Activity of erythrocyte sodium–hydrogen exchange in normal pregnancy

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1. Pregnancy is associated with a 30–50% rise in cardiac output and a 50% increase in blood volume. The contribution of changes in the activity of primary and secondary active transporters to these haemodynamic adaptations remains unknown. For the first time, we measured sodium–hydrogen exchange activity over the course of normal pregnancy.

2. Eighteen healthy pregnant women were studied at 14, 24 and 33 weeks of gestation and compared with 18 non-pregnant healthy women. None of the pregnancies was complicated by hypertension. At each antenatal visit, body weight and blood pressure were recorded, blood and 24 h-urine samples were taken to control renal function and metabolic equilibrium, maternal glucose tolerance was evaluated by oral glucose test and glycated haemoglobin testing, and erythrocyte sodium–hydrogen antiport was also measured.

3. Erythrocyte antiport activity values were 10.0 ± 3.0, 9.6 ± 2.9 and 8.4 ± 3.5 mmol h⁻¹ (litre of cells)⁻¹ in the three gestational trimesters respectively, significantly higher at each trimester than in control women [6.8 ± 2.5 mmol h⁻¹ (litre of cells)⁻¹]. The clearances of urea and creatinine were constantly elevated in pregnant women; at each trimester their serum concentrations were lower than in non-pregnant women. Serum potassium significantly decreased during pregnancy. Serum total cholesterol and triacylglycerol levels, already above the normal range from the first trimester, further increased until the third trimester. The area under the glycaemic curve became larger during pregnancy, and the area under the insulinaemic curve increased to a lesser extent. There was a significant association between antiport activity and serum triacylglycerol levels.

4. The observed hyperactivity of the transporter, peaking at the fourteenth week of gestation, may be a contributing factor to the haemodynamic adjustments attending upon normal pregnancy.

INTRODUCTION
The contribution of changes in the activity of primary and secondary active transporters to cardiovascular and haemodynamic adaptations during pregnancy remains unknown [1]. Few studies have dealt with sodium–lithium countertransport in normal and hypertensive pregnancy [1–5]. However, the physiological role of sodium–lithium countertransport and its relationship to the well known sodium–hydrogen exchange remain enigmatic [6].

For the first time, we measured erythrocyte sodium–hydrogen exchange activity in each trimester of normal gestation and searched for any correlation with several clinical indices of maternal circulatory, renal and metabolic functions.

METHODS
The study population consisted of 18 pregnant (mean age 32 ± 4 years, pre-pregnancy body mass index 23 ± 3 kg/m², 12 primigravidas and 6 parous) and 18 non-pregnant (33 ± 8 years, body mass index 22 ± 2 kg/m²), healthy normotensive women. Pregnant women were studied at 14 ± 2, 24 ± 2 and 33 ± 1 weeks of gestation. At each antenatal visit, body weight and blood pressure were recorded, blood samples were taken to measure urea, creatinine, glucose, sodium, potassium and lipoproteins, and 24 h urine collections were taken to assay urea, creatinine, glucose, sodium and potassium. Maternal glucose tolerance was evaluated by 100 g 3-h oral glucose tolerance test (the area under the glycaemic curve, as g min dl⁻¹, and the area under the insulinaemic curve, as m-units min ml⁻¹, were calculated geometrically) and by measuring glycated haemoglobin (HbA1c, %). Ethics committee approval for the study and the informed consent of all participants were obtained.

The serum and urinary urea, creatinine, glucose, cholesterol, triacylglycerols, sodium and potassium were measured using a multichannel autoanlyser...
was quantified as amiloride-sensitive H+ efflux from high-density lipoprotein-cholesterol was determined after sodium/phosphotungstate/magnesium chloride precipitation (reagents obtained from Menarini SpA, Milano, Italy). HbA1c was assayed using a Bio-Rad Diamat fully automated haemoglobin analyser system.

Erythrocyte sodium–hydrogen antiport activity was quantified as amiloride-sensitive H+ efflux from acid-loaded cells, as described in detail elsewhere [7–9]. Briefly, erythrocytes were isolated by three washes with cold isotonic saline solution (5 mmol/l sodium phosphate, pH 7.4) at 1320 g for 5 min at 4°C. Packed cells (0.200 ml) were added to 3.8 ml of a solution containing 150 mmol/l NaCl, 1 mmol/l KCl, 1 mmol/l MgCl2 and 10 mmol/l glucose, and were incubated at 37°C for 5 min under magnetic stirring. The resultant actual cell volume in the suspension was 4.10 + 0.54% instead of the expected 5% (based on adding 0.200 ml of packed cells to 3.8 ml of isotonic solution); antiport activity was thus normalized for the real haemocrit in the incubation mixture. The pH of the cell suspension was brought to 6.35–6.45 within about 10 min by a 0.2 mol/l HCl solution in 150 mmol/l NaCl. In a parallel experiment, amiloride (4,4'-di-isothiocyanostilbene-2,2'-disulphonate (DIDS) (0.2 mmol/l final concentration) and brought the pH of the medium to 7.95–8.00 by the addition of a 0.05 mol/l NaOH solution in 150 mmol/l NaCl. In a parallel experiment, amiloride (0.5 mmol/l final concentration) was added with DIDS. Thereafter, the first minute proton efflux was registered. The rate of Na–H exchange, expressed as mmol h–¹ (litre of cells)–¹, derives from the difference in the rates of medium acidification in the absence (ΔpH1) and presence (ΔpH2) of amiloride, corrected for the buffering capacity of the incubation medium (b), the cell volume in the suspension (m) and the incubation time (t): [(ΔpH1 − ΔpH2) × b × m⁻¹ × t⁻¹]. The buffering capacity was measured every day of the experiment and gave a value of 0.261 ± 0.046 μequiv. of hydrogen ions per pH unit. The intra-assay coefficients of variation of proton efflux differences with and without amiloride, and the buffering capacity of the incubation medium were 5.3, 6.6 and 3.6% respectively [9].

Statistical analysis included unpaired t-test to compare group means (non-normally distributed variables were log-transformed) and one-way analysis of variance for repeated measurements.

RESULTS

The mean results for pregnant and control women are summarized in Table 1. All pregnant women had normal and uneventful gestation. No pregnancy was complicated by hypertension: mean blood pressure values, calculated as diastolic blood pressure + pulse pressure/3 are given (Table 1). The mean gestational weight gain was 13 ± 4 kg, mean gestational age at delivery was 39 ± 2 weeks and the mean neonatal weight was 3.3 ± 0.5 kg.

Erythrocyte sodium–hydrogen antiport rate was higher in pregnant than in control women, the highest values being observed in the first trimester (fourteenth week of gestation). Serum urea and creatinine did not change during gestation; however, at each trimester, the serum concentrations were lower in pregnant than in non-pregnant women. Urea and creatinine clearances were constantly elevated in pregnant in comparison with non-pregnant women. Serum sodium remained steady in the normal range,

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<th>Table 1. Clinical characteristics of the study population. Comparison between 18 pregnant women, studied in each trimester of gestation, and 18 non-pregnant control women, by unpaired Student's t-test; *P &lt; 0.05, **P &lt; 0.01, ***P &lt; 0.001; intra-trimester comparison by analysis of variance; $P &lt; 0.05, ¶P &lt; 0.01, §P &lt; 0.001. Abbreviations: HDL, high density lipoprotein; AUGC, area under the glycemic curve; AUC, area under the insulinemic curve.</th>
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<td><strong>Pregnant women</strong></td>
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<td>Mean blood pressure (mmHg)</td>
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women from the second to the third trimester. Urinary excretion rates of sodium and potassium increased to a lesser extent. No correlation was found between antiport activity and maternal clinical parameters, except for a weak significant positive association with serum triacylglycerol levels (taking into account values for control and pregnant women; $r = 0.4$, $P = 0.001$; coefficient of correlation 22.2, standard error 6.4), nor between blood pressure and insulin.

**DISCUSSION**

Pregnancy causes a 30–50% rise in cardiac output (peaking between weeks 12 and 20), a close to 50% increase in plasma volume, a 25% increase in erythrocyte mass, and a 30–50% increase in glomerular filtration rate and renal plasma flow (peaking between weeks 16 and 24). Owing to the increased renal function, blood urea and creatinine levels decrease.

Previous studies have found an overactivity of sodium-lithium countertransport in pregnant women, with no difference between normal and hypertensive pregnancies [10–11]. However, under physiological conditions, in the absence of lithium, what the role of the erythrocyte sodium–lithium exchanger is, and which is the transport system assayed for the sodium–lithium countertransport, remain unclear. Unlike sodium–lithium countertransport, the sodium–hydrogen exchanger is involved in normal cell physiology by regulating sodium, hydrogen and intracellular pH. Thus, we believed that it would be better to study the effect of normal pregnancy on sodium–hydrogen exchange activity before evaluating the role of this transporter in the pathogenesis of pregnancy-induced hypertension. The results of our study provide clear evidence that erythrocyte sodium–hydrogen exchange is elevated during normal normotensive pregnancy. The highest values were observed in the first trimester, at about week 14 of gestation; thereafter, the activity of the exchanger progressively decreased, still remaining higher than in non-pregnant controls. Although apparently consistent with the elevation in sodium–lithium countertransport observed during pregnancy [1–5], the overactivity of the sodium–hydrogen antiport does differ greatly from the other proton transporter in the pattern of increase: indeed, a longitudinal study [4] demonstrated that sodium–lithium countertransport was maintained maximally elevated from the first trimester until 38 weeks. In contrast, the sodium–hydrogen antiport showed a peak at 14 weeks and then progressively decreased.

The lack of concordance is not surprising, taking into account the poor correlation found between the two transport systems [12]. Our findings fit well with the lowered intracellular pH reported by Bardicef et al. [13] in 22 third-trimester pregnant women compared with 33 non-pregnant controls. This physiological intracellular acidosis in pregnancy could precondition the sodium–hydrogen exchanger and enhance proton efflux.

No association emerged between fasting serum insulin (or post-load insulin secretory response) and either blood pressure or antiport activity, contrary to the current opinion relating insulinaemia to gestational blood pressure [14], as well as to the suggested stimulatory effect of insulin on the exchanger [15]. The weak correlation with serum triacylglycerols may reflect the supposed influence of plasma lipids on cell membrane composition and the functional properties of its cation transport systems [16].

So far, the pregnancy-related humoral factors that account for the increase in antiport activity remain unidentified, as do the mechanisms that maintain normal arterial blood pressure, despite a marked volume expansion combined with an antiport activity near to the range of essential hypertensive subjects [7]. The peak of antiport activity contemporary with that of cardiac output suggests a contributing role of sodium–hydrogen exchange in plasma volume expansion at the beginning of gestation. In pregnancy, both plasma renin and angiotensinogen concentrations are increased; high renin secretion just occurs during expansion of the extracellular volume.
Among a number of vasoactive agents and growth factors regulating sodium–hydrogen exchanger activity, angiotensin II has been shown to activate the sodium–hydrogen exchanger, inducing an intracellular alkalization [18]. Based on present knowledge, we hypothesize a conceivable relationship between the expansion of maternal extracellular volume, the elevation in plasma angiotensin I1 and knowledge, we hypothesize a conceivable relation-
ger activity, angiotensin I1 has been shown to activate the sodium–hydrogen exchanger, inducing an

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REFERENCES