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Insulin Level, RBC Na^+ Transport and Blood Pressure in Cushing's Syndrome†

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=Abstract=**To test the hypothesis that hyperinsulinemia and/or abnormalities of RBC Na^+ transport are concerned in the pathogenesis of hypertension in Cushing's syndrome, we investigated the relationship between insulin level, RBC Na^+ transport and blood pressure in patients with Cushing's syndrome which is frequently associated with hyperinsulinemia, abnormalities of RBC Na^+ transport and hypertension. Both systolic and diastolic pressure were significantly higher in Cushing's syndrome than in normal subjects. Fasting serum insulin level was higher and both serum glucose and insulin responses after a 75g glucose load were significantly increased in patients with Cushing's syndrome as compared with normal subjects. Both RBC Na^+ concentration and passive Na^+ permeability were significantly lower but Vmax of Na^+, K^+-pump was significantly higher in patients with Cushing's syndrome than in normal subjects, while Vmaxs of Na^+-K^+ cotransport and Na^+-Li^+ countertransport were similar in the two groups. In multiple stepwise regression analysis for patients with Cushing's syndrome, fasting serum insulin level was directly correlated with both systolic and diastolic pressures (r=0. 52, p=0. 01; r=0. 51, p=0. 02, respectively). On the other hand,RBC Na^+ transport parameters showed little correlation with either systolic or diastolic pressures. These results suggest that hyperinsulinemia may contribute to the hypertension in Cushing's syndrome, but that the abnormalities of RBC Na^+ transport seen in Cushing's syndrome are not causally related to hypertension.**

Key Words: *Cushing's syndrome, Insulin, RBC Na^+ transport, Blood pressure*

INTRODUCTION

Cushing's syndrome, a condition of chronic glucocorticoid excess, is frequently associated with hypertension but the mechanism by which hypertension is produced still remains unclear (Boscaro *et al.* 1989; Gomez-Sanchez 1986; Ritchie *et al.* 1990; Saruta *et al.* 1986).

It has been known for a long time that glucocorticoids antagonize the action of insulin. Insulin resistance and compensatory hyperinsulinemia in Cushing's syndrome are clinically well recognized (Johnston *et al.* 1982; Rizza *et al.* 1982).

For several years recently many epidemiologic and clinical studies have shown that

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insulin resistance and compensatory hyperinsulinemia may have a role in the pathogenesis of hypertension (Ferrannini *et al.* 1987; Manicardi *et al.* 1986; Modan *et al.* 1985; Rose *et al.* 1986; Shen *et al.* 1988). The hypertensinogenic effects of insulin in promoting renal sodium resorption and enhancing sympathetic nervous system activity have been identified and thoroughly studied in several laboratories (DeFronzo 1981; Lansberg 1986, 1987). However, the evidence that insulin can actually raise blood pressure in the long-term is lacking (Mbanya *et al.* 1988; O'Hare 1988).

Both intracellular Na^+ concentration and cellular Na^+ transport are altered in essential hypertension (Blaustein 1984; Haddy 1983; Hilton 1986; Saito *et al.* 1984), and abnormalities in cellular Na^+ transport are also found in Cushing's syndrome (Ng *et al.* 1988; Wambach *et al.* 1982).

In view of these suggested associations between either hyperinsulinemia or abnormalities of RBC Na^+ transport and hypertension, the present study was undertaken to examine whether or not the very common state of hypertension in Cushing's syndrome may be related to hyperinsulinemia and the abnormalities of RBC Na^+ transport which are frequent companions of Cushing's syndrome. We investigated the relationship between insulin level, RBC Na^+ transport and blood pressure in patients with Cushing's syndrome.

MATERIALS AND METHODS

The present study was approved by the human study committee of Seoul National University Hospital and written informed consent was obtained from all study subjects. The participants in the study were 18 patients with Cushing's syndrome (Cushing's disease 13. adrenal adenoma 4. adrenal carcinoma 1; 5 men and 13 women, mean age 36 yrs) and 18 normal subjects (6 men and 12 women, mean age 42 yrs) who satisfied the following conditions ; no family history of diabetes mellitus and hypertension, no known diseases, a normal physical examination, blood pressure less than 140/90 mmHg, no drug consumption, normal results from an oral glucose tolerance test according to conventional criteria (National Diabetes Data Group 1979).

Venous blood was drawn after an overnight fast, and serum glucose and insulin levels were measured 30, 60, 90 and 120 minutes after a 75g oral glucose load. An oral glucose tolerance test couldn't be done in one patient with Cushing's syndrome because she had been on insulin injection. Blood pressure was measured at both arms twice with a mercury sphygmomanometer with subjects sitting for at least 5 minutes. The diastolic pressure was recorded at the disappearance of Korotkoffs sounds (phase 5). The mean of four readings was used in the analysis. Anthropometric measurements used in this study were body mass index (BMI ; Kg/M^2) and waist-to-hip circumference ratio (WHR ; Cm/Cm). WHR, an index of body fat distribution, was determined by measurement with a tape of waist circumference at the umbilical level and hip circumference at the greater trochanter level of the femur. Areas under the glucose and insulin curve were calculated by the following formula; area ={1/2(fasting value +120-minute value) + 30-minute value + 60-minute value + 90 -minute value} x 30 minutes.

Glucose was measured by glucose oxidase method. Insulin and cortisol were measured by RIA using commercial kits.

= Measurement of RBC Na^+concentration and RBC Na^+ transport =

RBC Na^+ concentration and RBC Na^+ transport were measured using the methods of Canessa *et al.* (1986) and Trevisan *et al.* (1986) with slight modification, as previously described in detail (Kim *et al.* 1990a). RBC has several well-characterized Na^+ transport systems. The ouabain-sensitive Na^+, K^+-pump is the main system maintaining the Na^+ electrochemical gradients across RBC membranes against the passive Na^+ diffusion. At least three additional transport systems have recently been described in human RBC membranes : so-called ouabain

-insensitive Na transport, 1) Na^+-K^+ cotransport (bumetanide or furosemide-sensitive Na^+ transport), 2) Na^+-Li^+ countertransport (Na^+-Na^+ countertransport), 3) passive Na^+ permeability.

This method is described briefly below.

1) Blood sampling and determination of initial electrolytes

10-15ml of blood was drawn by venipuncture with a heparinized plastic syringe. The cells after removal of the plasma and buffy coat were stored at 4℃ in a preservation solution containing (mmol/L) : 140 KCl, 10 NaCl, 2.5 KHPO4, pH 7. 4. The determination of RBC Na^+ concentration and RBC Na^+ transport was carried out 5-10 hrs after blood sampling. The preservation procedure described above has been documented as not influencing the RBC Na^+ transport rate for a period of 3 days (Trevisan *et al*. 1986). RBC were washed 4 times with 15ml cold choline chloride washing solution. An aliquot of cells was diluted with an equal volume of washing solution (fresh cell 50% suspension). The hematocrit of the suspension was determined twice. 0.1ml of fresh cell 50% suspension was added to 10ml of 0. 02% acationox (fresh cell suspension 1:101 dilution). This dilution is used for Na^+ determination. RBC Na^+ concentration was calculated using the following formula ;

$$\text{RBC } Na^+ \text{ concentration (mmol/L RBC)} = \frac{Na^+ \text{ concentration}(\mu\text{mol/L}) \times 101(\text{dilution factor})}{\text{Hct (\%)} \times 10}$$

2) Cell loading

1 ml of packed, washed cells was added to 5ml of cold (4℃) nystatin loading solution containing 4μl of freshly prepared nystatin solution (5mg nystatin in 1.3 ml of dimethylsulphoxide). The ionophore nystatin has a high partition coefficient for membrane lipids. The insertion of nystatin into the membrane is favored by low temperature (4℃). Under these conditions the membrane becomes permeable to cations and the nystatin holes will equilibrate internal and external cation concentrations. The cells were incubated for 20 minutes at 4℃ and protected from light. Cells were then centrifuged at 4℃ for 5 minutes, and the supernatant was removed and replaced with 5ml of cold (4℃) loading solution containing no nystatin for another 10 minutes. This loading procedure resulted in intracellular Na^+, K^+ and Li^+ concentrations of about 70 mmol/L, 70 mmol/L and 10 mmol/L respectively. These conditions became maximal activation (Vmax) of the Na^+, K^+-pump, the outward Na^+-K^+ cotransport and Na^+-Li^+ countertransport.

3) Nystatin removal

Nystatin is removed by raising the temperature to 32-37℃ and adding albumin which also binds ionophore released from the membrane. After incubation, the loaded cells were spun at room temperature and washed 4 times with a warm nystatin washing solution. The cell suspension was incubated in a 37℃ water bath for 5 minutes after the first and the second washes.

4) Removal of extracellular cations and final cell electrolytes determination

After removal of nystatin, the loaded cells were washed 4 times with cold choline chloride washing solution. The packed washed cells were then suspended with an equal volume of cold choline chloride washing solution. The hematocrit of the cell suspension was then determined twice. A 1/101 dilution was made using 0.02% acationox for Na^+ and Li^+ determination and a 1/1010 dilution was made for K^+ and hemoglobin determination. The hemoglobin content (g/L) in loaded cells didn't differ from that of the original cells by more than 2% in the present study.

5) Efflux measurement

- Vmax of Na^+, K^+-pump -

0.3ml of cell suspension was added to each of two tubes containing 7ml of pump media with and without ouabain. After the addition of the cell suspension to each tube, each flux medium was divided into 5 tubes (1.5ml for each tube). 2 tubes were centrifuged at 4℃, immediately (0-minute), and the supernatant was

then aspirated and saved for the Na^+ determination. 3 tubes were incubated at 37℃ for 20 minutes. The tubes were capped before starting and vortexed during incubation. Vmax of Na^+, K^+-pump was calculated by subtracting the Na^+ efflux in the medium with ouabain from the Na^+ efflux in the medium without ouabain.

- Vmax of Na^+-K^+ cotransport -

0.3ml of cell suspension was added to each of two tubes containing 7ml of cotransport media with and without furosemide. The furosemide (32mg) was dissolved fresh in 1 M Tris-Base 100μl. The furosemide concentration of this solution is 1 M. In order to have a final concentration of 1mM in the medium, this solution has to be diluted 1/1000 (in the media). After the addition of the cell suspension to each tube, each flux medium was divided into 5 tubes, 2 tubes are for the 0-minute time and 3 tubes for the 60-minute time. Vmax of Na^+-K^+cotransport was calculated by subtracting the Na^+ efflux in the medium with furosemide from the Na^+ efflux in the medium without furosemide.

- Vmax of Na^+-Li^+ countertransport -

0.8 ml of cell suspension was added to 8ml countertransport media with and without Na^+. After addition of the cell suspension to each tube, each flux medium was divided into 5 tubes, 2 tubes for the 0-minute time and 3 tubes for the 60-minute time. Vmax of Na^+-Li^+ countertransport was calculated by subtracting the Li^+ efflux in the medium without Na^+ from the Li^+ efflux in the medium with Na^+.

- passive Na^+ permeability -

Passive Na^+ permeability was calculated from the Na^+ efflux rate in the medium containing both ouabain and furosemide during measurment of Vmax of the Na^+-K^+ cotransport.

- Analysis -

Na^+, K^+ and Li^+ were measured using atomic absorption spectrophotometry.

= Follow-up =

Eight patients with Cushing's syndrome were reexamined within 1-2 months after trans-sphenoidal pituitary surgery (n=2), unilateral adrenalectomy (n=4), o'p'-DDD (n=1) and ketoconazole treatment (n=1) when their hypercortisolism was corrected. Six patients were taking prednisolone 5mg daily after surgery.

= Statistical analysis =

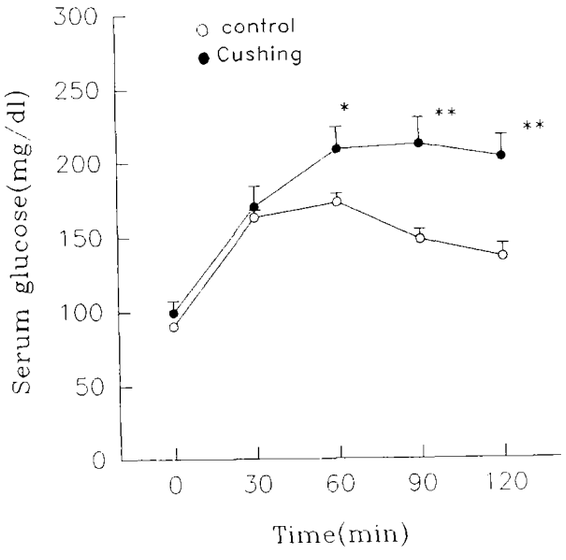
Results are expressed as mean ±S. D. Statistical analysis was done, using an SPSS statistical package. The serum glucose and insulin responses in the two groups during oral glucose tolerance test were compared in a two-way analysis of variance. Between-group differences in mean values were compared using student's paired or nonpaired t-tests. The simple and multiple regression analysis was performed according to standard methods. The results of two patients were not included in the simple and multiple regression analysis because one patient had been on insulin injection and another had a very high fasting serum glucose level and concomitant very low insulin level. A P value of less than 0. 05 was regarded as significant.

RESULTS

BMI, WHR, systolic and diastolic blood pressures, serum cholesterol and triglyceride levels were significantly higher in Cushing's syndrome than in normal subjects as shown in Table 1 ($p<0.05$) . Serum K^+ level which is an index of mineralocorticoid activity tended to be low in Cushing's syndrome but was not significantly different in the two groups (3.8 ± 0.6 vs. 4.1 ± 0.3 mmol /L).

The serum glucose and insulin levels after a 75g oral glucose challenge are shown in Fig. 1. During fasting, serum glucose levels were similiar in the two groups (99 ± 33 vs. 90 ± 10 mg/dl) but serum insulin level was significantly higher in patients with Cushing's syndrome as compared with normal subjects (19.5 ± 1.4 vs. 11.3 ± 4.0 U/ml, $p<0.01$). After 75g oral glucose load, both the serum glucose and insulin levels were significantly higher in patients with Cushing's syndrome (two-way analysis of variance, $p < 0.001$)

Fig. 2 and 3 show RBC Na^+ concen-



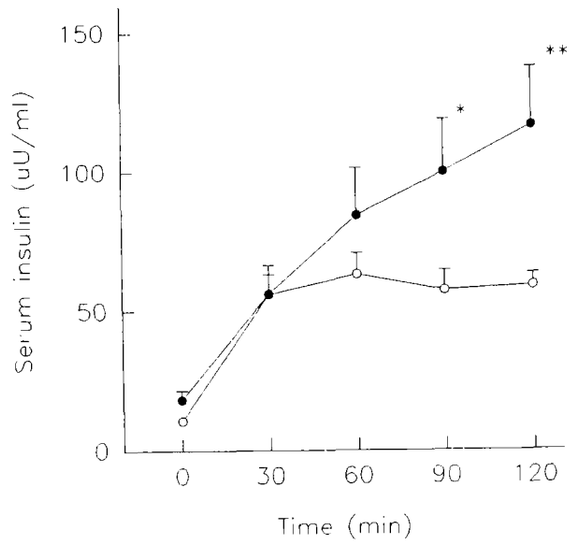


Fig. 1. Response of serum glucose and insulin to 75gm glucose in patients with Cushing's syndrome and normal subjects. Bar denotes 1 S. D.
*p 〈 0.05, **p 〈 0.01

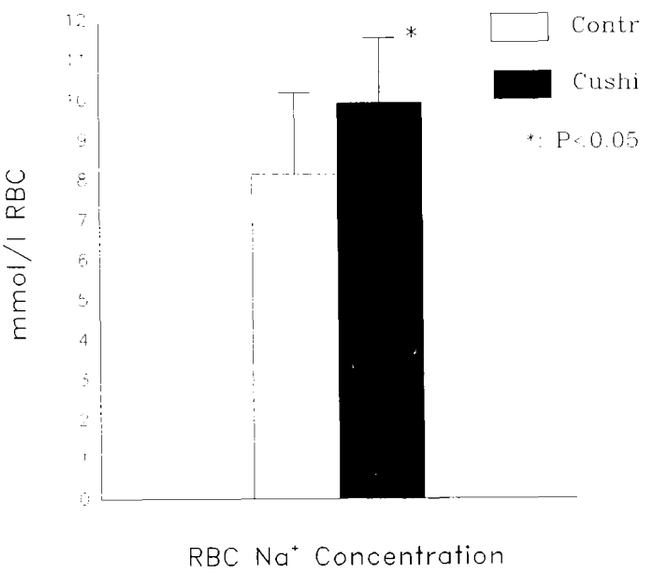


Fig. 2. RBC Na^+ concentration.
Bar denotes 1 S.D.

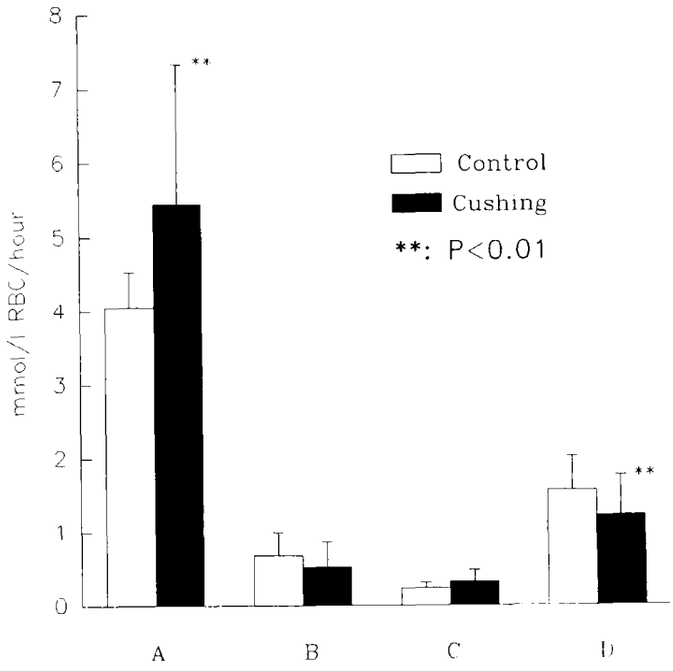


Fig. 3. RBC Na^+ transport parameters.
Bar denotes 1 S. D.
A: Vmax of Na^+-K^+ pump
B: Vmax of Na^+-K^+ cotransport
C: Vmax of Na^+-Li^+ countertransport
D: Passive Na^+ permeability

tration and RBC Na^+ transport parameters in the two study groups. RBC Na^+ concentration and passive Na^+ permeability were significantly lower in patients with Cushing's syndrome than in normal subjects (8.1 ± 2.0 vs. 9.9 ± 1.6 mmol/L RBC, p 〈 0.05 ; 1.40 ± 0.27 vs. 1.65 ± 0.26 mmol/L RBC/hr, p 〈 0.01, respectively) while Vmax of Na^+, K^+-pump was significantly higher in Cushing's syndrome (5.77 ± 1.31 vs. 4.05 ± 0.78 mmol/L RBC/hr, p 〈 0.01). However, the two groups had similar Vmaxs of Na^+-K^+ cotransport and Na^+-Li^+ countertransport (0.59 ± 0.28 vs. 7.28 ± 0.28 mmol/L RBC/hr ; 0.31 ± 0.16 vs. 0.23 ± 0.08 mmol/L RBC/hr, respectively).

Simple and multiple regression analyses were used to examine the relationship between blood pressure as dependent variable and

other independent variables. The results of simple regression analysis are shown in Table 2, WHR, 24-hr urine free cortisol, and fasting serum insulin were significantly associated with systolic blood pressure ($r=0.61$, $p < 0.05$; $r=-0.54$, $p < 0.05$; $r=0.64$, $p < 0.01$, respectively) and WHR, fasting serum insulin and insulin area with diastolic blood pressure ($r=0.59$, $p < 0.05$; $r=0.63$, $p < 0.01$; $r=0.52$, $p < 0.05$, respectively). The results of multiple regression analysis are shown in Table 3. When all the other independent variables were taken into consideration only fasting plasma insulin level and WHR significantly correlated with systolic and diastolic pressure ($r=0.52$, 0.47, $p=0.01$, 0.02 ; $r=0.51$, 0.45, $p=0.02$, 0.03 respectively).

Table 4 shows the results of multiple regression analysis for RBC Na^+ concentration and RBC Na^+ transport parameters when other independent variables, including age, BMI, WHR, blood pressure, 24-hr urine cortisol, serum K, fasting serum glucose and insulin levels, and glucose and insulin responses during oral glucose tolerance test were considered, only serum triglyceride and fasting serum glucose levels were strongly associated with Vmax of Na^+-K^+ cotransport ($r=0.61$, $p=0.01$; $r=-0.42$, $p=0.05$, respectively) and serum HDL-cholesterol level with passive Na^+ permeability ($r=0.58$, $p=0.02$).

Changes in clinical data, glucose and insulin response during oral glucose tolerance test, and RBC Na^+ transport parameters after treatment of Cushing syndrome are shown in Table 5. BMI, WHR, systolic and diastolic blood pressure and 24-hr urine free cortisol decreased significantly after treatment. Fasting serum glucose and insulin levels,and the glucose and insulin responses during oral glucose tolerance test tended to decrease, but RBC Na^+ concentration, Na^+-K^+ cotransport and passive Na^+ permeability didn't change significantly after hypercortisolism was corrected.

DISCUSSION

The present study suggests that insulin may be an important factor in blood pressure regulation in Cushing's syndrome, but that the abnormalities of RBC Na^+ transport seen in Cushing's syndrome have no relation to either hypertension or hyperinsulinemia .

More than 70% of patients with Cushing's syndrome had hypertension (Saruta *et al.* 1986). However the mechanism of hypertension is far from being understood. Several factors have been suggested to play a pathogenetic role in the hypertension of Cushing's syndrome (Boscaro *et al.* 1989; Gomez-Sanchez 1986; Ritchie *et al.* 1990).

It is clear that cortisol causes insulin resistance and compensatory hyperinsulinemia. Thus the ability of cortisol hypersecretion to initiate or augment diabetes mellitus has been noted in patients with Cushing's syndrome (Johnston *et al.* 1982; Rizza *et al.* 1982).

In the present study, the patients with Cushing's syndrome were found to have elevated blood pressure ,fasting hyperinsulinemia and both enhanced glucose and insulin response to 75g oral glucose load (Table 1, Fig. 1). Of 18 patients with Cushing's syndrome, 14 had hypertension (systolic blood pressure ≥ 160 mmHg and/ or diastolic blood pressure ≥

Table 1. Characteristics of study subjects

Characteristics	Cushing(n=18)	Control(n=18)
Age(years)	34 ± 11	42 ± 8
Sex(M/F)	5/13	6/12
BMI(Kg/M^2)	25.7 ± 4.38*	22.6 ± 2.1
WHR(Cm/Cm)	0.97 ± 0.11**	0.84 ± 0.06
SBP(mmHg)	152 ± 12**	114 ± 8
DBP(mmHg)	102 ± 10**	76 ± 5
Cholesterol(mg/dl)	220 ± 32**	157 ± 25
Triglyceride(mg/dl)	197 ± 77*	131 ± 71
HDL-chol.(mg/dl)	45 ± 6	39 ± 10
Serum Na^+(mmol/l)	141 ± 2	139 ± 2
Serum K^+(mmol/l)	3.8 ± 0.6	4.1 ± 0.3

Values are mean ±S.D.
BMI: body mass index
WHR: waist-to-hip circumference ratio
SBP: systolic blood pressure
DBP: diastolic blood pressure
HDL-chol: high density lipoprotein cholesterol
*:$p<0.05$ **:$p<0.01$ vs. control

95 mmHg) and 12 had impaired glucose tolerance or diabetes mellitus (data not shown).

Based on epidemiologic and clinical data, it would appear that insulin resistance and compensatory hyperinsulinemia are directly and possibly even causally related to hypertension (Ferrannini 1991; Modan *et al.* 1985; Slater 1991). Obesity was the first condition widely recognized to link insulin resistance and hyperinsulinemia to hypertension (Kaplan 1985; Lucas *et al.* 1985; Modan *et al.* 1985; Rose *et al.* 1986). Patients with high blood pressure are hyperinsulinemic on average (Ferrannini *et al.* 1987; Kim *et al.* 1990b; Singer *et al.* 1985), and some studies have reported a significant correlation between plasma insulin level and blood pressure (Lucas 1985; Manicardi *et al.* 1986). Finally, dietary alterations that produce insulin resistance and hyperinsulinemia in normal rats also led to the development of hypertension (Hwang *et al.* 1987). Two-well established effects of insulin, enhanced renal sodium resorption and stimulation of sympathetic nervous system activity, further suggest potential mechanisms that may relate hyperinsulinemia to hypertension (DeFronzo 1981; Lansberg 1986, 1987). However, the relationship between insulin levels and hypertension is not straightforward and insulin has not yet been demonstrated to directly cause a sustained rise in blood pressure (Mbanya *et al.* 1988; O'Hare 1988). Grugni *et al.* (1990) have reported that there was no correlation between insulin levels and hypertension in a group of obese subjects. Hall *et al.* (1990) failed to observe a rise in blood pressure when normal dogs were given a chronic insulin infusion with or without norepinephrine. They speculated that additional factors besides hyperinsulinemia and increased catecholamine levels must therefore play a role in the pathogenesis of obesity hypertension. In the present study, we have confirmed the finding that there is a significant correlation between insulin level and blood pressure, independent of age, obesity and other possible compound variables (Table 2, 3). Although a variety of factors operate, the role of hyperinsulinemia has received little attention in contributing to the rise in blood pressure in Cushing's syndrome. The present study is the first one that shows evidence for the associ-

Table 2. Correlation coefficients among various parameters in Cushing's syndrome (n=16)

	systolic BP	diastolic BP
Age	0.12	-0.15
BMI	0.20	0.22
WHR	0.61*	0.59*
24-hr urine free cortisol	-0.54*	-0.39
Serum K^+	0.27	0.21
Cholesterol	0.38	0.13
Triglyceride	0.25	0.29
HDL-cholesterol	0.23	0.45
Fasting serum glucose	0.20	0.23
2-hr postload serum glucose	-0.24	-0.18
Glucose area	-0.16	-0.12
Fasting serum insulin	0.64**	0.63**
2-hr postload serum insulin	0.40	0.48
Insulin area	0.47	0.52*
RBC Na^+ concentration	-0.20	-0.29
Vmax of Na^+-K^+ pump	-0.21	-0.05
Vmax of Na^+-K^+ cotransport	0.12	0.06
Vmax of Na^+-Li^+ countertransport	-0.08	-0.03
Passive Na^+ permeability	0.06	0.21

*: $p<0.05$; **: $p<0.01$

BMI: body mass index

WHR: waist-to-hip circumference ratio

Table 3. Multiple stepwise regression analysis for blood pressure in patients with Cushing's syndrome (n=16)

	Beta	T	p-value
Systolic BP			
1. Fasting Serum Insulin	0.52	2.19	0.01
2. WHR	0.47	2.67	0.02
Diastolic BP			
1. Fasting Serum Insulin	0.51	2.75	0.02
2. WHR	0.45	2.45	0.03

BP: blood pressure

WHR: waist-to-hip circumference ratio

ation of insulin level and blood pressure in Cushing's syndrome. In contrast to fasting serum insulin level, 2-hr postload insulin level and insulin area had no significant correlation with blood pressure. It has been generally recognized that hyperinsulinemia was observed in hypertensive subjects after they ingested glucose, whereas fasting insulin level was normal (Ferrannini *et al.* 1987; Manicardi *et al.* 1986; Rose *et al.* 1986; Singer *et al.* 1985), but in some studies elevated fasting insulin level has been noted as well (Lucas *et al.* 1985; Modan *et al.* 1985; Rocchini 1991).

While these data of insulin and blood pressure do not prove cause and effect, they support the concept that blood pressure regulation in Cushing's syndrome may be controlled in part by the tendency to secrete an increased amount of insulin. However the possibility that the association between insulin level and blood pressure in Cushing's syndrome may result from unrecognized compound variables which influence both insulin level and blood pressure can't be excluded. The mechanism by which hyperinsulinemia causes hypertension was not pursued in the present study. Further studies are needed to define the true role that hyperinsulinemia plays in blood pressure homeostasis in Cushing's syndrome.

In addition we found WHR, rather than BMI, was directly related to blood pressure in Cushing's syndrome when insulin and other compound variables were controlled (Table 2, 3). This result is consistent with the reports by Kaplan(1989) and Shear *et al.* (1987) who described how upper body obesity (central body fat deposit), measured by subscapular skinfold thickness or WHR, even in the absence of significant overall obesity was strongly related to hypertension. Our finding that both fasting serum insulin level and degree of obesity had a significant correlation with blood pressure is compatible with independent influences of hyperinsulinemia and obesity on blood pressure and agree with the findings of Manicardi *et al.* (1986) and Rose *et al.* (1986) who reported that obesity and hyperinsulinemia were independently associated with hypertension. On the contrary Lucas *et al.* (1985) observed that weight was not significantly related to blood pressure when the correlation was adjusted for age, serum glucose level and serum insulin level in obese women. These contradictory results may be due to the differences in the study subjects and the methods of measurement of obesity.

In the present study, as previously reported by Boscaro *et al.* (1989), we found no significant correlation with blood pressure and 24-hr urine cortisol or serum K level which is an index of glucocorticoid or mineralocorticoid activity (Table 2, 3). These results support the evidence that the hypertensinogenic class of steroid hormones which can be distinguished from glucocorticoid and mineralocorticoid may have a role in the pathogenesis of hypertension in Cushing's syndrome (Boscaro *et al.* 1989; Gomez-Sanchez 1986; Ritchie *et al.* 1990).

Despite some inconsistency in results, intacellular Na^+ concentration and cellular Na^+ transport are altered in essential hypertension. Since many of the cellular Na^+ transport systems that are present in kidney cells, sympathetic neurons or vascular smooth muscle cells are also present in RBCs and WBCs which are easily accessible and conveniently studied, much attention has recently been devoted to the study of Na^+ transport in RBCs and WBCs in patients with hypertension (Blaustein 1984; Haddy 1983;Hilton 1986). Among the abnormalities of RBC Na^+ transport that have received considerable attention in hypertension are the decreased Na^+, K^+-pump activity and the resulting elevated RBC Na^+ concentration, and increased Vmax of Na^+-Li^+ countertransport (Canessa *et al.* 1980; Saito *et al.* 1984) However there still exits some controversy about whether these abnormalities are merely genetic markers or if they may play a role in the pathogenesis of hypertension. It has also been reported that Na^+, K^+-ATPase activity is altered in patients with Cushing's syndrome and glucocorticoid-induced hypertension in rats (Ng *et al.* 1988; Jonses *et al.* 1982;Wambach *et al.*

1982), but its significance in the pathogenesis of hypertension is unknown.

We demonstrated that RBC Na^+, K^+-pump is more active and RBC Na^+ concentration is lower in Cushing's syndrome (Fig. 2, 3) as previously shown in human WBCs and RBCs (Ng *et al.* 1988; Wambach *et al.* 1982) . In contrast Boscaro *et al.* (1989) reported that there was no difference in Na^+, K^+-pump activity between Cushing's syndrome and normal subjects. The differences between the report by Boscaro *et al.* and the other reports including the present study may in part relate to the methods of measurement of Na^+, K^+-pump activity. The present study also showed that glucocorticoid excess state could lead to a reduction of passive Na^+ permeability but didn't affect Vmaxs of Na^+-K^+ cotransport and Na^+-Li^+ countertransport. We measured Na^+-K^+ cotransport and Na^+-Li^+ countertransport in Cushing's syndrome for the first time. These results are consistent with those of Ng *et al.* (1988) who reported that Na^+, K^+-pump activity was increased but RBC Na^+ concentration and ouabain-insensitive Na^+ transport were reduced in Cushing's syndrome. Ouabain-insensitive Na^+ transport is made up of Na^+-K^+ cotransport, Na^+-Li^+ countertransport and passive Na^+ permeability. However, in constrast to the findings of Ng *et al.* (1988) that there was a significant correlation between Na^+, K^+ pump activity and 24-hr urine cortisol, the correlation between them was not found in the present study (Table 2, 3). The reason for this discrepancy is unclear. As cortisol excess in Cushing's syndrome is associated with both decreased RBC Na^+ concentration and increased sodium pump activty the mechanism(s) leading to hypertension in Cushing's syndrome differs from that in essential hypertension where it has been generally recognized that intracellular Na^+ concentration is raised and Na^+, K^+-pump activity is decreased (Blaustein 1984; Haddy 1983; Hilton 1986). Our data that there was no significant correlation between RBC Na^+ transport and blood pressure in Cushing's syndrome (Table 2,3,4) also indicate that abnormalities of RBC Na^+ transport seen in Cushing's syndrome may not be causally related to hypertension.

Insulin has been shown to affect cellular Na^+ transport, in vivo and in vitro(Houston 1988; Tedde *et al.* 1988). It has also been suggested that insulin actions on cellular Na^+ transport may be involved in the causation of hypertension (Rocchini 1991; Tedde *et al.* 1988). However, we found no correlation of RBC Na^+ transport with insulin level in Cushing's syndrome, suggesting that hyperinsulinemia does not influence RBC Na^+ transport in Cushing's syndrome although permissive effect cannot be excluded(Table 4). Ng *et al.* (1988) found no correlation of Na^+, K^+-pump activity with fasting serum insulin level and suggested that the effect on the leukocyte sodium pump in Cushing's syndrome is unlikely to be secondary to insulin elevation.

The significant correlations of serum triglyceride and fasting serum glucose level with Na^+-K^+ cotransport and HDL-cholesterol with passive Na^+ permeability were observed in the present study(Table 4). There is evidence from cross-sectional studies that plasma lipid levels affect the cellular ion transport (Adragna *et al.* 1985; Hunt *et al.* 1986; Kim *et al.* 1990a; Ko *et*

Table 4. Multiple stepwise regression analysis for RBC Na^+ transport in patients with Cushing's syndrome (n=16)

	Beta	T	p-value
RBC Na^+ concentration			
No variable included			
Vmax Na^+-K^+ cotransport			
1. triglyceride	0.61	2.87	0.01
2. fasting serum glucose	-0.42	-2.18	0.05
Vmax Na^+-K^+ pump			
No variable included			
Vmax Na^+-Li^+ countertransport			
No variable included			
Passive Na^+ permeability			
1. HDL-chol.	0.58	2.69	0.02

HDL-chol: high density lipoprotein cholesterol

al. 1991). It may be assumed that fatty acid or cholesterol incorporated into RBC membrane interacts with membrane components which are involved in Na^+ transport. In contrast to the positive correlation of HDL-cholesterol with passive Na^+ permeability and the absence of correlation between plasma lipid level and Na^+-Li^+ countertransport seen in the present study, Hunt *et al.* (1986) found a negative correlation between HDL-cholesterol and passive Na^+ permeability and a positive corrlation between tryglyceride and Na^+-Li^+ countertransport, and Adragna *et al.* (1985) reported a negative correlation between HDL-cholesterol and Na^+-Li^+ countertransport. The reason for this discrepancy is not readily apparent, but may relate to the differences in study subjects and methods. The negative correlation of fasting serum glucose level with Vmax of Na^+-K^+ cotransport in the present study was unexpected. This can't be explained.

The present study shows that when the Cushing's patients were successfully treated, weight and blood pressure decreased significantly, and the glucose and insulin responses to oral glucose load tended to decrease whereas RBC Na^+ transport did not change significantly(Table 5) . This finding is not surprising considering that an alteration in RBC membrane Na^+ transport originates during erythropoiesis and remains during the life of the cell (about 120 days). The patients were reexamined within 1-2 months after treatment in the present study.

In conclusion, the results of the present study indicate that hyperinsulinemia is closely linked to hypertension in Cushing's syndrome, but that the abnormalities of RBC Na^+ transport seen in Cushing's syndrome are not related to hypertension or hyperinsulinemia

Table 5. Comparison before and after treatment of Cushing's syndrome

	Before treatment(n=8)	After treatment(n=8)
BMI(kg/M^2)	24.7±5.4	22.4±5.5**
WHR(Cm/Cm)	0.97±0.12	0.89±0.11*
Systolic BP(mmHg)	154±11	121±16**
Diastolic BP(mmHg)	103±10	83±11*
24-hr urine free cortisol(μg/day)	614±606	40±36*
Serum glucose(mg/dl)		
Fasting	90±11	83±10
2-hr postload	187±36	137±55*
Glucose area(mg.min×10^2/dl)	193±17	174±37
Serum insulin(μU/ml)		
Fasting	18.8±11.1	13.9±7.5
2-hr postload	106.4±48.2	95.9±81.3
Insulin area(μU.min×10^2/ml)	85.1±50	80±51
RBC Na^+ concentration (mmol/l RBC)	7.53±2.13	8.70±1.74
Vmax of Na^+-K^+ cotransport(#)	0.40±0.22	0.35±0.21
Vmax of Na^+-K^+ pump(#)	5.03±2.44	4.59±1.12
Vmax of Na^+-Li^+ countertransport(#)	0.27±0.15	0.24±0.10
Passive Na^+ permeability(#)	1.15±0.49	1.46±0.33

*: $p<0.05$ **: $p<0.01$ vs before and after treatment
BMI: body mass index
WHR: waist-to-hip circumference ratio
BP: blood pressure
#: mmol/L RBC/hour

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