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The Effect of Diabetes, Gestational Diabetes or Pre-eclampsia on Urinary Protein and Mineral Excretion during Pregnancy

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Abstract

Thirteen healthy control, 13 pre-eclamptic, 7 diabetic(DM) and 12 gestational diabetic(GDM) pregnant women participated in a study of the interrelationships between the levels of protein, calcium, magnesium, phosphorus, zinc and copper in urine. Urinary protein, magnesium and copper levels were significantly higher($p<0.0005$, $p<0.0003$, $p<0.005$ respectively) in pre-eclamptic women than those of control, DM and GDM women. Urinary zinc excretion in pre-eclamptic women(1.61mg/g creatinine) was higher than that of DM women(0.81mg/g creatinine); urinary zinc losses of control and GDM women were between the other two groups. The GDM women excreted significantly more phosphorus in their urine in comparison to control and preeclamptic women ($p<0.002$), but this was not seen in DM women. Among the DM women, urinary protein excretion was positively correlated with glycosylated hemoglobin($r=0.940$) and fasting blood glucose concentration($r=0.889$). Urinary zinc excretion also was correlated with glycosylated hemoglobin($r=0.853$) and fasting blood glucose($r=0.956$). In the GDM and pre-eclamptic women there were also significant correlations between urinary calcium and magnesium($r=0.857$, $r=0.749$ respectively) and between urinary protein and copper($r=0.638$, $r=0.778$ respectively).

Key words: diabetes mellitus(DM), gestational diabetes mellitus(GDM), pre-eclampsia, proteinuria, urinary mineral excretion

INTRODUCTION

Studies of nutrient metabolism in women experiencing metabolic disorders during pregnancy are relatively limited, and consequently, it is not known if the dietary requirement for nutrients is altered by these metabolic conditions. There is, however, considerable evidence that both insulin-dependent diabetes mellitus(IDDM) and non-insulin dependent diabetes mellitus(NIDDM) in nonpregnant adults are associated with an increased urinary excretion of protein and essential minerals(1-6).

While there have been a number of studies evaluating the mineral status of diabetic subjects, these studies have yielded inconsistent results. For example, blood calcium levels in diabetic subjects have been reported to be high (1-2) or unchanged(3-4). Hypomagnesemia and hypermagnesiuria have been noted particularly when the diabetes was poorly controlled in some(3,7-9), but not all (5,10) studies. Hyperzincuria has been found in IDDM(6, 11) and NIDDM(12), but both low(11,13) and high(6,14) concentrations of plasma zinc have been reported in diabetic subjects compared to control. Serum and plasma levels of copper have been found increased(3,11) and unchanged(15) in diabetics. With careful control of blood glucose concentrations, more and more IDDM pregnant women are carrying their pregnancies to term. It is important, therefore, to improve our understanding of the nutrient requirements of these women. Other metabolic disorders that are common among women during pregnancy include gestational diabetes mellitus(GDM) and pre-eclampsia. Since both of those disorders also alter renal function, it is reasonable to propose that the urinary excretion of essential nutrients is altered in women with those conditions as well.

Although the significance of proteinuria is well-documented for pregnancy complicated by pre-eclampsia or diabetes, "asymptomatic" proteinuria is associated with a number of adverse pregnancy outcomes and serious long-term maternal morbidity(16). Pre-eclampsia is associated with hypocalciuria(17). Moreover, 1,25-dihydroxy-

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vitamin D_3 is reduced in pre-eclampsia and may lead to hypocalciuria by causing decreased intestinal absorption of calcium, and increased distal tubular resorption of calcium. The cause of reduced 1,25-dihydroxyvitamin D_3 in pre-eclampsia is unknown and may be due to either diminished renal or placental production of the hormone (18). Blood glycosylated hemoglobin was higher and serum magnesium concentration was lower in the pregnancies that ended in adverse fetal outcome, compared with the other successful pregnancies. Impaired magnesium status may contribute to the high spontaneous abortion and fetal malformation rate in DM pregnant women(19). Although a decrease in serum zinc and magnesium has been observed during pregnancy in both DM and control subjects, zinc was not different between insulin-treated DM patients and control at the term of pregnancy, whereas serum magnesium was lower at term both in DM and in insulin-treated women with GDM(8).

The purpose of this study, therefore, was to determine the effect of DM, GDM, and pre-eclampsia during pregnancy on the urinary excretion of protein, calcium, phosphorus, magnesium, zinc and copper.

MATERIALS AND METHODS

Subjects and sample collection

Four groups of pregnant women were studied. Group 1 consisted of 13 control pregnant women, group 2 was 13 pre-eclamptic pregnant women, group 3 was 2 IDDM and 5 NIDDM pregnant women, and group 4 consisted of 12 women with GDM. Women attending a special prenatal clinic for diabetic patients at the University of California at San Francisco were recruited for the study. The study was approved by the University of California Committee for Protection of Human Subjects and informed consent was obtained from all participants. With the exception of the pre-eclamptic women, a single spot urine sample was obtained from the women at the time of their prenatal visit for analysis of protein and selected minerals. Urine samples from patients with pre-eclampsia were obtained from a bank of urine that had been collected in conjunction with a large-scale study of pre- eclampsia. All women were in the last trimester of pregnancy when the samples were collected. The average age of the women and the average time of gestation when studied are summarized for the four groups in Table 1.

An aliquot of the spot urine sample routinely collected at the time of the visit was labeled and stored in a trace-element-free tube and refrigerated. Information about the age of the women, duration of pregnancy, number of children, duration of diabetes, concentration of hemoglobin, hematocrit, glycosylated hemoglobin and fasting blood glucose at the time of the visit was obtained for each woman from the medical chart. A summary of the subject's usual dietary intake, estimated from a 24-hr recall by a dietitian, was also obtained from the patient's medical charts. Spot urine samples were also obtained from a group of control pregnant women who did not have any of the clinical symptoms of DM, GDM, or pre-eclampsia. Those samples were collected at the time of the prenatal visit, labeled and stored by the attending nurse in trace-element-free tubes and refrigerated. All urine samples were picked up at the end of the day of each prenatal clinic, transported on dry ice and stored at -20°C until analysis.

Sample analysis

Urinary protein concentration was determined by a Bradford protein assay(Bio-rad, Richmond, California, 94804, U.S.A.)(20). The levels of urinary calcium, magnesium, zinc, and copper were measured by atomic absorption spectrophotometry. Prior to analysis, urine samples were evaporated to dryness at 100°C on a hot plate, ashed for 5 hours in a low temperature asher(Branson/IPC, Model #S-4075, RF power 600 watt, Hayward, CA, U.S.A.). Urinary calcium and magnesium levels were determined after dilution(1:20-300 for calcium and 1:400-1000 for magnesium) with 0.27% lanthanum chloride in 0.125N hydrochloride; standards were prepared in like manner.

Table 1. Maternal age and gestational age of the subjects at the time of the study

Group	Control (n=13)	Pre-eclampsia (n=13)	DM[1] (n=7)	GDM[2] (n=12)
Maternal age(yr)	30.6±6.4	27.5±5.0	34.0±5.2	33.3±5.5
Gestational age(wk)	37.4±3.6	36.3±4.5	30.1±4.3	36.3±3.1

Data are expressed as Mean±SD
[1]Diabetic pregnant women
[2]Gestational diabetes

For determination of zinc and copper, urine samples were diluted to 1:2-10 for zinc and 1:0.2 for copper with 0.125N hydrochloride. Standards were also prepared in 0.125N hydrochloride. Urinary calcium, magnesium, zinc and copper were analyzed by flame atomic absorption spectrophotometry(Thermo Jarell Ash-22, Franklin, MA). Urinary creatinine and urinary phosphorus concentrations were determined by an automated technique(Gemeni, Electro-Nucleonics Inc., Isuine, CT 92718-2512). The creatinine analysis was based on a modified Jaffe reaction(21) and the phosphorus determination was based on the Fisk and Subbarrow method(22).

Statistical analysis

Statistical analyses were done using a Statview computer program(Abacus Concepts, Inc., Berkeley, CA). All values are expressed as mean and standard deviations. Tests for analysis of variance were followed by Duncan's multiple-range test. Pearsons correlation coefficients and stepwise multiple regression were also done. All p-values <0.05 were considered to be statistically significant.

RESULTS

The DM and GDM groups reported eating diets providing about 1790kcal/day and 1920kcal/day, respectively, with about 40% of the energy from carbohydrate, 35% from fat, and 25% from protein(Table 2). The fasting blood glucose, hemoglobin and glycosylated hemoglobin concentrations and hematocrits did not differ between the DM and GDM women(Table 3). The concentrations of protein, calcium, magnesium, phosphorus, zinc and copper per g urinary creatinine are indicated in Fig. 1~6 for the four groups of women. There was considerable variation in the urinary excretion of protein and minerals within each group. For example, the coefficient of variation(CV) in urinary magnesium excretion among the control women was 274%; within the pre-eclamptics the CV for urinary magnesium was 91%, and it was 51% and 45%, respectively, for the DM and GDM groups. The variation within the control group was greater than that in the other three groups with an average CV for all 6 measurements of 107±84%. The overall CV for the pre-eclamptics was 79±20%, and it was 50±11% and 51±7% in the DM and GDM groups.

Although there was considerable variation within groups, there still were significant differences between groups. In comparison with control women, pre-eclamptic women excreted significantly more protein($p<0.0005$), magnesium($p<0.0003$) and copper($p<0.005$)(Fig.1-3). There was no difference in urinary calcium and zinc excretion(Fig.4,5). Urinary protein excretion among the pre-eclamptic women was positively correlated with urinary copper($r=0.749$)

Table 2. Mean daily dietary intake in DM and GDM women

Group	DM[2] (n=7)	GDM[3] (n=12)
CHO[1](g)	170.3± 9.1	183.6± 17.9
Protein(g)	106.0± 19.0	110.3± 30.6
Fat(g)	68.0± 11.0	75.5± 17.9
Energy(kcal)	1789.5±204.5	1920.0±263.1
CHO/Energy(%)	39.7± 3.4	39.6± 3.9

Data are expressed as mean±SD
Means in the same line does not significantly different ($p<0.05$)
[1]Carbohydrate
[2]Diabetic pregnant women
[3]Gestational diabetes

Table 3. Fasting blood glucose, glycosylated hemoglobin, hemoglobin, and hematocrit in DM and GDM women

Group	DM[1] (n=7)	GDM[2] (n=12)
Hemoglobin(g/dl)	11.3± 1.8	11.8± 1.0
Hematocrit(%)	34.2± 4.8	35.2± 2.6
Glycoslylated hemoglobin(%)	6.9± 1.7	6.3± 1.4
Fasting blood glucose(mg/dl)	108.2±48.1	96.8±20.9

Data are expressed as mean±SD
Means in the same line does not significantly different ($p<0.05$)
[1]Diabetic pregnant women
[2]Gestational diabetes

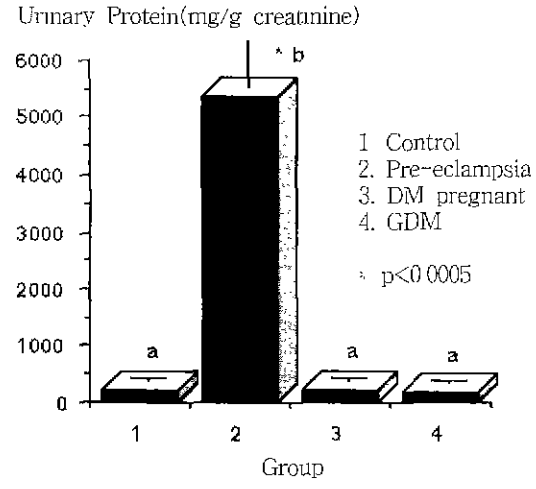

Fig. 1. Urinary protein in normal, pre-eclamptic, DM, GDM pregnant women.

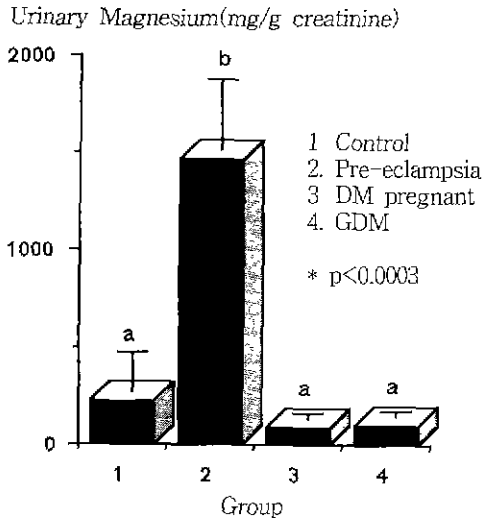

Fig. 2. Urinary magnesium in normal, pre-eclamptic, DM, GDM pregnant women.

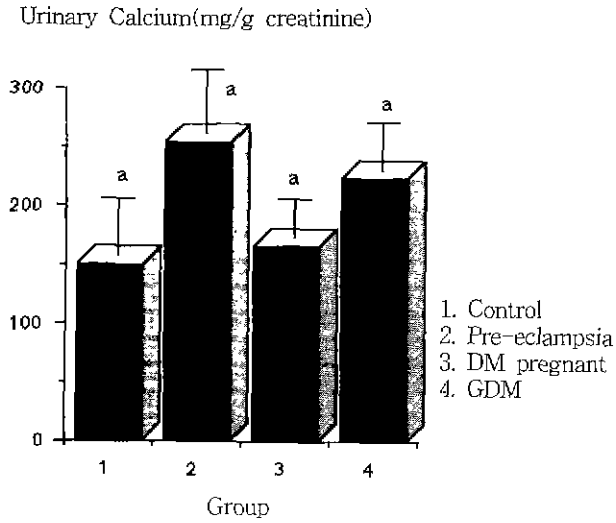

Fig. 4. Urinary calcium in normal, pre-eclamptic, DM, GDM pregnant women.

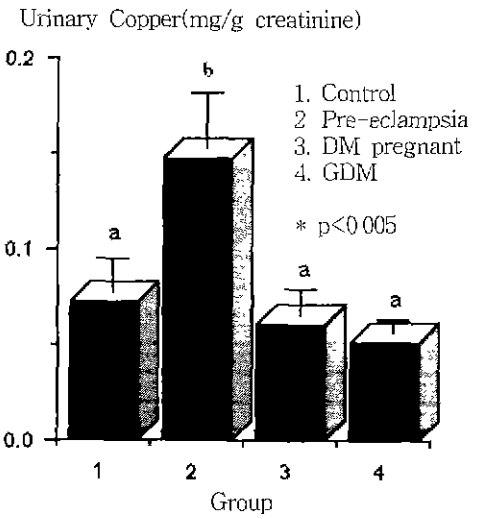

Fig. 3. Urinary copper in normal, pre-eclamptic, DM, GDM pregnant women.

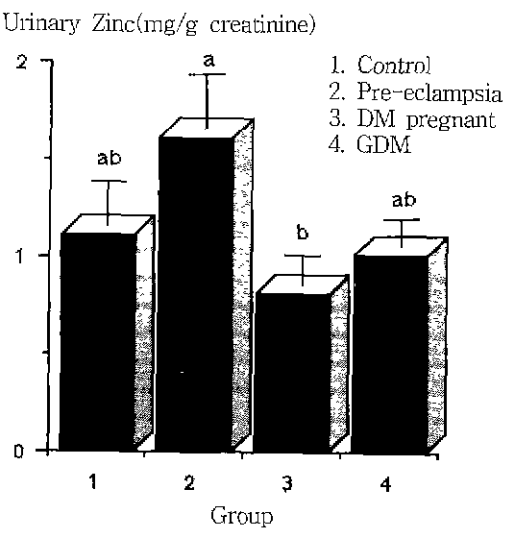

Fig. 5. Urinary zinc in normal, pre-eclamptic, DM, GDM pregnant women.

and calcium excretion was correlated with urinary magnesium(r=0.778)(Table 4).

The results of the diabetic women differed from those of the pre-eclamptic women. In comparison with the control pregnant women, there were no significant differences in urinary protein, calcium, magnesium, zinc, and copper excretion among either the DM or the GDM women. The GDM women excreted significantly more phosphorus in their urine in comparison with control women($p<0.002$), but this was not seen in DM pregnant women(Fig.6). Among the DM pregnant women, the higher the urinary protein excretion the higher the glycosylated hemoglobin (r=0.940), urinary zinc(r=0.723), and the fasting blood glucose concentration(r=0.889). The urinary zinc excretion also was correlated with the glycosylated hemoglobin concentrations(r=0.853) and the fasting blood glucose concentration(r=0.956)(Table 5). These relationships between fasting blood glucose, urinary protein and zinc were not

Table 4. Correlation coefficients among urinary excretion of protein, calcium, magnesium, phosphorus, zinc and copper in pre-eclamptic pregnant women

	U-Ca	U-Mg	U-P	U-Zn	U-Cu
U-protein	-0.543	0.171	-0.294	0.041	0.749*
U-Ca		0.778*	0.065	0.289	0.134
U-Mg			-0.036	0.185	0.331
U-P				-0.063	-0.275
U-Zn					0.343

*p<0.05

Table 5. Correlation coefficients among fasting glucose and glycosylated hemoglobin in serum and urinary excretion of protein, calcium, magnesium, phosphorus, zinc and copper in diabetic pregnant women

	U-Ca	U-Mg	U-P	U-Zn	U-Cu	Hb A_{1c}[1)]	FBG[2)]
U-protein	-0.022	0.149	-0.304	0.723*	0.398	0.940**	0.889**
U-Ca		0.636	0.213	0.068	0.681	-0.385	-0.170
U-Mg			0.600	-0.177	0.540	-0.244	-0.292
U-P				-0 488	-0.166	-0.156	-0.376
U-Zn					0.155	0.853**	0.956**
U-Cu						-0.188	0 014
Hb A1c							0.952**

[1)]Glycosylated hemoglobin
[2)]Fasting blood glucose
*p<0.05, **p<0.01

Table 6. Correlation coefficients among fasting glucose in serum, gestational age and urinary excretion of protein, calcium, magnesium, phosphorus, zinc and copper in gestational diabetic women

	U-Ca	U-Mg	U-P	U-Zn	U-Cu	FBG[1)]	Ges age[2)]
U-protein	-0.121	-0.123	0.131	0.109	0.638*	-0 493	0 521
U-Ca		0.857***	0.063	0.549	0.182	0.224	-0.002
U-Mg			0.062	0.469	0.064	0.237	-0.175
U-P				-0.324	0.289	-0 526	-0.630*
U-Zn					0.049	0.086	0.277
U-Cu						-0.475	0.752**
FBS							-0.511

[1)]Fasting blood sugar
[2)]Gestational age
*p<0.05, **p<0.01, ***p<0.001

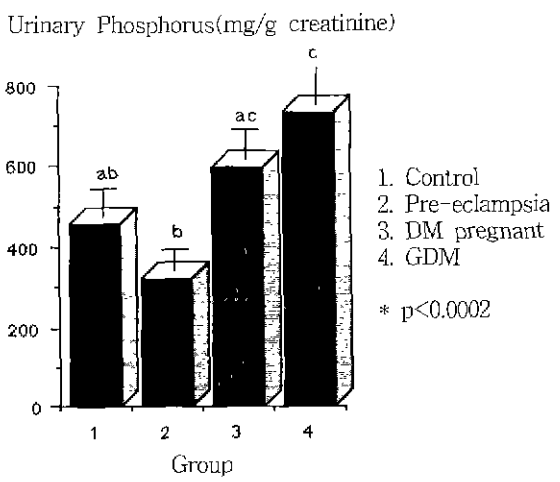

Fig. 6. Urinary phosphorus in normal, pre-eclamptic, DM, GDM pregnant women.

seen in the GDM group. Instead, urinary calcium and magnesium(r=0.857), urinary protein and copper(r=0.638), and gestational age and urinary copper(r=0.752) were positively correlated in that group(Table 6).

DISCUSSION

The urinary excretion of protein and minerals varied widely among the subjects within each group. This was especially true between the control and pre-eclamptic women. The higher variation between those two groups was due to the presence of very low as well as high values whereas the diabetic groups tended to be more consistent. These data suggest that renal function varies widely among women who are apparently healthy during their pregnancies and have no clinical symptoms of diabetes or pre-eclampsia Also, not all urinary constituents varied in the same manner. For example, the CV in urinary phosphorus excretion ranged from 31 to 60% in the four groups, whereas the CV for urinary magnesium ranged from 45 to 274%. This suggests that the various minerals are handled differently by the kidney. The urinary excretion of protein, magnesium and copper was significantly higher in the pre-eclamptic women compared to the controls and phosphorus excretion was significantly lower. Pre-eclampsia is a common disorder of pregnancy characterized by hypertention and varying degrees of vasoconstriction, intravascular coagulation and hepatic, renal, cerebral and placental dysfunction(23, 24). The reduction of glomerular filtration rate caused the increase of albumin excretion in women with severe pre-eclampsia.

The increase in clearance of albumin relative to that of creatinine is compatible with increased glomerular permeability to albumin and or decreased tubular albumin reabsorption(25,26).

Although diabetes is associated with increased urinary losses of protein and other minerals in non-pregnant women and adult men(3,5,6,15,27), there was no evidence that this difference occurred during pregnancy. The excretion of all of these constituents is greater in pregnancy than that of non-pregnant women presumably due to the increased glomerular filtration rate in pregnancy(28), and the presence of diabetes did not increase the urinary excretion of protein and minerals further.

Within the group of DM pregnant women(n=7), a positive correlation was found between the severity of their disease, as measured by fasting blood glucose concentration and glycosylated hemoglobin, and the urinary excretion of protein and zinc. However, relationship was not found with urinary calcium, magnesium, phophorus or copper.

Although there were no differences in the urinary excretion of protein and minerals between the GDM and DM women, renal function may appear to differ between the two groups. No relationship between the severity of the disease as measured by and urinary protein or mineral excretion was seen in the GDM group. Instead, there were positive correlations between urinary calcium and magnesium and between urinary protein and copper. The protein/creatinine ratio was highly correlated with total protein excretion($r=0.977$, $p<0.0001$). The correlation was not affected by pregnancy or pre-eclampsia(25).

In summary, the results of this study show that pre-eclampsia may alter renal function and lead to an increase in urinary protein, magnesium and copper. Urinary protein losses were about 20 times those of the control women, and the magnesium and copper losses were about 6-fold and 2-fold greater, respectively. Total urinary losses of protein and copper losses were small in comparison with usual dietary intakes, however. Total urinary protein was about 5g/g creatinine out of a usual dietary protein of about 70g/day; copper losses averaged about 0.15mg/g creatinine out of a usual intake of about 1-2mg/day. Urinary magnesium losses were high, approximately 1500mg/day. Among the diabetic women, renal losses of nutrients do not appear to be enhanced over those of control pregnant women except, possibly in those DM women who are poorly controlled.

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