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NOTE

Measurement of Erythrocyte Na,K-ATPase Activity in Normal Pregnant Women

MASAYOSHI YOSHIMURA, MITSUSHIGE NISHIKAWA, HIDENORI OGASAWARA, MASATERU HORIMOTO, NORIO YOSHIKAWA, ISAMU SAWARAGI* AND MITSUO INADA

*Second Department of Internal Medicine and *Department of Obstetrics and Gynecology, Kansai Medical University, Osaka 570, Japan*

Abstract. To investigate the peripheral metabolic status during normal pregnancy, we measured the number of erythrocyte Na,K-ATPase units as well as the cation transport activity of the pump from 32 normal pregnant women and 12 normal controls. The number of pump units determined by maximal ouabain binding to erythrocyte in normal pregnancy was significantly higher than that in normal controls (mean±SEM: 0.52±0.03 *vs.* 0.39±0.04 pmol/10^9RBC, $P<0.05$). The total cation transport activity of the pump measured by ^{86}Rb uptake also significantly increased during pregnancy (98.9±6.4 *vs.*73.1±5.4 nmol/10^9 RBC, $P<0.01$). However, the mean cation transport activity per pump unit, which was presumed to be an indicator of the peripheral metabolic status, was unchanged in any of three trimesters when compared with that in normal controls. Serum FT_4 levels measured by two different methods were significantly lower in the third trimester than in the first trimester ($P<0.01$). In conclusion, erythrocyte Na, K-ATPase activity per pump unit is normal in pregnant women, suggesting that the peripheral metabolic status in pregnancy seems to be normal. Increases in both the number and function of the pump may be influenced by factors other than thyroid function.

Key words: Na, K-ATPase, Pregnancy, Thyroid hormone, Metabolic status, hCG.

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DURING pregnancy, the serum concentrations of T_4 and thyroxine binding protein (TBG) increase in association with a subnormal concentration of serum albumin [1]. Therefore, it has been recognized that FT_4 reflects the thyroid functional status more accurately in pregnant women [2]. However, recent studies have revealed lower FT_4 concentrations in late pregnancy which are independent of the changes in TBG or serum albumin [3, 4]. We have reported relatively high FT_4 in the first trimester, presumably stimulated by hCG [5]. Increased FT_4 during early pregnancy may be the consequence of the thyrotropic activity of a placental stimulator that overrides the normal operation of the hypothalamic/pituitary/thyroid feedback system [6]. Thus, the thyroid status of normal pregnant women has been a controversial subject.

It has been proposed that the activity of Na, K-ATPase is a major indicator of thyroid thermogenesis [7], and thyroid hormones are known to enhance the Na, K-ATPase activity in rodent tissues including heart [8], liver [9], skeletal muscle [10], and kidney [11]. However, the number of pump units measured by ouabain binding (B) in human erythrocyte has been reported to be re-

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Correspondence to: Dr. Masayoshi YOSHIMURA, West Los Angeles VA Medical Center, Endocrinology Research Laboratory, Building 114, Room 200, Wilshire and Sawtelle Blvds. Los Angeles, CA 90073, USA

duced in hyperthyroidism [12–15], and increased in primary hypothyroidism [12, 15] and nonthyroidal illness [15, 16]. This paradoxical phenomenon is considered to relate to a possible effect of thyroid hormones on the rate of degradation of the pump from erythrocyte membranes [12, 15, 17]. We have recently reported that total cation transport activity of the pump determined by ^{86}Rb uptake (U) was unchanged in hyperthyroidism, and the cation transport activity per pump unit (U/B) significantly correlated with the serum T_3 and T_4 levels, indicating that U/B is a useful index of the peripheral metabolic status [17].

The aim of this study is to estimate the peripheral metabolic status in normal pregnant women by measuring erythrocyte Na,K-ATPase.

Subjects and Methods

Subjects

Study subjects were 32 normotensive pregnant women who had neither goiter nor signs of thyrotoxicosis (7 in the first trimester, 11 in the second, and 14 in the third trimester). Their age ranged from 20 to 41 years (mean, 27±1, 28±1 and 29±2 years in each trimester). Control subjects were 12 normal volunteers whose age ranged from 20 to 35 years (mean, 26±2 years). No abnormalities in erythrocyte morphology were noted in the study subjects.

Measurement of erythrocyte membrane Na,K-ATPase

Erythrocyte membrane Na,K-ATPase activity was measured according to the methods of De-Luise *et al.* with some modifications [18]. Briefly, 10 m*l* of heparinized blood was obtained from each subject. The plasma was separated, and the buffy coat discarded. To determine erythrocyte Na,K pump numbers, aliquots of the cell were washed 3 times with 150mM NaCl containing 30 mM HEPES and 10 mM dextrose, pH 7.5, and finally resuspended so that the cell concentration would be 10% (vol/vol). 200 μl aliquots of the cell suspension were incubated with 0.5 pM of ^{3}H-ouabain (New England Nuclear Co., Boston, MA, specific activity 2.0 MBq/μg) and unlabelled ouabain ranging in concentration from 0 to 100 nM. After incubation for 2 h at 37°C with agitation, cells were washed 3 times with 1 m*l* of ice-cold 140 mM choline chloride and the radioactivity associated with the cell membranes was eluted with 5% trichloroacetic acid (TCA) and counted in a liquid-scintillation spectrometer. Maximal ouabain binding capacity was calculated and expressed as picomoles of ouabain bound per 10^9 erythrocytes.

To measure the cation transport activity of the pump, aliquots of the cell were washed 3 times with 150 mM NaCl and then resuspended in the buffer (150 mM NaCl, 10 mM Tris HCl, 2 mM RbCl, 10 mM dextrose, pH 7.4). Two hundred μl aliquots were incubated with approximately 10^5 cpm of $^{86}RbCl$ (New England Nuclear Co., specific activity 0.18 MBq/μg) and 25 μl of the buffer in the presence or absence of excess (100 μM) ouabain. Sixty min-incubation at 37°C was carried out and the cells were then washed 3 times with 1 m*l* of 150 mM NaCl. Intracellular radioactivity released by 5% TCA was counted and the cellular uptake of ^{86}Rb specifically mediated by the Na,K-ATPase was calculated as the difference between the radioactivity in the presence of absence of excess ouabain. Rb uptake was expressed as nanomoles of Rb taken up per 10^9 erythrocytes.

The erythrocyte count was performed in a hematocytometer.

Estimation of serum FT_4, TSH and hCG concentrations

To measure FT_4, we used two different methods utilizing radioimmunoassay: an Amerlex free T_4 RIA kit (Amersham Japan Co., Tokyo, Japan, normal range: 0.87–1.96 ng/d*l*) (FT_4(A)) and a kit obtained from Diagnostic Products Co. (DPC Japan Co., Tokyo, Japan, normal range: 0.7–2.1 ng/d*l*) (FT_4(D)). TSH was measured with a RIA-gnost hTSH kit (Hoechst Japan Co., Tokyo, Japan, normal range: 0.3–3.6 μU/m*l*), a high sensitive immunoradiometric assay. In the TSH kit, there was no cross reactivity with hCG even at levels of 10^6 IU hCG/*l*. hCG was measured by a specific time-resolved immunofluorometric assay (Delfia hCG kit, Pharmacia Co., Tokyo, Japan).

Statistics

Comparison of the mean values in the two groups was analyzed by Student's *t*-test, and linear regression was used to determine the relation between two parameters. All data were expressed

as the mean±SEM.

Results

Measurement of erythrocyte membrane Na,K-ATPase

Erythrocyte membrane Na,K-ATPase activity in normal pregnant women and in control subjects is shown in Fig. 1. The Na,K-pump number represented by maximal ouabain binding capacity (B) in normal pregnant women ranged from 0.25 to 0.93 pmol/10^9RBC. Five out of 32 pregnant women had higher pump number values than the mean+2SD value for control subjects. The mean pump number was significantly higher in pregnant women than in control subjects (0.52±0.03 *vs.* 0.39±0.04 pmol/10^9RBC, $P<0.05$, Fig. 1-a). The cation transport activity of the pump estimated by ouabain-sensitive ^{86}Rb uptake (U) in

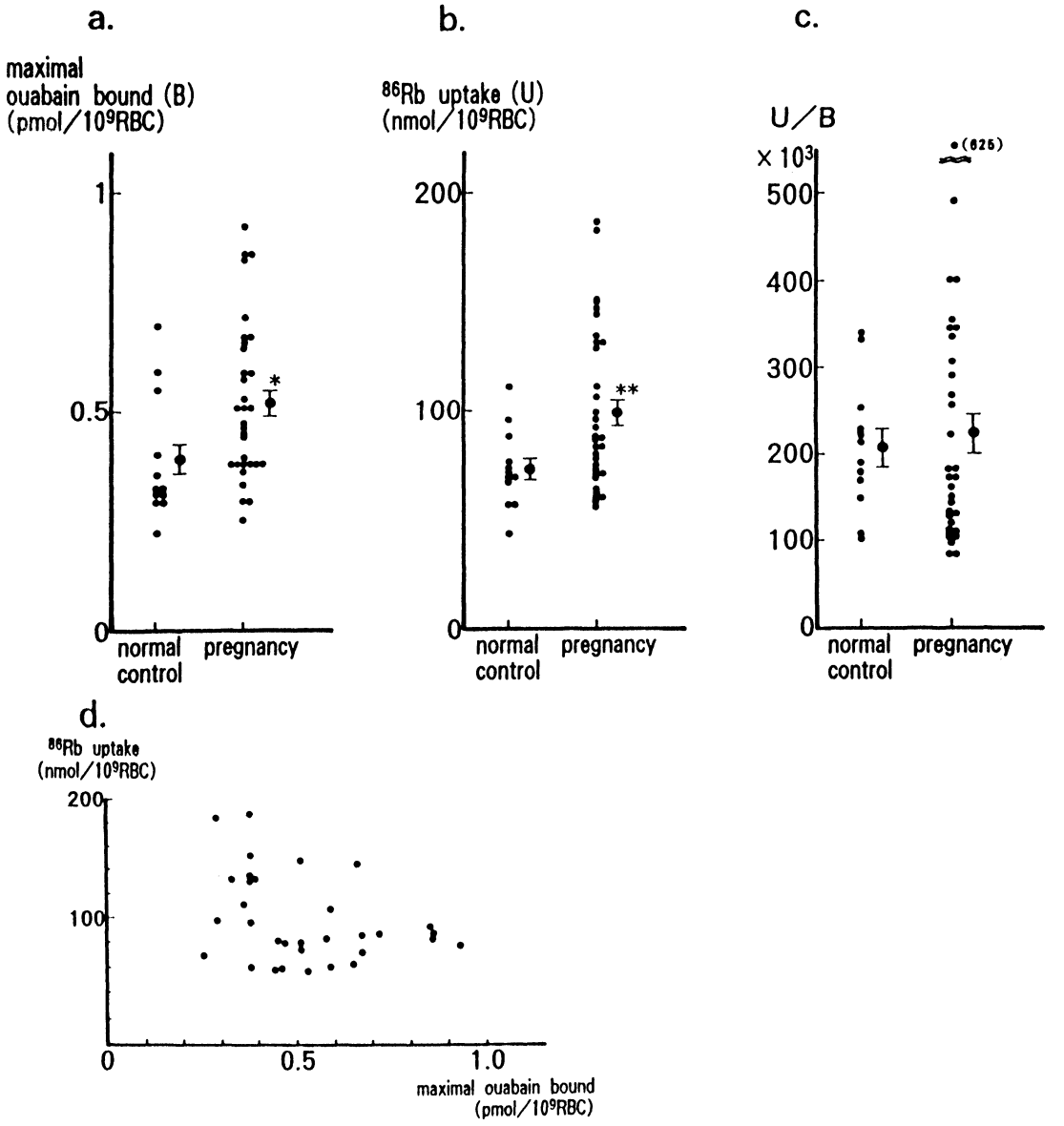

Fig. 1. Individual values for the Na-K pump number represented by maximal ouabain binding (B). (a), the cation transport activity of the pump estimated by ouabain sensitive ^{86}Rb uptake (U). (b), and the cation transport activity per pump unit (U/B) (c) in erythrocyte. These data are expressed as the mean (large dot) ± SEM (vertical bar). *, $P<0.05$, **, $P<0.01$. No significant correlation between B and U was found in normal pregnant women (d).

Table 1. Na, K-ATPase activity during normal pregnancy

	1st trimester (n=7)	2nd trimester (n=11)	3rd trimester (n=14)	normal control (n=12)
B	0.52±0.07	0.53±0.05	0.52±0.05	0.39±0.04
			*	
U	91.4±11.7	82.4±7.8	115.6±10.8	73.0±0.3
		*		*
U/B ($\times 10^3$)	202.4±45.9	177.0±28.9	265.2±42.1	206.4±22.0

Mean±SEM. *, $P<0.05$. B, maximal ouabain binding (pmol/10^9 RBC); U, ^{86}Rb uptake (nmol/10^9 RBC).

pregnant women ranged from 56.5 to 187.4 nmol/10^9RBC, and was increased in 9 out of 32 subjects compared with the mean+2SD value for the control. The mean value was also significantly higher than that in control subjects (98.9±6.4 *vs.* 73.1±5.4 nmol/10^9RBC, $P<0.01$, Fig. 1-b). When cation transport activity per pump unit was calculated as U/B, it ranged from 102.4×10^3 to 340.2×10^3 in control subjects. In normal pregnancy, it ranged from 96.4×10^3 to 625×10^3. Thus, no statistically significant difference in the mean cation transport activity per pump unit (U/B) for the two groups was observed ($[221.2\pm23.6]\times10^3$ *vs.* $[206.4\pm22.0]\times10^3$, Fig. 1-c).

In normal pregnant women, no significant correlations were found between B and U (Fig. 1-d). There were no significant changes in B or U/B in any of the three trimesters. However, U in the third trimester was higher than that in the second trimester ($P<0.05$, Table 1).

Serum FT_4, TSH and hCG concentration

Measurements of the hormones are shown in Table 2. In a comparison of FT_4(A) and FT_4(D), results by these two methods significantly corre-

Table 2. Changes in hormone concentrations during normal pregnancy

	1st trimester (n=7)	2nd trimester (n=11)	3rd trimester (n=14)	normal control (n=12)
			**	
			**	
FT_4(A) (ng/dl)	1.1±0.07	0.8±0.08	0.7±0.04	1.2±0.06
		*		
		***		
FT_4(D) (ng/dl)	1.2±0.07	1.0±0.14	0.9±0.05	nd
		**		
TSH (μU/ml)	1.7±0.7	1.1±0.2	1.4±0.2	1.6±0.2
hCG ($\times10^3$ IU/l)	90 ±25.0	19 ±2.4	23 ±4.8	nd
		*		
		*		

Mean±SEM. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. nd: not done. FT_4 (A), FT_4 measured by Amerlex FT_4 kit; FT_4 (D), FT_4 measured by DPC kit.

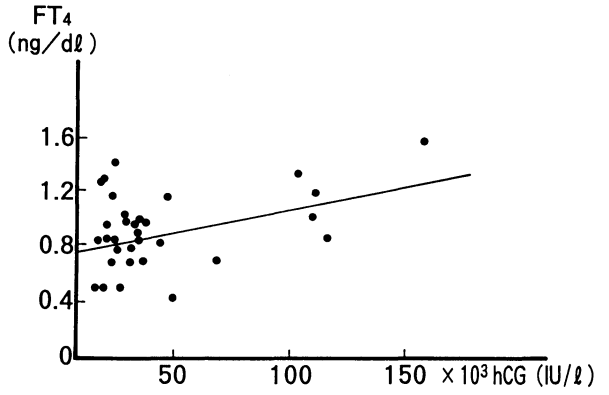

Fig. 2. Correlation between FT_4 measured by Amerlex FT_4 kit and hCG levels in normal pregnancy. A statistically significant positive correlation was observed between FT_4 and hCG levels ($r=0.43$, $P<0.05$).

lated ($y[FT_4(D)]=0.79\times[FT_4(A)]+0.33$; $r=0.55$, $P<0.001$), but the mean value for the former was significantly lower than that for the latter ($P<0.01$). FT_4 decreased during pregnancy, and FT_4 in the third trimester was significantly lower than in the first trimester in both methods ($FT_4(A)$: $P<0.001$, FT_4 (D): $P<0.01$). In the second trimester, $FT_4(A)$ was also lower than in the first trimester ($P<0.05$). There was no significant correlation between U/B and FT_4.

hCG levels were significantly higher in the first trimester than in the other two ($P<0.05$). There was a statistically significant positive correlation between hCG and FT_4 (A) in normal pregnancy ($r=0.43$, $P<0.05$, Fig. 2). Only one out of 32 pregnant women had a low serum TSH level (0.28 μU/m*l*), her hCG level being the second highest in the group. However, the mean TSH value remained unchanged in each of the three trimesters.

Discussion

In normal pregnancy, it was previously reported that the number of Na,K-ATPase pump units increased in the third trimester compared with that in control subjects [19–21], whereas the pump function was reported to be increased [21–23] or unchanged [20, 24]. In the present study, increases in both the number and function of the pump were observed in normal pregnant women. Gallery *et al.* insisted that pregnancy is a condition characterized by sodium retention and volume expansion and that an early increase in the number of pump units resulted in a fall in [Na]i, which in turn inhibited the pump function, limiting a further fall in [Na]i [21].

An increase in the red cell mass during pregnancy has been reported [25] and we previously ascertained that the number of pump units in young erythrocytes is higher than that in old ones [15]. However, in this assay, neither the number nor function of the pump have any correlation with the mean corpuscular volume (data not shown). Therefore, it is conceivable that an increase in the number of pump units is not affected by a change in the metabolism of erythrocytes during pregnancy.

There is considerable evidence that hCG has weak but intrinsic thyrotropic activity [26–28]. We reported that thyroid stimulating activity exists in the sera of normal pregnant women, which reflects hCG itself [5]. The thyrotropic activity of hCG extracted from the sera of normal pregnant women was also elucidated [29]. Thus, the results showing that there was a positive correlation between FT_4 and hCG levels, and that the mean FT_4 value increased in the first trimester when hCG concentrations are highest, offer further evidence of hCG-induced thyrotropic activity in normal pregnancy. These assumptions are also compatible with the result showing that the cation transport activity of erythrocyte Na,K-ATPase per pump unit did not change during pregnancy, because the thyrotropic activity of hCG is not sufficient to induce overt hyperthyroidism [5]. The decline in hCG may lead to a decrease in FT_4 in late pregnancy, because no subsequent increase in TSH was observed in this study.

The reason we measured FT_4 with two analog assays was to avoid potentially discrepant results due to differences in the binding characteristics of commercially prepared radiolabeled analog T_4 molecules. It has been reported, for example, that the affinity to albumin and TBG might differ for the $FT_4(A)$ and $FT_4(D)$ analog tracers [30]. In the present study, lower FT_4 levels in late pregnancy were found in both methods, and this agrees with previous reports [3, 4]. However, there was no significant difference between U/B in normal pregnant women and control subjects. And no correlation was found between U/B and FT_4 during pregnancy. These results suggest that the peripheral metabolic status throughout pregnancy is normal, because U/B is presumed to reflect the

status of peripheral thyroid function [17]. Moreover, it is possible that the decline in FT_4 levels in late pregnancy may not reflect the real metabolic status. Therefore, it is worth measuring erythrocyte Na,K-ATPase activity in pregnant women when there seems to be a discrepancy between the thyroid hormone levels and the metabolic status *in vivo*.

In conclusion, the cation transport activity of erythrocyte Na,K-ATPase per each pump unit as an index of the peripheral metabolic status was unchanged during normal pregnancy compared with that in control subjects. Increases in both the number and function of the pump may be influenced by other factors such as changes in the electrolyte transport system during pregnancy, which require more complete elucidation.

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