of gestational age-specific reference intervals for 36 laboratory blood tests, calculated in accordance with the recommendations of the IFCC for statistical treatment of reference values (22). We used an unprecedented number of women ($n=391$) without any complications during pregnancy, delivery, or in the early postpartum period. Some components of the blood samples can show minor differences in stability at shorter time periods than those indicated in Table 1. However, a pragmatic stability time was selected in order to provide equivalent processing time and storage conditions to those found in daily practice.

Results from nearly half of the 801 women were omitted before establishing the reference intervals. This major reduction of potentially healthy pregnant participants could be a concern. However, it was important to consider only women with totally uncomplicated pregnancies in order to be sure that the results would not be influenced by any pathological condition. In addition, we compared the mean values and distribution of results at each gestational period for the 391 women with the values obtained for the women who were excluded and found only minor sporadic differences between the two groups (data not shown). Thus, we could have used data from all 801 women and obtained essentially the same reference intervals as shown in Table 2. The present study population was similar to that of the excluded women, and to all women delivering at our hospital during the same time period with respect to age, parity, and BMI.

Only a few analytical quantities remained unchanged during pregnancy. Some changes were minor and remained within the non-pregnant reference intervals. Unfortunately, we had too few samples collected before gestational week 13, and thus we cannot describe changes seen early in pregnancy. Some parameters are reported to change very early as can be suspected from our first time period of weeks 13–20 for albumin (ALB) (Figure 1). Some of our observations were in agreement with those reported previously (1, 3, 6, 7, 10, 17, 19, 21). At delivery and during the early postpartum period, essentially all parameters changed, as reported by other investigators (6, 25). In the following discussion, we focus on instances in which our results differ from recently published reference values for pregnant women.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) did not differ from the non-pregnant reference

Table 1 Performed tests, abbreviations and analytical details.

Test (abbreviation)	CV	Method	Traceability	Stability
Alanine aminotransferase (ALT)	3.0	Vitros 165 5281	IFCC method	96
Albumin (ALB)	1.9	Vitros 819 6057 198 8211	NIST SRM 927c	144
Alkaline aminotransferase (ALP)	3.6	Vitros 105 3180	IFCC method	96
Aspartate aminotransferase (AST)	2.2	Vitros 843 3815	IFCC method	168
Bilirubin (BIL)	4.9	Vitros 838 3051 161 2365	Jendrassic-Grof – Doumas method	24
Cholesterol (CHOL)	4.6	Integra 03039773190	Calibrator f.a.s. (ID-MS)	144
Cholesterol high-density lipoprotein (HDL)	3.5	Integra 03038637322	C.f.a.s. Lipids CDC (dextran sulfate & Abell-Kendall)	144
Cholesterol low-density lipoprotein (LDL)	NA	Calculated	NA	144
Cholesterol very-low-density lipoprotein (VLDL)	NA	Calculated	NA	144
C-reactive protein (CRP)	15.6	Integra 20764930322	CRM 470	24
Creatinine (CREA)	1.1	Vitros 814 1947	NIST SRM 912a	24
Hematocrit (HCT)	2.6	ADVIA 2120	None	4
Hemoglobin (Hb)	1.4	ADVIA 2120	ICSH – NCCLS H-15A	72
Iron (IRON)	1.6	Vitros 151 5808 192 4547	NIST SRM 937	24
Iron binding capacity saturation (TIBC)	NA	Calculated	None	NA
Lactate dehydrogenase (LDH)	1.5	Vitros 838 4489	Pyruvate/lactate – Buhl method	8
Leukocyte count (WBC)	2.9	ADVIA 2120	Reference method T03-3685-52	48
Basophil count (BASO)	73.6[a]	ADVIA 2120	None	72
Eosinophil count (EOS)	14.6	ADVIA 2120	None	24
Lymphocyte count (LYMP)	4.2	ADVIA 2120	None	24
Monocyte count (MONO)	8.8	ADVIA 2120	None	10
Neutrophil count (NEUT)	2.0	ADVIA 2120	None	72
Mean corpuscular hemoglobin (MCH)	2.1	ADVIA 2120	None	48
Mean corpuscular hemoglobin concentration (MCHC)	2.1	ADVIA 2120	None	144
Mean erythrocyte corpuscular volume (MCV)	1.3	ADVIA 2120	NCCLS H-7A2	4
Platelet count (PLT)	4.6	ADVIA 2120	Class A hemocytometer and phase contrast microscopy	24
Potassium (K)	1.4	Vitros 815 7596	NIST SRM 918a	24
Red blood cell count (RBC)	1.7	ADVIA 2120	Reference method T03-3685-52	48
Sodium (Na)	0.7	Vitros 837 9034	NIST SRM 919a	24
Thyroid stimulating hormone (TSH)	6.7	Immulite L5KRT	WHO 2nd IRP 80/558	24
Thyroxine free (FT4)	14.9	Immulite L5KF4	Internal	24
Transferrin (TRAN)	3.7	Integra 03015050122	CRM 470	24
Triglyceride (TRIG)	4.8	Integra 20767107322	Calibrator f.a.s. (ID-MS)	144
Triiodothyronine total (T3)	15.6	Immulite L5KT3	Internal	24
Urea nitrogen (BUN)	6.5	Vitros 810 2204	NIST SRM 912a	24
Uric acid (URIC)	1.9	Vitros 194 3927	NIST SRM 913a	24

Total analytical imprecision for the method used to calculate the reference intervals given for each test as an average variation coefficient (CV%) of three levels of internal controls through 1 year ([a]only high level control). Instruments, reagents, and traceability are indicated. Samples with pre-analytical processing times longer than the stability in blood (in hours) were excluded from the calculations.

Table 2 Gestational age-specific reference intervals.

Test, unit	Normal value	13–20 weeks	21–28 weeks	29–34 weeks	35–42 weeks	Partus	Partus +1	Partus +2
ALT, μkat/L	0.17–0.75[a]	n = 539 [0] **0.13** **0.62** (0.12–0.15) (0.53–0.67)	n = 367 [0] **0.15** **0.54** (0.12–0.15) (0.50–0.73)	n = 173 [1] **0.14** **0.60** (0.12–0.16) (0.48–0.80)	n = 361 [1] **0.05** **0.63** (0.05–0.10) (0.53–0.72)	n = 314 [1] **0.05** **0.76** (0.05–0.08) (0.60–1.06)	n = 299 [2] **0.10** **0.70** (0.08–0.12) (0.67–0.89)	n = 185 [1] **0.13** **0.97** (0.11–0.16) (0.75–1.35)
ALB, g/L	36–48[a]	n = 539 [0] **32.4** **43.1** (31.6–33.0) (42.7–44.0)	n = 367 [0] **31.0** **40.5** (30.5–31.5) (40.1–41.1)	n = 173 [0] **30.2** **39.9** (29.3–30.8) (38.7–40.4)	n = 362 [0] **30.0** **39.8** (29.4–30.3) (38.9–40.5)	n = 317 [0] **29.3** **41.0** (28.3–29.9) (39.9–41.9)	n = 304 [0] **25.5** **37.6** (24.9–26.5) (36.9–37.8)	n = 189 [0] **26.7** **38.5** (25.5–27.7) (37.6–41.0)
ALP, μkat/L	0.58–1.75[a]	n = 539 [0] **0.66** **1.53** (0.62–0.68) (1.45–1.62)	n = 367 [0] **0.73** **1.99** (0.70–0.77) (1.85–2.23)	n = 173 [0] **0.94** **2.66** (0.89–0.97) (2.15–2.79)	n = 361 [0] **1.47** **4.49** (1.30–1.58) (4.22–5.09)	n = 314 [0] **1.82** **6.15** (1.80–1.95) (5.28–6.78)	n = 299 [0] **1.60** **4.68** (1.45–1.65) (4.33–4.94)	n = 185 [0] **1.48** **4.29** (1.33–1.61) (3.84–6.11)
AST, μkat/L	0.25–0.58[a]	n = 539 [0] **0.28** **0.67** (0.28–0.28) (0.58–0.73)	n = 367 [0] **0.27** **0.57** (0.25–0.28) (0.55–0.67)	n = 173 [0] **0.27** **0.67** (0.26–0.28) (0.56–1.19)	n = 362 [0] **0.27** **0.68** (0.27–0.28) (0.67–0.81)	n = 317 [2] **0.30** **0.87** (0.28–0.32) (0.80–0.93)	n = 304 [1] **0.37** **1.29** (0.35–0.40) (1.22–1.39)	n = 188 [1] **0.38** **1.22** (0.36–0.40) (1.11–1.42)
BIL, μmol/L	5–25[a]	n = 532 [0] **2** **15** (2–2) (12–16)	n = 367 [0] **2** **12** (2–2) (10–13)	n = 173 [0] **2** **12** (2–2) (10–14)	n = 358 [0] **2** **13** (2–2) (11–14)	n = 251 [0] **2** **12** (2–2) (10–15)	n = 260 [0] **2** **15** (2–2) (12–17)	n = 163 [0] **2** **13** (2–2) (10–14)
CHOL, mmol/L	2.9–6.1[a]	n = 539 [0] **3.7** **6.9** (3.5–3.8) (6.7–7.1)	n = 367 [0] **4.1** **7.8** (3.9–4.4) (7.6–8.2)	n = 173 [0] **4.5** **8.1** (4.3–4.7) (7.8–8.2)	n = 363 [0] **4.4** **8.8** (4.2–4.7) (8.5–8.8)	n = 316 [0] **4.5** **9.5** (4.2–4.8) (9.0–9.7)	n = 305 [0] **3.7** **8.2** (3.5–4.0) (8.0–8.4)	n = 189 [0] **4.2** **8.3** (3.8–4.4) (7.8–8.4)
HDL, mmol/L	1.0–2.7[a]	n = 539 [0] **1.4** **2.9** (1.3–1.4) (2.8–3.0)	n = 367 [0] **1.4** **3.0** (1.2–1.4) (2.9–3.3)	n = 173 [0] **1.4** **3.0** (1.4–1.4) (2.9–3.4)	n = 362 [0] **1.2** **2.9** (1.1–1.3) (2.8–3.1)	n = 318 [0] **1.2** **3.2** (1.2–1.2) (3.1–3.2)	n = 304 [0] **1.1** **3.0** (1.0–1.2) (2.9–3.3)	n = 189 [0] **1.0** **2.9** (0.9–1.2) (2.8–3.2)
LDL, mmol/L	1.2–4.3[a]	n = 539 [0] **1.2** **4.0** (1.1–1.3) (3.9–4.2)	n = 367 [0] **1.3** **4.7** (1.1–1.6) (4.5–5.1)	n = 173 [0] **1.4** **4.8** (1.4–1.7) (4.4–5.1)	n = 356 [0] **1.6** **5.6** (1.4–1.8) (5.1–5.9)	n = 311 [0] **1.6** **5.9** (1.4–1.8) (5.6–6.5)	n = 303 [0] **1.2** **5.0** (0.9–1.5) (4.7–5.4)	n = 187 [0] **1.4** **5.0** (1.2–1.7) (4.7–5.5)
VLDL, mmol/L	<0.5[b]	n = 539 [0] **0.3** **1.1** (0.3–0.4) (1.1–1.2)	n = 367 [0] **0.4** **1.4** (0.4–0.4) (1.3–1.6)	n = 173 [0] **0.4** **1.6** (0.4–0.5) (1.5–2.0)	n = 356 [0] **0.6** **2.0** (0.5–0.6) (1.9–2.1)	n = 311 [0] **0.6** **2.0** (0.5–0.6) (1.8–2.1)	n = 303 [0] **0.5** **1.8** (0.5–0.6) (1.7–1.9)	n = 187 [0] **0.5** **1.9** (0.5–0.6) (1.8–2.1)
CRP, nmol/L	<10[b]	n = 523 [0] **10** **237** (10–10) (200–295)	n = 362 [0] **10** **309** (10–10) (189–539)	n = 167 [1] **10** **224** (10–10) (152–324)	n = 352 [0] **10** **210** (10–10) (171–324)	n = 248 [0] **10** **424** (10–10) (268–636)	n = 260 [0] **38** **979** (29–48) (810–1085)	n = 163 [1] **48** **872** (38–57) (742–971)
CREA, μmol/L	50–90[a]	n = 532 [0] **45** **73** (43–45) (71–74)	n = 367 [0] **42** **72** (41–44) (69–75)	n = 173 [0] **42** **74** (41–44) (72–79)	n = 358 [0] **44** **84** (43–45) (76–87)	n = 252 [0] **44** **89** (43–45) (86–93)	n = 260 [0] **46** **92** (44–49) (86–100)	n = 163 [0] **46** **86** (45–50) (85–88)
HCT, 1	0.35–0.46[a]	n = 520 [0] **0.32** **0.41** (0.31–0.32) (0.41–0.42)	n = 358 [0] **0.31** **0.40** (0.31–0.31) (0.40–0.41)	n = 171 [0] **0.30** **0.40** (0.29–0.31) (0.39–0.41)	n = 352 [0] **0.31** **0.42** (0.30–0.32) (0.41–0.43)	n = 129 [0] **0.30** **0.45** (0.28–0.32) (0.42–0.46)	n = 217 [0] **0.29** **0.41** (0.27–0.30) (0.41–0.42)	n = 139 [0] **0.28** **0.42** (0.26–0.30) (0.41–0.43)
Hb, g/L	117–153[a]	n = 531 [0] **113** **147** (111–113) (145–148)	n = 366 [0] **111** **143** (108–113) (142–146)	n = 174 [0] **109** **145** (103–111) (137–149)	n = 361 [0] **110** **147** (106–114) (147–148)	n = 310 [0] **108** **156** (101–111) (153–163)	n = 298 [0] **97** **147** (95–103) (145–148)	n = 184 [0] **95** **149** (93–103) (145–156)
IRON, μmol/L	9–34[a]	n = 532 [0] **7** **33** (7–8) (32–35)	n = 367 [0] **7** **33** (6–8) (30–35)	n = 172 [0] **5** **39** (4–7) (30–62)	n = 358 [0] **7** **38** (6–7) (35–42)	n = 251 [0] **5** **30** (4–7) (28–31)	n = 260 [0] **4** **27** (4–6) (26–31)	n = 163 [0] **4** **21** (3–5) (18–23)
TIBC, %	10–50[a]	n = 532 [1] **9** **47** (8–10) (45–50)	n = 367 [0] **8** **43** (7–8) (39–48)	n = 173 [0] **5** **41** (4–7) (36–51)	n = 358 [2] **6** **36** (5–6) (34–42)	n = 251 [0] **5** **32** (4–6) (27–37)	n = 260 [0] **5** **32** (4–5) (29–36)	n = 163 [1] **5** **23** (3–5) (218–28)
LDH, μkat/L	1.8–3.4[a]	n = 531 [1] **1.9** **3.0** (1.8–1.9) (3.0–3.2)	n = 362 [1] **1.9** **3.0** (1.8–2.0) (2.9–3.1)	n = 170 [0] **1.9** **3.2** (1.8–1.9) (3.1–4.1)	n = 355 [0] **2.0** **3.5** (2.0–2.1) (3.3–3.8)	n = 166 [0] **2.1** **4.5** (2.1–2.3) (4.0–4.7)	n = 226 [0] **2.6** **5.4** (2.3–2.8) (5.28–6.1)	n = 145 [0] **2.5** **5.2** (2.5–2.8) (5.0–5.5)
WBC, $\times 10^9$/L	3.5–8.8[a]	n = 527 [0] **5.9** **13.8** (5.7–6.1) (12.9–14.1)	n = 366 [0] **6.5** **14.8** (6.1–6.8) (14.5–16.1)	n = 174 [0] **6.2** **14.9** (5.6–6.6) (14.0–18.0)	n = 361 [0] **6.3** **14.9** (6.0–6.7) (14.4–15.8)	n = 289 [0] **8.2** **25.8** (7.9–8.5) (24.9–27.3)	n = 289 [0] **7.5** **21.4** (6.9–8.3) (20.2–22.7)	n = 179 [0] **7.2** **18.6** (6.9–8.1) (17.5–19.4)

(Table 2 continued)

Test, unit	Normal value	13–20 weeks	21–28 weeks	29–34 weeks	35–42 weeks	Partus	Partus +1	Partus +2
BAS, $\times 10^9$/L	0.00–0.20[b]	n = 527 [0] **0.01** **0.07** (0.01–0.01) (0.07–0.08)	n = 362 [0] **0.01** **0.06** (0.01–0.01) (0.05–0.07)	n = 172 [0] **0.01** **0.07** (0.01–0.01) (0.06–0.08)	n = 356 [0] **0.01** **0.08** (0.01–0.01) (0.07–0.10)	n = 300 [0] **0.01** **0.14** (0.01–0.01) (0.13–0.15)	n = 286 [0] **0.01** **0.16** (0.01–0.02) (0.12–0.17)	n = 179 [0] **0.02** **0.15** (0.01–0.02) (0.12–0.16)
EOS, $\times 10^9$/L	0.00–0.50[b]	n = 522 [0] **0.03** **0.39** (0.03–0.04) (0.36–0.41)	n = 363 [0] **0.04** **0.40** (0.03–0.04) (0.35–0.44)	n = 174 [0] **0.03** **0.43** (0.03–0.04) (0.30–0.48)	n = 354 [0] **0.03** **0.41** (0.03–0.03) (0.30–0.46)	n = 245 [0] **0.01** **0.35** (0.01–0.01) (0.32–0.48)	n = 255 [0] **0.02** **0.44** (0.01–0.02) (0.31–0.50)	n = 161 [0] **0.04** **0.55** (0.04–0.08) (0.49–0.62)
LYMP, $\times 10^9$/L	0.70–4.80[b]	n = 522 [0] **1.08** **3.00** (1.02–1.12) (2.84–3.14)	n = 363 [0] **1.16** **3.06** (1.14–1.21) (2.89–3.26)	n = 174 [0] **1.11** **2.63** (0.79–1.16) (2.50–3.20)	n = 354 [0] **1.17** **3.17** (1.11–1.22) (3.00–3.30)	n = 246 [0] **0.76** **4.04** (0.71–0.87) (3.57–4.35)	n = 255 [0] **1.23** **4.05** (1.08–1.36) (3.76–4.26)	n = 161 [0] **1.09** **3.69** (0.55–1.32) (3.46–4.24)
MONO, $\times 10^9$/L	0.00–1.10[b]	n = 521 [0] **0.26** **0.72** (0.24–0.27) (0.69–0.77)	n = 358 [0] **0.27** **0.77** (0.25–0.30) (0.72–0.87)	n = 171 [0] **0.26** **0.94** (0.25–0.30) (0.78–1.02)	n = 353 [0] **0.30** **0.99** (0.27–0.32) (0.91–1.04)	n = 181 [0] **0.29** **1.17** (0.26–0.37) (1.08–1.35)	n = 226 [0] **0.30** **1.25** (0.25–0.36) (1.20–1.41)	n = 144 [0] **0.30** **0.97** (0.22–0.33) (0.86–1.07)
NEUT, $\times 10^9$/L	1.80–7.40[b]	n = 528 [0] **3.85** **10.48** (3.71–4.04) (10.02–11.05)	n = 363 [0] **4.41** **11.59** (4.22–4.57) (11.15–12.66)	n = 174 [0] **4.00** **11.62** (3.11–4.53) (10.95–15.91)	n = 357 [0] **4.30** **11.84** (3.91–4.45) (10.83–12.92)	n = 300 [0] **4.82** **23.42** (4.25–5.64) (22.17–25.36)	n = 286 [0] **5.29** **18.10** (4.51–5.91) (17.15–19.01)	n = 179 [0] **5.39** **14.73** (4.67–5.69) (13.85–16.81)
MCH, pg/cell	27.0–33.0[a]	n = 527 [0] **27.7** **33.8** (27.4–29.0) (33.8–33.8)	n = 366 [0] **29.0** **33.8** (29.0–29.0) (33.8–35.2)	n = 174 [0] **27.4** **33.8** (27.4–29.0) (33.8–34.8)	n = 361 [0] **27.4** **33.8** (25.9–29.0) (33.8–33.8)	n = 289 [0] **27.4** **33.8** (27.4–29.0) (33.8–33.8)	n = 289 [0] **27.8** **35.4** (25.8–29.0) (33.8–35.4)	n = 179 [0] **26.6** **34.6** (25.8–28.2) (33.8–35.4)
MCHC, g/L	317–357[a]	n = 531 [0] **337** **372** (335–339) (372–375)	n = 366 [0] **337** **371** (335–340) (369–372)	n = 174 [0] **339** **374** (337–340) (370–377)	n = 362 [0] **335** **375** (332–338) (371–377)	n = 314 [0] **335** **372** (330–337) (371–374)	n = 303 [0] **334** **374** (327–336) (371–375)	n = 188 [0] **332** **371** (328–335) (367–372)
MCV, fL	82–98[a]	n = 520 [0] **80** **95** (77–81) (94–96)	n = 358 [0] **83** **96** (81–83) (95–98)	n = 171 [0] **80** **96** (77–82) (96–96)	n = 352 [0] **80** **96** (76–81) (96–97)	n = 128 [0] **77** **95** (69–80) (94–96)	n = 217 [0] **78** **96** (77–81) (95–100)	n = 140 [0] **80** **97** (76–82) (95–99)
PLT, $\times 10^9$/L	145–390[a]	n = 524 [0] **173** **406** (167–179) (384–419)	n = 366 [0] **169** **401** (153–182) (386–426)	n = 174 [0] **153** **406** (142–170) (392–472)	n = 358 [0] **151** **392** (137–160) (371–409)	n = 250 [0] **147** **383** (128–163) (376–399)	n = 261 [0] **137** **360** (132–155) (350–369)	n = 163 [0] **138** **387** (116–164) (362–398)
K, mmol/L	3.5–4.4[a]	n = 528 [1] **3.2** **4.2** (3.1–3.2) (4.1–4.2)	n = 359 [0] **3.2** **4.2** (3.1–3.3) (4.0–4.2)	n = 170 [0] **3.2** **4.1** (3.1–3.3) (4.0–4.1)	n = 352 [0] **3.2** **4.2** (3.2–3.4) (4.1–4.2)	n = 129 [0] **3.1** **4.5** (3.0–3.3) (4.2–4.6)	n = 216 [0] **3.1** **4.3** (3.0–3.2) (4.2–4.5)	n = 140 [1] **3.2** **4.3** (3.0–3.3) (4.2–4.3)
RBC, 10^{12}/L	3.90–5.20[a]	n = 527 [0] **3.56** **4.78** (3.53–3.61) (4.71–4.85)	n = 366 [0] **3.50** **4.57** (3.45–3.53) (4.51–4.69)	n = 174 [0] **3.38** **4.64** (3.21–3.50) (4.47–4.84)	n = 361 [0] **3.62** **4.78** (3.56–3.66) (4.70–4.86)	n = 289 [0] **3.56** **4.88** (3.46–3.69) (4.83–5.02)	n = 289 [0] **3.26** **4.80** (3.16–3.32) (4.61–4.88)	n = 179 [0] **3.13** **4.78** (3.03–3.33) (4.67–4.95)
Na, mmol/L	137–144[a]	n = 532 [1] **135** **142** (135–136) (141–142)	n = 367 [0] **135** **142** (135–136) (141–142)	n = 173 [0] **135** **142** (134–136) (141–142)	n = 358 [0] **135** **141** (134–135) (141–141)	n = 252 [0] **132** **141** (132–133) (140–141)	n = 260 [0] **134** **141** (132–135) (141–142)	n = 163 [0] **133** **143** (129–135) (142–144)
TSH, mIU/L	0.4–4.0[b]	n = 532 [0] **0.3** **4.1** (0.1–0.4) (3.6–4.4)	n = 367 [0] **0.5** **4.3** (0.4–0.7) (3.8–4.8)	n = 173 [0] **0.4** **3.7** (0.3–0.6) (3.4–4.9)	n = 358 [0] **0.6** **4.0** (0.3–0.7) (3.6–4.9)	n = 252 [0] **0.9** **6.3** (0.8–1.0) (5.8–7.6)	n = 260 [0] **0.7** **5.2** (0.7–0.8) (4.7–6.2)	n = 163 [0] **1.0** **6.2** (0.4–1.2) (5.3–6.4)
FT4, pmol/L	10.3–23.2[b]	n = 532 [0] **11.9** **18.7** (11.5–12.1) (18.1–19.2)	n = 367 [0] **11.1** **17.2** (10.7–11.4) (16.5–17.8)	n = 173 [0] **9.4** **17.8** (8.3–11.0) (16.2–18.4)	n = 358 [1] **9.7** **16.1** (9.5–10.0) (16.0–16.3)	n = 252 [1] **10.5** **16.5** (9.8–10.7) (16.1–16.9)	n = 260 [0] **9.0** **15.9** (8.7–9.8) (15.3–18.3)	n = 163 [0] **9.2** **16.3** (8.9–9.9) (15.7–17.0)
TRAN, μmol/L	24–48[b]	n = 532 [0] **27** **51** (26–30) (50–53)	n = 367 [0] **31** **58** (31–34) (56–61)	n = 173 [0] **36** **61** (34–37) (60–63)	n = 358 [0] **38** **68** (37–40) (37–71)	n = 252 [0] **38** **70** (37–39) (67–78)	n = 260 [0] **33** **64** (31–35) (61–67)	n = 163 [1] **34** **61** (33–36) (58–69)
TRIG, mmol/L	0.45–2.60[a]	n = 539 [0] **0.76** **2.49** (0.72–0.81) (2.38–2.59)	n = 367 [0] **0.87** **3.15** (0.79–0.96) (2.97–3.49)	n = 173 [0] **0.98** **3.59** (0.83–1.04) (3.21–4.30)	n = 362 [0] **1.33** **4.72** (1.22–1.44) (4.40–5.04)	n = 318 [0] **1.25** **4.94** (1.01–1.33) (4.58–5.38)	n = 304 [0] **1.22** 4.06 (1.02–1.33) (3.88–4.46)	n = 189 [0] **1.19** **4.57** (1.06–1.29) (4.00–4.97)

(Table 2 continued)

Test, unit	Normal value	13–20 weeks	21–28 weeks	29–34 weeks	35–42 weeks	Partus	Partus +1	Partus +2
T3, nmol/L	1.0–2.6[b]	n = 532 [0]	n = 367 [0]	n = 173 [0]	n = 358 [0]	n = 252 [0]	n = 260 [0]	n = 163 [0]
		1.5 **3.1**	**1.6** **3.1**	**1.5** **3.3**	**1.5** **3.3**	**1.6** **3.6**	**1.4** **3.2**	**1.5** **3.5**
		(1.4–1.5) (3.0–3.2)	(1.5–1.6) (3.0–3.2)	(1.4–1.6) (3.1–3.7)	(1.4–1.5) (3.1–3.5)	(1.5–1.7) (3.3–3.7)	(1.3–1.5) (3.0–3.4)	(1.4–1.7) (3.4–3.7)
BUN, mmol/L	3.2–8.1[a]	n = 532 [0]	n = 367 [0]	n = 173 [0]	n = 358 [0]	n = 252 [0]	n = 260 [0]	n = 163 [0]
		1.9 **4.8**	**1.8** **4.2**	**1.7** **4.3**	**1.8** **4.9**	**1.9** **5.1**	**1.4** **4.7**	**1.7** **4.8**
		(1.8–2.0) (4.7–4.9)	(1.6–2.0) (4.1–4.4)	(1.6–1.8) (4.0–5.7)	(1.6–1.9) (4.5–5.3)	(1.8–2.1) (4.9–5.5)	(1.3–1.7) (4.5–5.1)	(1.6–1.9) (4.5–5.1)
URIC, µmol/L	155–350[a]	n = 532 [0]	n = 367 [0]	n = 173 [0]	n = 358 [0]	n = 252 [0]	n = 260 [0]	n = 163 [0]
		110 **267**	**120** **296**	**124** **330**	**150** **390**	**170** **437**	**185** **450**	**190** **459**
		(100–110) (250–270)	(110–130) (270–334)	(110–134) (310–373)	(140–170) (390–420)	(160–180) (417–457)	(180–190) (430–485)	(170–201) (430–480)

Reference intervals (SI units) defined by the 2.5th and 97.5th percentiles (fat) with 90% confidence interval (in parentheses below). The number of observations are listed with number of detected outliers (square brackets). The interval is calculated for gestational age (weeks): 13–20, 21–28, 29–34, and 35–42, at delivery and day 1 and 2 postpartum. Non-pregnant normal values are listed according to [a](23, 24) or [b]local recommendation. The test abbreviation is listed in Table 1.

intervals during pregnancy. This is in agreement with other studies (3, 19). However, some authors have reported variations during pregnancy, although these variations are within the non-pregnant reference intervals (1, 7, 10), and some have reported lower levels than those reported here (1, 7, 16). Though not stated, the older papers undoubtedly used assays lacking the addition of pyridoxal phosphate, and this might account for the observed differences. Ethnicity, dietary, or lifestyle could also play a role. In the present work, we observed an increase in alkaline phosphatase (ALP) and a slight increase in lactate dehydrogenase (LDH) (Figure 1), consistent with other studies (1, 16, 19). At delivery and during the postpartum period, ALT, AST, ALP, and LDH activities increased above the upper percentile for non-pregnant women. The postpartum increase in ALT and AST activity has been reported previously, with explanations, such as tissue trauma, exercise, and changes in blood volume (25). However, even the women in the present study with uncomplicated deliveries showed similar results.

During pregnancy, we found stable C-reactive protein (CRP) values, but at higher concentrations compared with non-pregnant women (Figure 1). Several authors have reported stable, but generally higher or rising CRP values during pregnancy (2, 15, 20). Larsson et al. reported a higher fluctuating level, especially around the time of delivery, and similar to our results (11), whereas others have shown stable values (14). The methods used to measure the analyte differ from study to study, and the early reports were probably not standardized against the international reference material (CRM 470) for serum proteins.

We confirmed a stable higher upper reference level for white blood cells (WBC) during pregnancy (Figure 1), as described in previous studies (2, 12). We observed that the WBC peaked at delivery, with a reference interval of 8.2–25.8 $\times 10^9$/L in uncomplicated deliveries, hampering the use of this parameter as a marker for infection during delivery. The same observation applies to the use of CRP.

A decrease in platelets was detected at day 1 postpartum, probably due to consumption during separation and delivery of the placenta. This is in contrast to Dahlstrøm et al. who found no change in platelets after vaginal delivery, however, their study included 11 participants only (4).

Stable, but slightly lower values for hemoglobin (Hb), hematocrit (HCT), and red blood cell count (RBC) were observed during pregnancy, in contrast to the results reported by others (13, 19). The Dutch cohort showed a decrease in all three components (22), although both Dutch and Danish women were supplemented with oral iron. The women included in our study were not iron deficient, judged by fairly stable normal levels of iron, transferrin (TRAN), and iron binding capacity (TIBC) (Figure 1). Interestingly, the study of Milman et al. that was conducted in our area more than 10 years ago showed an increase in Hb and HCT, and a fairly stable RBC in spite of the fact that women were supplemented with less iron (20–80 mg/day) compared to what our women received (50–70 mg/day) (13). One possibility is that compliance has changed.

Table 3 Equations predicting the value of quantity (possibly transformed) as a function of gestation week during pregnancy (weeks 13–40) and as a function of days at delivery (coded as 1), 1st, post-partum day (coded as 2), and 2nd, post-partum day (coded as 3).

Test (Y)	Estimated equation: $Y = \text{intercept} + B \times t + C \times t \times t$[a]					
	Pregnancy (weeks 13–40)			Delivery, day 1 and 2 post-partum		
	Intercept	B	C	Intercept	B	C
Ln(ALT)	–1.4761	0.01743[b]	0.00039[b]	–1.4164	0.1004[b]	ns
ALB	48.427	–0.8285[b]	0.01213[b]	42.8311	–9.9024[b]	2.164[b]
Ln(ALP)	0.2774	–0.04766[b]	0.001697[b]	1.649	–0.5324[b]	0.09583[b]
1/AST	2.024	0.04921[b]	–0.00096[b]	3.3521	–1.4403[b]	0.2965[b]
Ln(BIL)	2.5688	–0.0979[b]	0.0001685[b]	1,2292	0.2578[b]	–0.09496[b]
CHOL	2.1382	0.2194[b]	–0.00265[b]	8.4098	–2.1053[b]	0.4499[b]
HDL	1.5281	0.05081[b]	–0.00102[b]	2.3653	–0.327[b]	0.06065[b]
Sqrt(LDL)	0.8347	0.05238[b]	–0.00070[b]	2.1707	–0.4153[b]	0.09056[b]
Ln(VLDL)	–1.0972	0.03388[b]	ns	0.2515	–0.2111[b]	0.04641[b]
Ln(CRP)	3.5275	ns	ns	0.6792	4.0387[b]	–0.8067[b]
Ln(CREA)	4.1971	–0.0152[b]	0.000324[b]	4.05	0.07413[b]	–0.01358[b]
HCT	0.4276	–0.00572[b]	0.000107[b]	0.4184	–0.0544[b]	0.01089[b]
HGB	150.14	–1.9215[b]	0.03628[b]	149.21	–20.6338[b]	4.1827[b]
Sqrt(IRON)	5.3321	–0.08043[b]	0.001338[b]	3.7688	0.1805[b]	–0.1317[b]
Ln(TIBC)	–0.4367	–0.07433[b]	0.001011[b]	–2.2319	0.3536[b]	–0.1194[b]
Ln(LDH)	1.0889	–0.01949[b]	0.000423[b]	0.559	0.6772[b]	–0.1427[b]
Ln(WBC)	1.9103	0.0221[b]	–0.00032[b]	2.6345	0.09184[b]	–0.05047[b]
Ln(BASO)	–3.7947	0.005754[b]	ns[c]	–3.1479	ns[c]	ns
Ln(EOS)	–2.6079	0.04049[b]	–0.00085[b]	–2.1146	–0.7584[b]	0.3134[b]
Ln(LYMPH)	0.7397	–0.01439[b]	0.000327[b]	0.1092	0.5763[b]	–0.1153[b]
Ln(MONO)	–1.0518	0.01139[b]	ns	–0.8327	0.5342[b]	–0.157[b]
Ln(NEUT)	1.4736	0.0319[b]	–0.00050[b]	2.4319	0.05416[b]	–0.05018[b]
MCH	28.9161	0.1824[b]	–0.00307[b]	31.369	ns	ns
MCHC	354.99	ns	ns	335.14	–1.101[b]	ns
MCV	79.7973	0.6379[b]	–0.01095[b]	87.2378	0.5269[b]	ns
Ln(PLT)	5.4513	0.009562[b]	–0.00022[b]	5.6363	–0.1925[b]	0.05002[b]
K	3.9913	–0.02919[b]	0.000571[b]	3.7017	ns	ns
RBC	5.1573	–0.08801[b]	0.001617[b]	4.7772	–0.6589[b]	0.132[b]
Na	138.41	ns	ns	135.78	1.0283[b]	ns
Sqrt(TSH)	1.1943	0.003584[b]	ns	2.0362	–0.5700	0.14659[b]
FT4	18.8806	–0.2845[b]	0.003368[b]	16.0155	–3.4858[b]	0.8424[b]
Ln(TRANS)	3.1524	0.03362[b]	–0.00035[b]	4.1805	–0.2872[b]	0.05295[b]
Ln(TRIG)	–0.3044	0.03413[b]	ns	1.0834	–0.2414[b]	0.05438[b]
T3	1.5347	0.05069[b]	–0.00082[b]	3.3307	–1.1812	0.3019[b]
Ln(BUN)	1.4703	–0.02908[b]	0.00499[b]	1.4343	–0.3719[b]	0.08701[b]
Ln(URIC)	5.081	–0.00568[b]	0.000446[b]	5.6072	0.02609[b]	ns

[a]B and C are the coefficients of the equation and t is the gestations week (during pregnancy) or days relative to delivery (delivery and postpartum period). For instance the predicted value of ln(ALP) in gestation week 18 is $\ln(ALP) = 0.2774 - 0.04766 \times 18 + 0.001697 \times 18 \times 18 = -0.030652$. Then the predicted value of $ALP = EXP(-0.030652) = 0.9698$. [b]$p < 0.05$. [c]When included in initial model the B or C coefficient was not significantly ($p < 0.05$) different from 0 and therefore not included in final model.

ALB concentrations in the present study were lower than the non-pregnant reference interval, with a slight decrease in mean values of about 9% from gestational weeks 13–20 to 35–42 (Table 2, Figures 1 and 2.). During early pregnancy, we observed nearly identical values at gestational weeks 13–20 as described by Larsson et al. for gestational weeks 7–17, although they found a more pronounced decrease as pregnancy progressed (10). The instrument used in the present study used a bromcresol green (BCG) method for measuring ALB, whereas Larsson et al. used bromcresol purple (BGP) (10); both studies used heparin plasma. The two methods rely on dye binding to ALB, but each dye does so by a different mechanism. Discrepancies between results obtained by the two methods have been described (26–28), probably due to a matrix effect. Fibrinogen increases during pregnancy by a factor of two (8) and this might account for the difference in measurement of ALB by the two methods (29, 30). Immunologically determined ALB concentrations during pregnancy decreased by nearly 30% between gestational weeks 6–13 and 37–42 (9). It is unclear which observation of ALB concentrations during pregnancy is correct (9–11).

Many authors and textbooks have described a 10%–15% decrease in ALB during pregnancy (8, 19, 31). Most authors

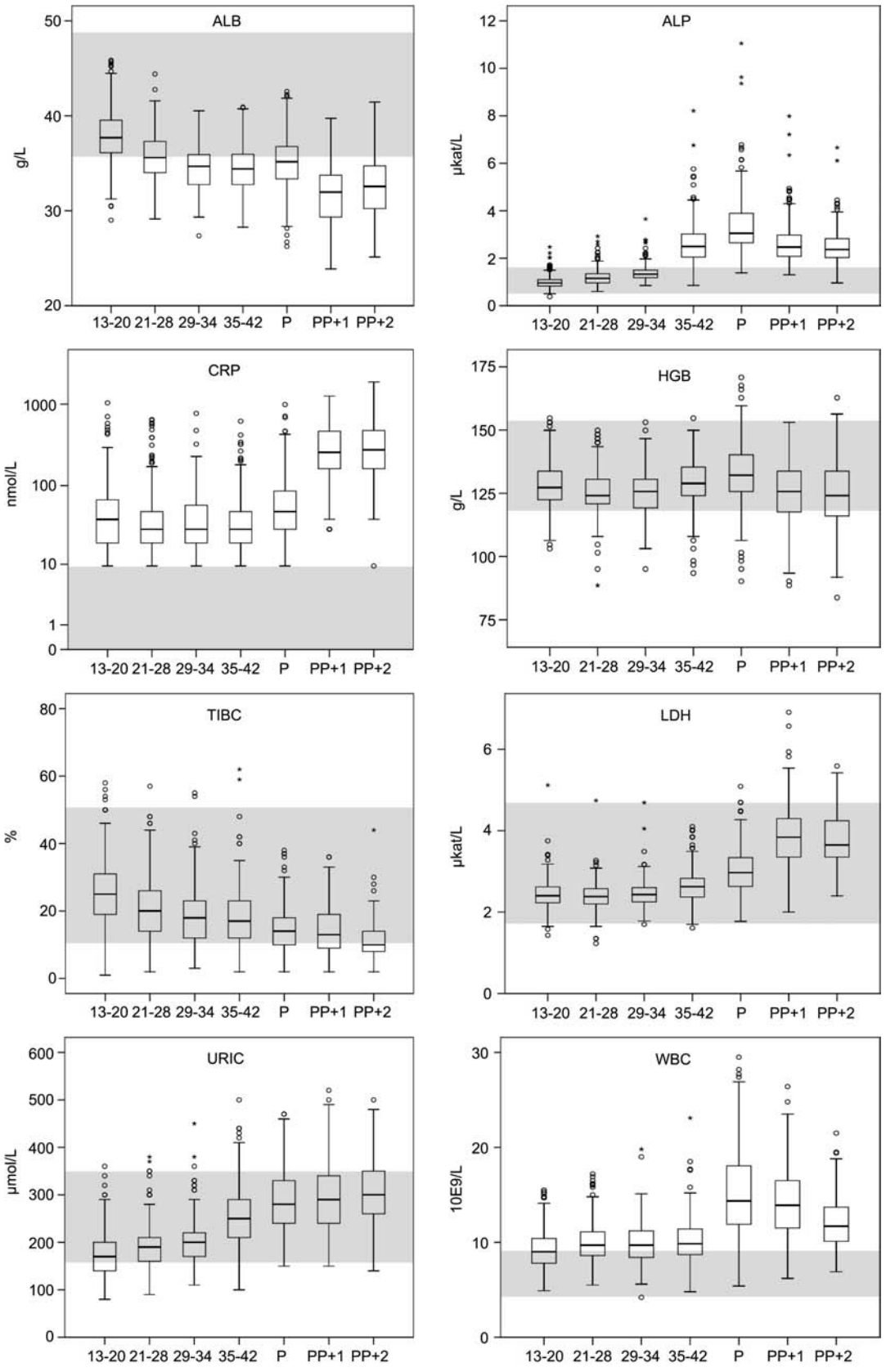

Figure 1 Gestational age-specific reference intervals.
Box plots show the range of data from the 25th to the 75th percentile, while the bar in the middle of each box plot represents the median value. The ends of the ''whiskers'' represent the highest and lowest values. Circles and asterisks indicate outliers (1.5×the interquartile range) and extreme values (3.0×the interquartile range) outside the central box. Shaded areas represent non-pregnant normal values.

Table 4 Suggested reference intervals.

Test	Unit	Normal value	13–20 weeks	21–28 weeks	29–34 weeks	35–42 weeks	Partus	Partus +1	Partus +2
ALT	µkat/L	0.17–0.75[a]	0.14–0.60				0.08–0.70		0.13–0.97
ALB	g/L	36–48[a]	32–43	30–40				25–38	
ALP	µkat/L	0.58–1.75[a]	0.66–1.53	0.73–1.91	0.94–2.66	1.47–4.49	1.82–6.15	1.60–4.50	
AST	µkat/L	0.25–0.58[a]	0.27–0.67				0.30–1.87	0.37–1.29	
BIL	µmol/L	5–25[a]	2–13						
CHOL	mmol/L	2.9–6.1[a]	3.7–9.5						
HDL	mmol/L	1.0–2.7[a]	1.0–3.0						
LDL	mmol/L	1.2–4.3[a]	1.2–6.0						
VLDL	mmol/L	<0.5[b]	0.3–2.0						
CRP	nmol/L	<10[b]	10–300				10–424	40–900	
CREA	µmol/L	50–90[a]	43–74				44–87		
HCT	1	0.35–0.46[a]	0.31–0.42						
HGB	g/L	117–153[a]	112–146				108–156	95–148	
IRON	µmol/L	10–50[a]	7–35				4–28		4–21
TIBC	%	10–50[a]	7–47		5–41		5–32		5–23
LDH	µkat/L	1.8–3.4[a]	1.9–3.1				2.1–5.1		
WBC	10^9/L	3.5–8.8[a]	6.1–14.1				8.2–25.8	7.2–21.4	
BAS	10^9/L	0.00–0.20[b]	0.01–0.07				0.01–0.15		
EOS	10^9/L	0.00–0.50[b]	0.02–0.41						
LYMP	10^9/L	0.70–4.80[b]	1.08–3.06				1.00–4.04		
MONO	10^9/L	0.00–1.10[b]	0.26–0.72	0.27–0.77	0.26–0.94	0.30–0.99	0.29–1.17	0.30–1.25	0.30–0.97
NEUT	10^9/L	1.80–7.40[b]	3.95–11.60	4.82–23.42	0.30–0.97	5.39–14.73	3.95–11.60	4.82–23.42	0.30–0.97
MCH	pg/cell	27–33[a]	27.4–34.6						
MCHC	g/L	317–357[a]	334–374						
MCV	fL	82–98[a]	78–96						
PLT	10^9/L	145–390[a]	167–406				150–390		
K	mmol/L	3.5–4.4[a]	3.1–4.2						
RBC	10^{12}/L	3.90–5.20[a]	3.45–4.70				3.56–4.88	3.13–4.80	
Na	mmol/L	137–144[a]	135–142						
TSH	mIU/L	0.4–4.0[b]	0.3–4.0				0.8–6.2		
FT4	pmol/L	10.3–23.2[b]	10.0–16.3						
TRAN	µmol/L	24–48[b]	27–52	31–58	36–61	38–68	38–70	33–64	34–61
TRIG	mmol/L	0.45–2.60[a]	0.76–2.49	0.87–3.15	0.98–3.59	1.33–4.72	1.25–4.94	1.22–4.06	1.19–4.57
T3	nmol/L	1.0–2.6[b]	1.5–3.5						
BUN	mmol/L	3.2–8.1[a]	1.7–4.9						
URIC	µmol/L	155–350[a]	110–267	120–296	124–330	150–390	170–437	185–450	190–459

*Normal non-pregnant values are listed according to [a](23, 24) or [b]local recommendation. The test abbreviation is listed in Table 1.

have explained the decrease in ALB due to ''hemodilution'', a phrase that has been used to describe the effect of plasma volume expansion (1, 9, 11). The increase in plasma volume during pregnancy cannot be detected before gestation week 6, and increases by 10%–15% at 6–12 weeks of gestation, expanding rapidly until 30–34 weeks, after which there is only a modest rise (32, 33). Unfortunately, we have too few observations in the early period. However, the larger proteins α_2-macroglobulin (9) and high-density lipoprotein (HDL) (Table 2) show no change during pregnancy. It is unlikely that the expansion in plasma volume reduces ALB concentrations only, and not the concentration of macromolecules of similar or larger size, unless synthesis or catabolism of ALB and other plasma macromolecules are also affected by pregnancy.

The total ALB mass increases during pregnancy (32–34), with an increased synthesis rate of 50% to ~9 g/day (34). This production rate should compensate for the increase in plasma volume of 310 mL seen between 6 and 12 weeks of gestation (32), since a plasma concentration of 35 g/L corresponds to about 10 g in 42 days. We suggest that increased catabolism of ALB provides essential amino acids to the fetus and placenta, exceeding the capacity of the liver to maintain a constant concentration of ALB during pregnancy. A lower level of plasma ALB during pregnancy may also represent a well controlled hormonal state that is beneficial for fetal development.

Potassium was stable throughout pregnancy, although the reference values for pregnant women were slightly lower than for non-pregnant women. This is in contrast to Larsson et al. who observed a minor increase, and a much higher 97.5th percentile during pregnancy with values above 5 mmol/L (10). This difference might be due to different pre-analytical conditions for the samples in the two studies. The potassium concentration in plasma may increase due to cellular leakage during storage before centrifugation. We eliminated samples that had more than 4 h time elapsed from collection of blood to separation of plasma. In addition, our samples were prepared without pre-analytical storage and freezing, and samples were analyzed immediately after separation of plasma. Larsson et al. do not provide information about these pre-analytic details (10).

The method we used to measure potassium is a variant of the direct ion selective electrode method, whereas Larsson et al. employed an indirect method (10). As judged by our external quality control system from Labquality, Helsinki, Finland, our method measures potassium ~0.15 mmol/L higher compared with the indirect methods. The indirect methods are sensitive to changes in plasma protein (35), resulting in over-estimation of potassium at lower protein concentrations, as observed during pregnancy. Our results are in accordance with van Buul et al., who found fairly stable potassium values using flame photometry, which is unaffected by interfering substances in the plasma (19).

We also observed stable levels of sodium that were very close to the reference interval for non-pregnant women, and in accordance with the findings obtained using flame photometry (19). Larsson et al. described only slightly lower concentrations of sodium than the present study during early pregnancy, but at partus they report a nearly 5 mmol/L lower 2.5 percentile than ours (10).

An alternative explanation for the discrepancy between the results is a difference in dietary intake of sodium and potassium between pregnant Swedes and pregnant Danes and Dutch. Pregnant women in Sweden are recommended to follow a low-sodium diet (The National Food Administration), and some even recommend using mineral salts that are relatively low in sodium and high in potassium. In Denmark, no such recommendations exist. The salt intake hypothesis assumes that pregnant women are more sensitive to salt intake than non-pregnant women. Such a difference in sensitivity to salt intake occurs in rats. Pregnant rats exposed to

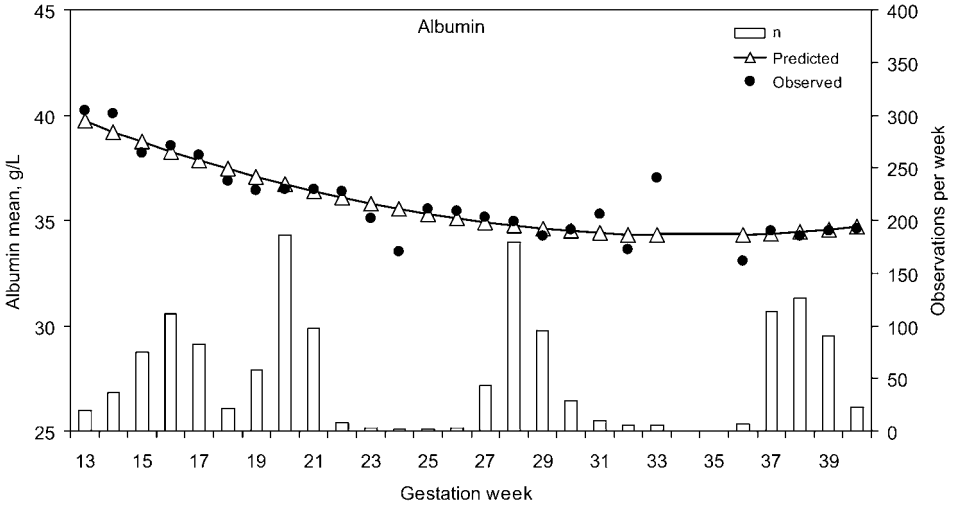

Figure 2 Observed and predicted mean albumin levels during pregnancy.
Estimated equation (triangle): albumin = 48.427 + 8285 × t + 01213 × t × t, t is the gestations week, observed mean (filled circles). Number of observations per gestation week are shown as columns.

a low-sodium diet show a decrease in sodium and an increase in potassium, whereas non-pregnant rats do not (36). Honda et al. reported similar results for rats fed standard fodder (37).

The observations concerning pronounced differences in plasma concentration of ALB and electrolytes between the present study and the study performed by Larsson et al. (10) stress the importance of establishing local reference values, since lifestyle habits, including dietary habits, may be reflected in the composition of plasma. Analytical methods may also influence results.

Determination of local reference intervals, as performed in the present study, is both cumbersome and expensive. For frequently performed tests, establishing reference intervals retrospectively from archived data is feasible, as shown in the Realab project (38). In our case, identification of the presence of pregnancy and the gestational age required some effort. We found virtually identical reference intervals to those obtained in the present study when the method was evaluated on a few tests with 5 years of data from a single ward where only women in late pregnancy are treated.

As most LIS report reference intervals based on age and gender only, we have designed a simple method to report the correct gestational age-specific reference values. A test is given a period suffix and defined as a separate test with the correct reference values. The test with its suffix appears on the ordering screen or sheet as a new test, although the analysis is performed exactly the same as usual. In this way, ''albumin'' may also appear as ''albumin gestation weeks 13–20'', etc. This procedure eliminates the need for pocket folders with reference values for pregnant women.

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Conflict of interest statement

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