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Muscle cell potassium, RNA and hydration in pregnancy and pre-eclampsia

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Summary

Thirty four pregnant women from 26 to 38 weeks gestation and 24 pregnant women with pre-eclampsia gave samples of muscle (rectus abdominis) at caesarean section. Muscle samples were analysed for H_2O, K^+, Mg^{2+} and Na^+. Cell extracellular H_2O was partitioned by the use of the Cl^- space. Also protein, nucleic acids and Zn^{2+} were determined. From 26 to 38 weeks gestation the concentration of K^+ per litre of cell water ($[K_i]$) slowly declined. The slope was significant. Points for patients with pre-eclampsia fell below the line and analysis of covariance showed that the two populations were different ($P < 0.001$). Patients A—J were regarded clinically as severe pre-eclamptics. Points for these patients, in general, fell between 1 and 2 SDs below the normal line. Since other cations per litre of muscle cell water did not change, questions are raised. Is the cation gap filled by amino acids or does vascular spasm cause a leakage of K^+ from muscle cells? Does hypotonicity eventually develop leading to water intoxication? The low oncotic pressure in pre-eclampsia (shown here), the negative free water clearance could all favour increased cell hydration (some evidence for this is presented here towards term).

Assessment of available information concerning creatinine excretion during normal pregnancy and K^{40}, K^{42} studies together with our own rodent studies leads us to believe that a significant increase in muscle mass occurs, but such may not be the case in pre-eclampsia since the reduction in RNA and Zn^{2+} concentrations in muscle would suggest excessive protein degradation. Our understanding of changes in body composition in pregnancy is incomplete — even more so in pre-eclampsia.

pre-eclampsia; cell K^+ and H_2O; RNA; Zn^{2+}; muscle mass.

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Introduction

Pre-eclampsia remains a major pathological problem during pregnancy for mother and fetus. Changes have been recorded in renal [17], adrenal medullary function [5,16], in sodium metabolism and in blood, extracellular and interstitial fluid volumes. Changes in intracellular fluid volume [12] have been suspected (see MacGillivray [11] for review).

In a previous publication [4] we accounted for additional cell growth and tissue potassium (K^+), 250—300 mM due mainly to fetal, placental and uterine growth and about 1.7 l of cell fluid [3]. It was found that from the 39th to the 40th week of gestation, the rectus abdominis muscle had less K^+, Mg^{2+}, intracellular fluid (non-chloride space), increased H_2O content and extracellular volume (Cl space) per kg of fat free fresh muscle tissue [4]. The concentrations of K^+ and Mg^{2+} per litre of cell water were normal. Comparisons were made with muscle from non-pregnant women.

Further studies on pregnant women [3] using ^2H_2O for measurement of body water [3] and the corrected Br^- space as the measure of extracellular volume using 6 h for Br^- to reach equilibrium [3,8], revealed a 5—6 l gain in extracellular volume and a 3 l gain in intracellular volume from 14 weeks to term.

The present work involves muscle analyses of normal pregnant women and women with pre-eclampsia during most of the third trimester (26th to 38th week of gestation). The major new findings are a progressive reduction in intracellular K^+ concentration per litre of cell water, $[K]_i$ with the passage of time during pregnancy, but points for women with severe pre-eclampsia fall well below the normal line.

Patients and methods

Twenty four patients diagnosed as having pre-eclampsia were admitted to the study. All patients except for one had an elevated diastolic pressure 90 mmHg, oedema, proteinuria 500 mg/day and all except one had plasma urate concentration determined. Those with diastolic pressure 100, together with gross pitting oedema, were regarded as severe (Table I, patients A—J), while the remainder were regarded as having moderate or mild pre-eclampsia. All but five patients were experiencing their first pregnancy.

Thirty-four pregnant women volunteered to give a muscle sample whilst undergoing caesarean section for conditions other than pre-eclampsia — mainly repeat elective sections — but preterm labour, premature rupture of membranes, placenta praevia, twins and breech presentations were all included in the study. As in previous work, the 2-g muscle sample was taken from the rectus abdominis muscle initially and prior to the use of retractors and the opening of the peritoneal cavity. At least l-g of muscle was used for analyses of Na^+, K^+, Mg^{2+}, Cl^-, H_2O and protein and if less than the requested amount was obtained it was not possible to undertake analyses for Zn^{2-}, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The methods for chemical analyses and calculation have been set out in detail previously

TABLE I

Clinical appraisal[a].

Patient	Clinical appraisal	Gestation (weeks)	Oedema	Blood pressure (max. diastolic)	Plasma urate (mM)
A	S	36	G	115	0.52
B	S	33	G	110	0.39
C	S	29	G	115	0.50
D	S	33	G	100	0.45
E	S	28	G	120	0.36
F	S	38	G	110	0.40
G	S	29	G	100	0.44
H	S	29	G	100	0.43
I	S	33	G	110	0.38
J	S	39	G	120	0.55
K	M	25	G	95	0.41
L	M	36	R	95	NA
M	M	37	R	110	0.41
N	M	28	R	90	0.58
O	M	32	R	100	0.54
P	M	30	R	100	0.42
Q	M	31	R	100	0.51
R	M	34	R	100	0.39
S	M	36	R	100	0.29
T	M	37	G	NA	0.36
U	M	32	R	105	0.57
V	M	31	G	85	0.48
W	M	34	G	90	0.41
X	M	38	R	120	0.52

[a]S = severe pre-eclampsia; G = gross; NA = not available; M = moderate pre-eclampsia; R = reduced.

[4]. Intracellular K^+ concentration per litre of cell water was calculated as:

$$[K]_i = \frac{K^+ \text{ content of muscle/kg}}{\text{non-chloride space of muscle/kg}}$$

where a kilogram of fat free fresh muscle is considered. The coefficient of variation was 2.04% for measurement of the non-Cl space and 2.54% for the measurement of K^+.

Oncotic pressure was measured by the colloid osmometer (Gonotec Osmomat 050). The results were expressed as mmHg.

Informed consent was obtained from all patients and the project was approved by the Ethics Committee of The Queen Victoria Hospital, Adelaide, South Australia.

Statistical calculations were carried out using analyses of covariance or by a two-tailed Student *t*-test. Such approaches have been documented [15].

Results

From the 26th week of gestation to the 38th there was a progressive decrease in the concentration of K^+ per litre of cell water within the muscle tissue. The black line

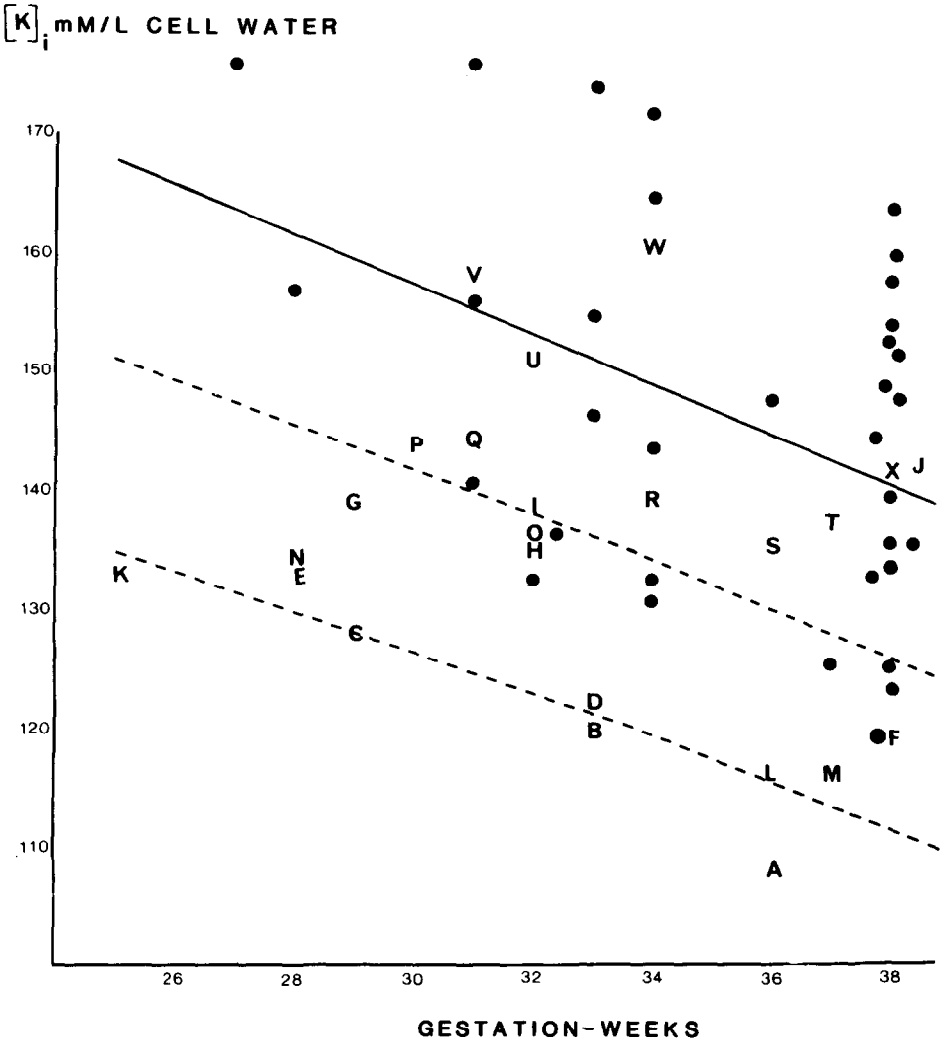

Fig. 1. The regression line (_____) for the concentration of K^+ per liter of cell water within the muscle is shown from 26 to 38 weeks gestation for 34 normal pregnant women (● indicate values for normals) One and two standard deviations below the normal line are shown by the broken lines. Values for $[K]_i$ for women with pre-eclampsia are shown as points A—X. Points for those assessed clinically as 'severe' (A—J) fall mainly between one and two standard deviations below the normal line. The equation for the normal line was $[K]_i = 211.9 - 2.08 \times$ gestation (in weeks), $r = 0.434$.

TABLE II

Rectus abdominis muscle per kilogram fat free fresh tissue.

	Normal			Pre-eclamptic			*P*
	Mean	S.D.	*n*	Mean	S.D.	*n*	
Protein (g)	183	16	34	180	14	24	NS
RNA (g)	1.22	0.23	24	0.94	0.26	15	< 0.002
ZINC (mg)	72.5	8.3	34	64.8	8.4	18	< 0.005
DNA (g)	0.75	0.08	24	0.76	0.16	15	NS
WATER (ml)	794	9	34	800	7	24	< 0.01

(Fig. 1) indicates the regression line and the black dots indicate data obtained from normal women during pregnancy. The slope of the line was significant ($r = 0.434$). Points for patients with pre-eclampsia are shown as A—X. Pre-eclampsia was assessed as being severe in patients A—J and moderate in K—X. The one and two standard deviations below the normal line are shown by the dotted lines in Fig. 1.

It is noteworthy that all the patients regarded as severe had points that fell between one and two standard deviations below the normal line, except point J, which was slightly above the normal line. The point I fell on the line for one SD and the points for patients A and B fell below the two SD line. Only three other points (for patients V, W and X) rose above the normal line. The data for 34 normal and 24 pre-eclamptic patients were analysed by analyses of covariance, gestation in weeks being used as the covariate. In effect, parallel regressions relating age to $[K]_i$ are computed for each group. If the assumption of parallelism is valid, the vertical displacement in $[K]_i$ level between the regression lines for the two groups measures the differing potassium levels independently of gestation.

The analysis showed a highly significant difference ($P < 0.001$) between the two groups. There was no significant regression on gestational age within the pre-

TABLE III

Term pregnant (no oedema).

	Litres							
	TBW		ECV		ICV		ICV/TBW	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control ($n = 12$)	39.4	3.1	17.8	1.5	21.6	2.1	54.7	2.5
Pre-eclampsia ($n = 7$)	39.3	4.3	16.8	2.5	22.5	2.0	57.5*	1.9

*$P < 0.025$, Student's *t*-test.

eclamptic group but the regressions on age for the two groups were not significantly different. Thus the assumption of the analysis of covariance is a valid one.

No such reductions with advancing gestation were found for $[Mg]_i$ in mmol/l for normal pregnant women (15.1 ± 4.4) nor for pre-eclamptics (15.9 ± 3.9). The same situation existed for $[Na]_i$ expressed as mmol/l in normal pregnant women, 10.0 ± 7.2 versus 12.3 ± 9.6 for pre-eclamptic patients.

As revealed in Table II, other chemical components of muscle tissue were reduced but no statistical difference could be found between patients with severe and moderate pre-eclampsia so the data were pooled. The RNA and Zn^{2+} were significantly reduced in concentration ($P < 0.005$) while water was increased ($P < 0.01$) due to an increase in the Cl space (15%). The plasma oncotic pressure in 23 pre-eclamptic patients was 16.4 ± 2.4 mmHg, much lower than the value of 21.2 ± 1.2 obtained for 30 normal pregnant women from 26 to 38 weeks gestation ($P < 0.001$).

Discussion

Forbes, in a recent publication [6], has reviewed changes in body composition during pregnancy including changes in potassium content. Using K^{40} measurements, values of 300—600 mM have been recorded while Forbes found an increment of 540 mM in 50 women. MacGillivray and Buchanan [13], using K^{42} reported an increment of 559 mM. From a consideration of all values Forbes concludes that at least 500 mM of potassium is added to the body during pregnancy. Our own data indicated that the fetus, placenta and uterine growth and expanded red cell mass and plasma volume account for 300 mM [4]. Forbes [6] considers that some 230 mM of K^+ remains to be accounted for in the mother and supports our thinking that muscle mass increases with a possible increase in intestinal mass. A small contribution of K^+ is made to increments in myocardial and kidney size. The suggestion that there is an increment in muscle mass during pregnancy is supported by the work of King et al. [10] who found an increase in creatinine excretion over a 10-week period suggesting a 2.8 kg increment in lean tissue (mainly muscle).

Work on pregnant rats showed a 14% increase in muscle mass at term when compared with virgin controls [3]. A reduction in the muscle protein/DNA units within the muscle mass was found. The decrease in protein/DNA was also shown in the human study [4]. In general, mammalian growth follows similar patterns. In normal mice, but particulary in those with isolated growth hormone deficiency, there is a significant replication of muscle nuclei during pregnancy and a gain in muscle mass [2].

In the human at least half of our estimated gain of 3 l [3] in intracellular water could be included in the growth of new muscle. The whole situation requires further exploration and, as stated by Forbes, is difficult to interpret. Clearly there would appear to be an excess of K^+ gained by the mother, unaccounted for and this may be incorporated into the growth of new muscle.

In pre-eclampsia the decrease in K^+ concentration in muscle, cell water without a change in $[Na^+]_i$ or $[Mg^{2+}]_i$ was greatest in patients regarded clinically as having a

severe condition. Such a finding raises questions. Is there a hypotonicity within the cell? Do amino acids fill the cation gap? It is known that muscle amino acids can act as cations during K^+ deficiency [1,18]. Moreover tissue hypoxia which can result from vasospasm is known to cause leakage of K^+ from myocardial muscle without any change in membrane potential or of the Na^+, K^+ ATPase system [7]. Such events could occur in skeletal muscle since vaso spasm exists in pre-eclampsia. Hypoxia might be expected to lead to the accumulation in tissues of hypoxanthine which in the primate is converted to urate [21]. Limited observations on four patients by other workers in 1958 did not support an excessive generation of urate in pre-eclampsia [20].

If K^+ extrusion from muscle cells continues, a state of hypotonicity could develop, particularly as antidiuretic factors, a negative free water clearance and a significant fall in oncotic pressure all occur [19]. Using techniques mentioned in the introduction, ^2H_2O to measure total body water (TBW) and the corrected Br^- space to assess extracellular volume and by difference, the intracellular volume (ICV), we studied seven patients with pre-eclampsia and 12 normally pregnant women. The patients were approaching term (36-40 weeks) and the seven with pre-eclampsia showed no obvious oedema. The body water was identical in both groups but in the pre-eclamptic group cellular hydration was prominent such that the ratio of ICV divided by TBW was higher ($P < 0.025$) (see Table III). Thus the tissue as to whether excess cell hydration can occur in pre-eclampsia with or without water intoxication has not been settled. Concern along these lines has only been expressed by MacGillivray and with respect to cell hydration [11] per se. Measurements made by us in 12 normally pregnant women and in 13 women with pre-eclampsia, but of only 26 to 34 weeks gestation, revealed excess water in the extracellular compartment in pre-eclampsia as has been the customary thinking and accepted by other workers [13].

Changes in protein metabolism in pre-eclampsia have long been appreciated. A reduction in the concentration of RNA and Zn^{2+} is compatible with the thinking. Indeed the group at St. Thomas' Hospital, London [14] have already shown reduced Zn^{2+} concentration in the rectus muscle of mothers delivering 'small for dates' babies. In pre-eclampsia it would appear that protein degradation exceeds protein synthesis which may partly explain the very low oncotic pressure in interstitial fluid.

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