UNIVERSITI SAINS MALAYSIA
PROJEK PENYELIDIKAN JANGKA PENDEK
LAPORAN AKHIR

ROLE OF MAGNESIUM IN ESSENTIAL HYPERTENSION

PENYELIDIK

PROF. MADYA DR. M.D. FAZLUL KARIM

PENYELIDIK BERSAMA

PROF. MADYA DR. HABINDARJEET SINGH
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PROF. MADYA DR. R. G. SIRISINGHE

BAHAGIAN PENYELIDIKAN & PEMBANGUNAN

CANSELORI

UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) **Nama Penyelidik:** Prof. Madya Dr. M.D Fazlul Karim

Nama Penyelidik-Penyelidik Lain ***(Jika berkaitan)*** :

1. Prof. Madya Dr. Harbindarjeet Singh
2. Dr. Abd. Rashid Abd. Rahman
3. Prof. Madya Dr. R.G. Sirisinghe

2) **Pusat Pengajian/Pusat/Unit :** Sains Perubatan , Department of Physiology

3) **Tajuk Projek:** Role of Magnesium in Essential Hypertension
"Mengkaji Peranan Magnesium Dalam Hipertensi"

4) (a) **Penemuan Projek/Abstrak**

*(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris** Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).*

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See Appendix 1 (attached).....

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(b) **Senaraikan Kata Kunci yang digunakan di dalam abstrak:**

Key words

Bahasa Malaysia	**Bahasa Inggeris**
.....	Magnesium, Hypertension, renin
.....	activity, Renin concentration,
.....	Aldosterone, plasma proteins,
.....	Serum, erythrocyte, sodium,
.....	calcium
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....

5) **Output Dan Faedah Projek**

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

This project has produced four abstracts, the copies of which are attached. Beside, a paper has been submitted for publication. The current status of magnesium in essential hypertension has already been stated in Appendix I (attached).

(b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.**
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

Research finding of this project have projected the basic information on the role of electrolytes, particularly magnesium, in untreated essential hypertensive patients among Malays.

(c) **Latihan Gunatenaga Manusia**

i) Pelajar Siswazah not applicables

ii) Pelajar Prasiswazah: not applicable

iii) Lain-Lain : trained Pn. Asiah Abu Bakar, a technologist in Physiology to estimate plasma renin activity (PRA) and plasma renin concentrate (PRC) by using radio immuno-essay method (RIA). she is further trained to estimate magnesium in serum and erythrocytes colorimetrically by using micro-flow spectrophotometer.

6. **Peralatan Yang Telah Dibeli:**

Following equipments has been purchased:

a) one sphygmomenometer

b) one bathroom weighing scale

c) calculator

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

untuk pengesahan &
tandatangan Dekan

DATO' PROFESOR MUSTAFFA EMBONG
DEKAN/PROFESOR PERUBATAN
PUSAT PENGAJIAN SAINS PERUBATAN
UNIVERSITI SAINS MALAYSIA
16150 KUBANG KERIAN
KELANTAN.

Appendix 1

Final report on the project entitled,"Role of Magnesium in essential hypertension"

Introduction

Beneficial role of magnasium supplementation in prevention of complications in coronary artery disease (Iseri,1984) in ischaemic heart disease (Altura, 1988) and in the treatment of patients with AMI (Abraham, 1990) has been well documented. One study showed that magnesium supplementation reduced arterial blood pressure in patients with hypertension (Dykner& Waster 1983). Renin profiling, nevertheless,has revealed higher and lower magnesium levels in low renin and high renin hypertensives respectively (Resnick LM et al 1983). The significance of this seems to be that the influence of magnesium intake on blood pressure may differ according to the underlying state of magnesium metabolism in hypertensive patients. These observations remain largely unconfirmed. Recently it has been reported that a disordered metabolism of magnesium and calcium ions is present in women with pregnency induced hypertension (Singh, HJ 1993)

So the objective of this study was to investigate the magnesium status in serum, erythrocyte and in urine of untreated hypertensive patients. Work was further extended to investigate the relationship between serum and urinary magnesium levels,plasma renin activity (PRA) and plasma renin concentration (PRC) in normotensive and age and sex-matched untreated hypertensives.

Methodology :

Subjects

Male and female, divided into two groups
Control group(n=34; male 17, female 17)
Hypertensive group (n= 34 ; male 17, female 17)
from 20 to 60 year of age
Race: 98% malay and 2% chinese

Selection of cases and blood pressure measurement

Patients were selected from out-patient department and also from blood bank.
These patients were not diagnosed and treated for hypertension before.
3 successive readings of arterial blood pressure were taken under identical conditions at an interval of 6 hrs.
Systolic BP>150 mmHg, Diastolic BP > 90 mmHg

Sample collection:

15 ml of venous blood obtained.
Mg2+,Ca2+,Na+, K+ and creatinine were estimated in serum.
Mg 2+ was also estimated in erythrocytes.
Plasma was used for determination of Renin activity (PRA)
and Renin concentration (PRC)
Serum was also used for estimation of aldosterone and total
protein concentration.
(Renin was estimated by the rate of generation of angiotensin 1 (A1).
A1 was determined using RIA

Urine collection

24 hr. urine was collected.
urinary excretion of all the above electrolytes (Mg2+, Ca2+, Na+,K)
and creatinine were estimated.

Magnesium determination:

Erythrocyte, serum and urinary magnesium were determined colorimetrically by using microflow spectrophotometer and commercially available reagents.

GFR

GFR was calculated by using endogenous creatinine clearance.

All the above tests were carried out in both normotensive and hypertensive subjects.

Results and Discussion:

No significant differences were evident in serum and erythrocyte magnesium concentration between the two groups.Serum sodium concentration was significantly higher in the hypertensive (146.9± 0.43 Vs 142.5±0.64) where as there were no significant differences in plasma aldosterone level between the two groups. 24 hr. urinary excretion of sodium and magnesium in hypertensive subjects were lower compare to that of the normotensisubjects, but the differences were not significant. Serum ionised calcium level was significantly lowerand total plasma protein were significantly higher in hypertensive subjects. Low serumionised calcium in hypertensive patients is probably due to more congugation of calcium with plasma protein. We do not however, have any explanation at this stage as to why total plasma protein rises in hypertensives. In addition, we were also unable to demonstrate a significant correlation between PRA and magnesium concentration. Interestingly however, serum magnesium concentration revealed a weak but significant inverse correlation with plasmarenin concentration [$r = -0.39$; $P < 0.05$]

Results of the above parameters are shown by bars comparing with the control in the figure 1 to figure 17. Significant differences have been shown by p value.

Summary of results:

a) No significant differences were evident in serum and erythrocyte magnesium and serum potassium concentration between the two groups.

b)Serum sodium concentration was significantly higher in untreated hypertensive patients.

c)Daily urinary excretion of sodium and magnesium were lower in patients with essential hypertension.

d)No significant differences were evident in urinary excretion of calcium and potassium between the two groups.

e) No significant differences were evident in serum aldosterone level between the two groups.

f) Serum ionised calcium was significantly lower in subjects with essential hypertension.

g) Total serum protein was significantly higher in subjects with essential hypertension.

h) No significant differences were evident in creatinine clearance between the two groups.

I) Plasma renin activity (PRA) did not show any correlation with serum magnesium and serum calcium concentration in hypertensive subjects.

j) Serum magnesium concentration showed a significant negative correlation with plasma renin concentration ($r=-0.39$; $p < 0.05$)

Conclusions

1. Our observations fail to confirm a definite relationship between magnesium and hypertension or between magnesium and renin activity.

2. The data however, confirms previous reports of decreased serum ionised calcium in some hypertensives,probably secondary to a raised serum protein concentration.

3. Indicates a probable disturbed calcium , sodium and perhaps magnesium metabolism in some hypertensives.

4. The precise nature of the disturbance is unclear and it is also not clear at this stage whether these observations are a consequence of raised blood pressure or a cause of the hypertension.

Acknowledgement:

We wish to thank Puan Asiah abu Bakar and Puan Noriah Othman for their technical assistance and support.

This study was supported by a University Sains Malaysia short term grant

References:

1.Iseri LT (1984) Magnesium in coronary artery disease. Drugs 28 (supple) 151

2. Altura BM (1988) Ischaemic heart disease and magnesium. Magnesium 7,57

3. Dykner T & Wester PO (1983) Effect of magnesium on blood pressure Br.Med.J 286, 1847.

4. Resnick LM et al (1983)Divalent cations in essential hypertension New Eng. J Med. 309,(15) ,888

5Abraham AS (1990) Treatment of patients with AMI with IV magnesium. Magnes Trace. Elem (Switzerland) 9 (4) 177

6.Singh HJ et al (1993) Serum level and urinary excretion of magnesium , calcium and electrolytes in mild pregnency induced hyprttension. Hypertensiom in Pregnency. B 121; 119-126

Fig.1

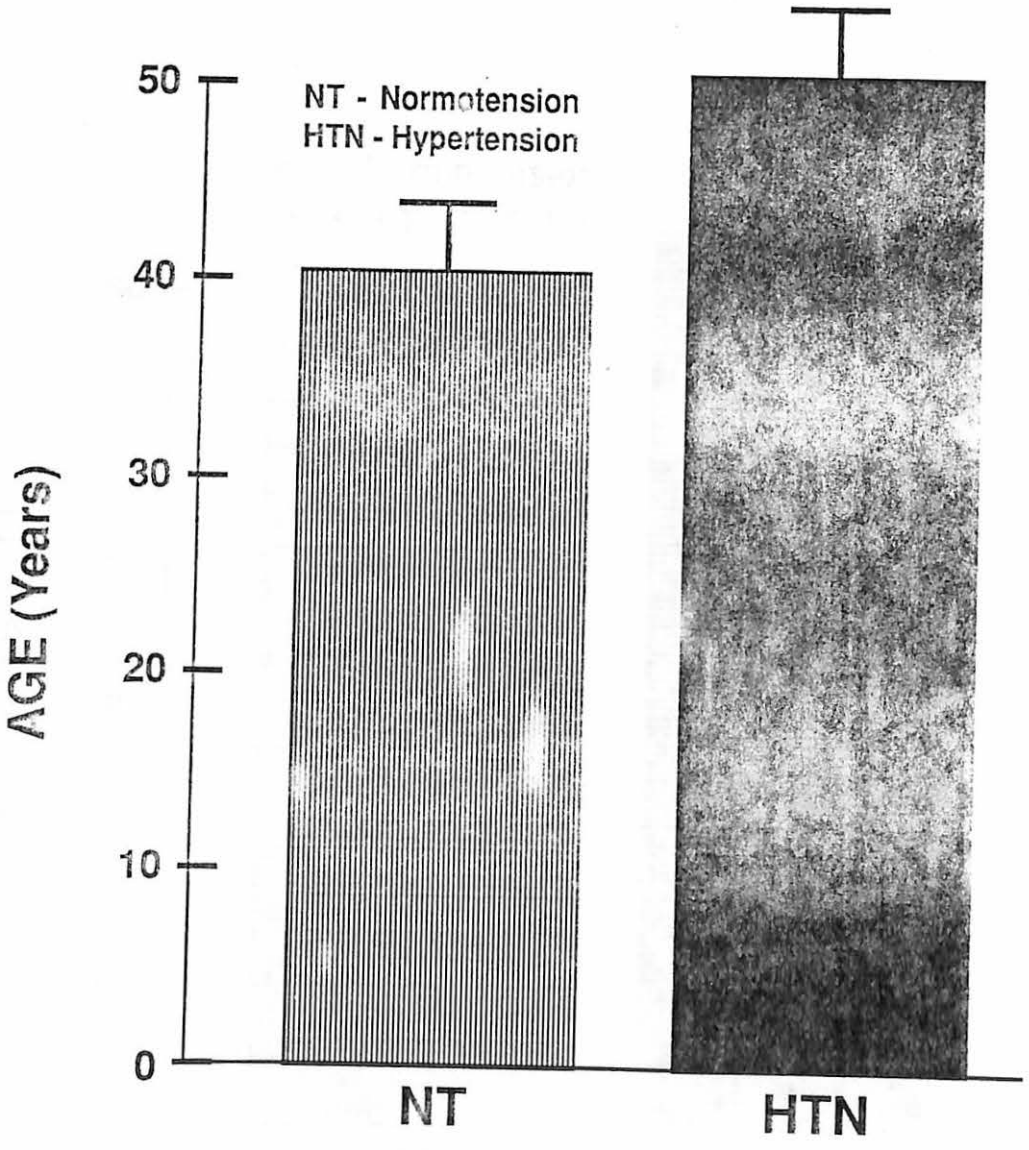
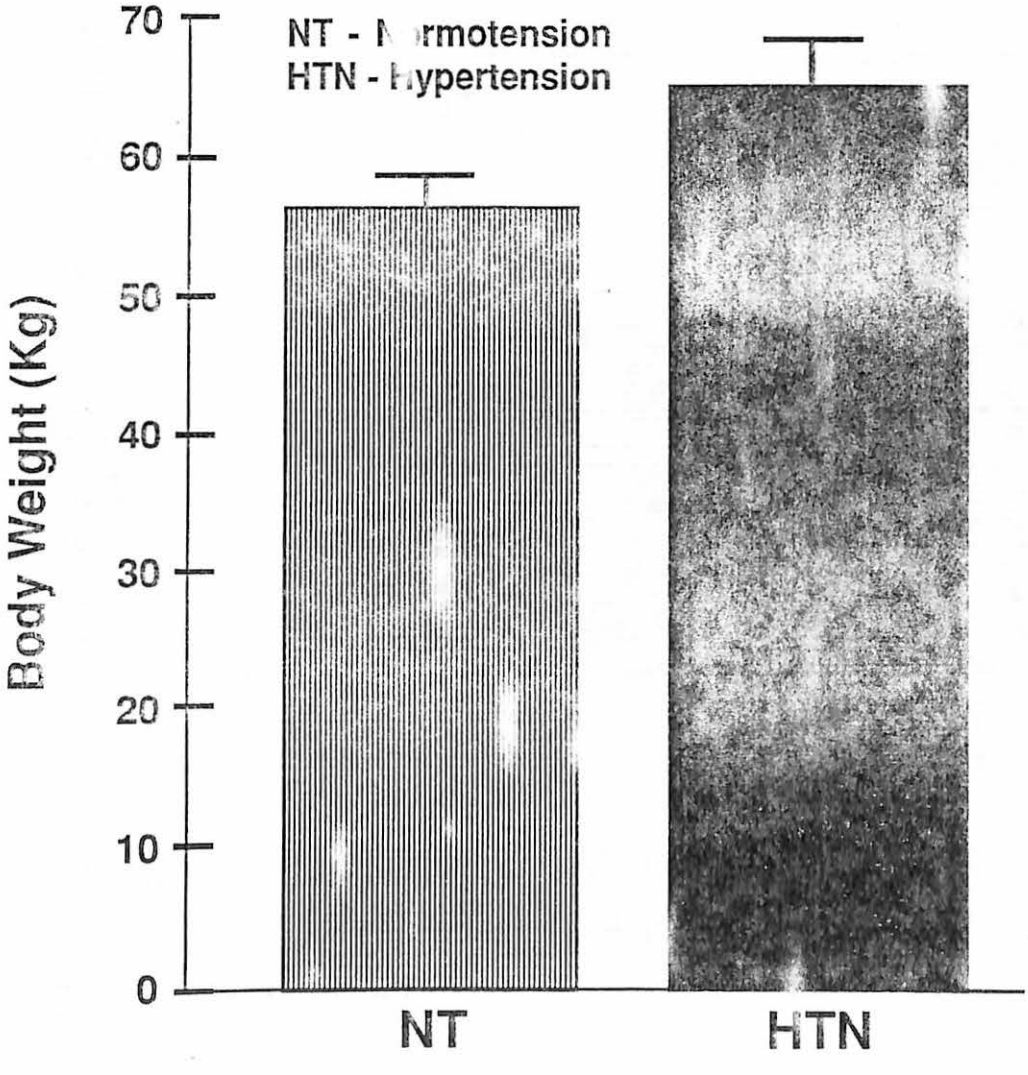

NT - Normotension
HTN - Hypertension
AGE (Years)
50
40
30
20
10
0
NT
HTN

Fig. 2


NT - Normotension
HTN - Hypertension
Body Weight (Kg)
70
60
50
40
30
20
10
0
NT
HTN

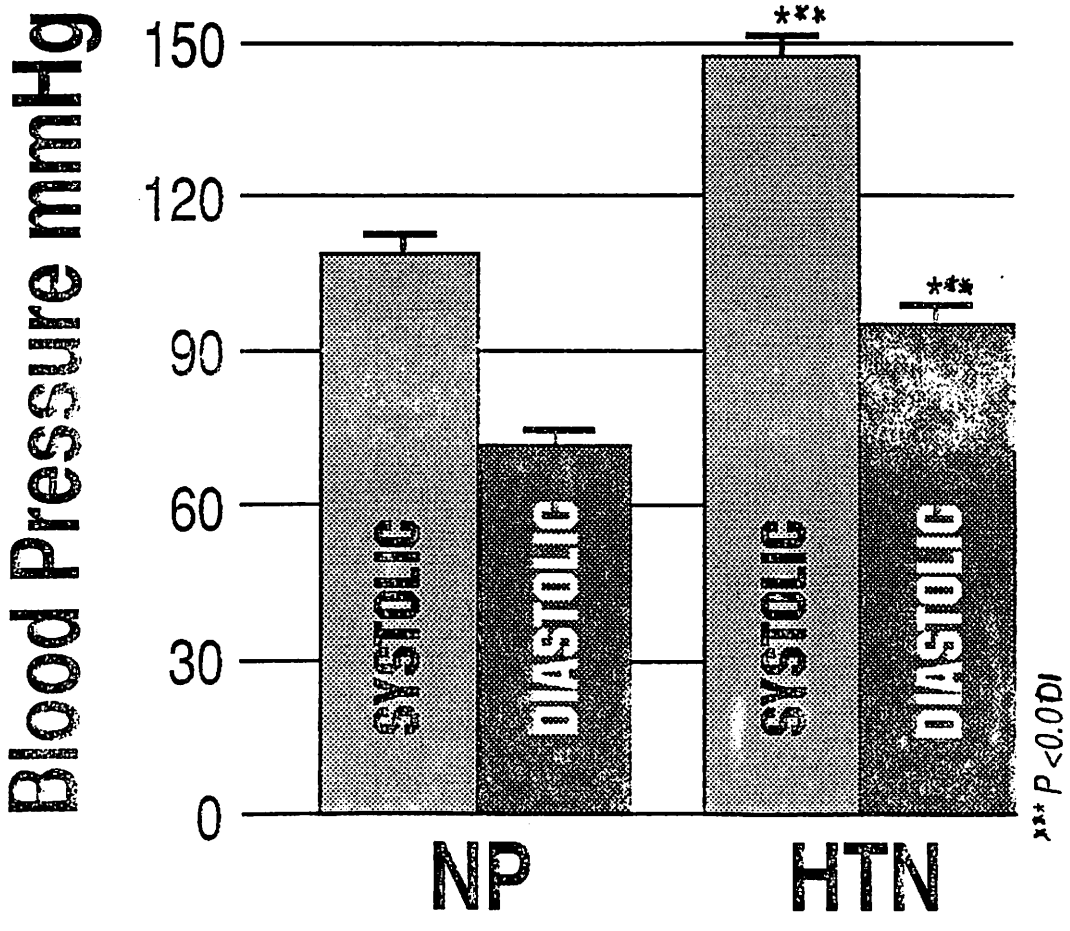


Fig.3

Mean systolic and diastolic blood pressures in normotensive (NP) and in hypertensive subjects (HTN).

*** P<0·001

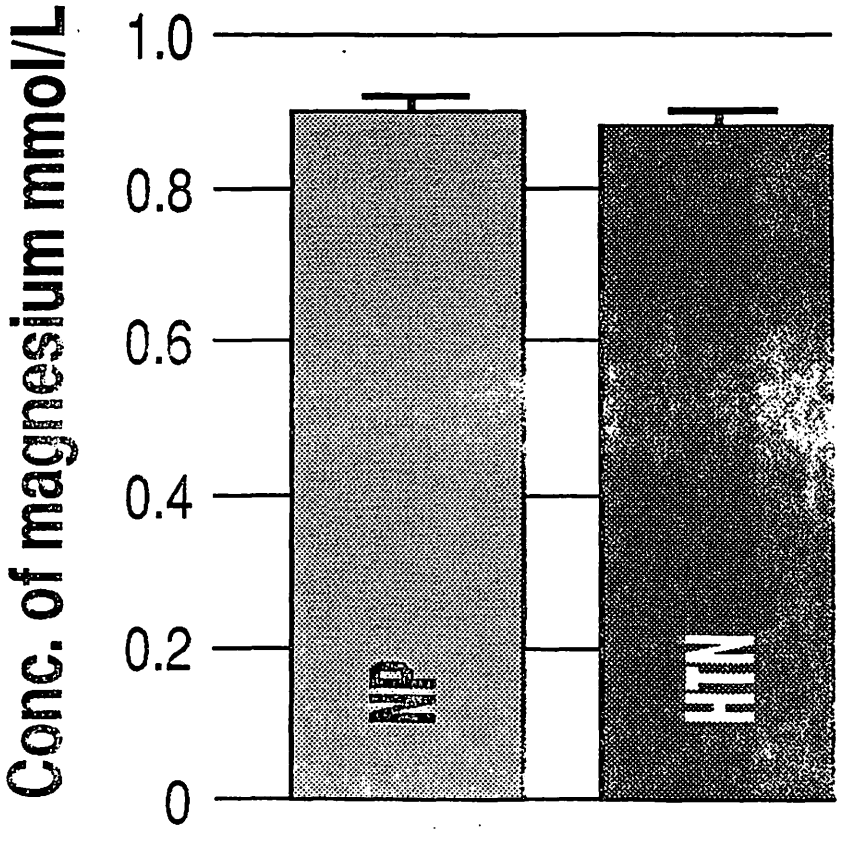


Fig.4

Serum magnesium concentrations in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

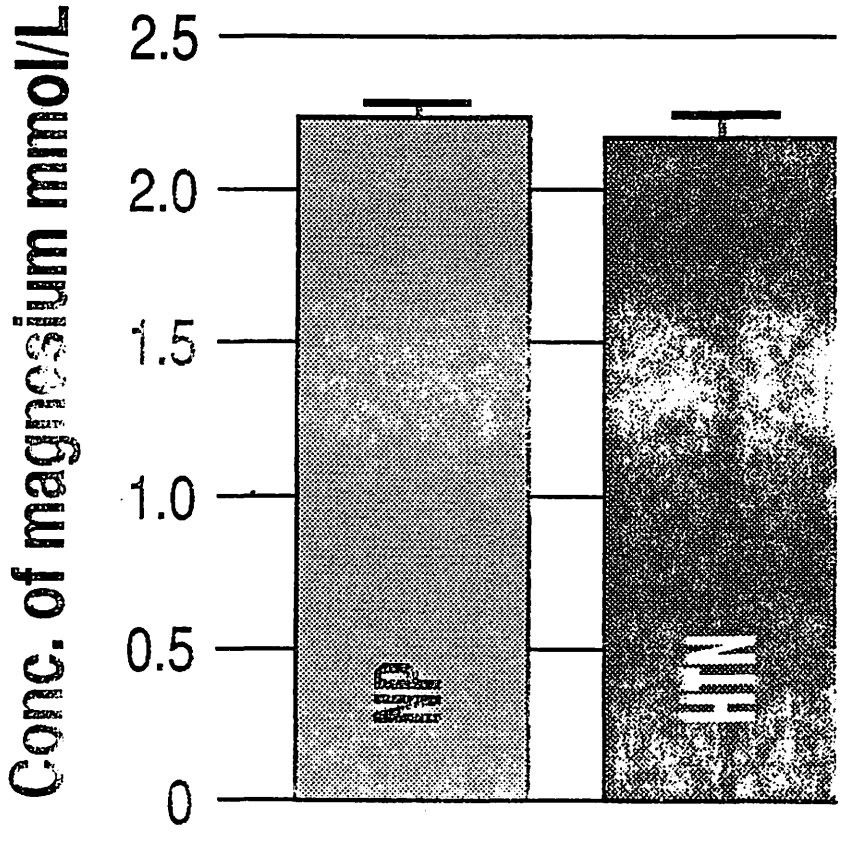


Fig.5

Erythrocyte magnesium concentrations in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

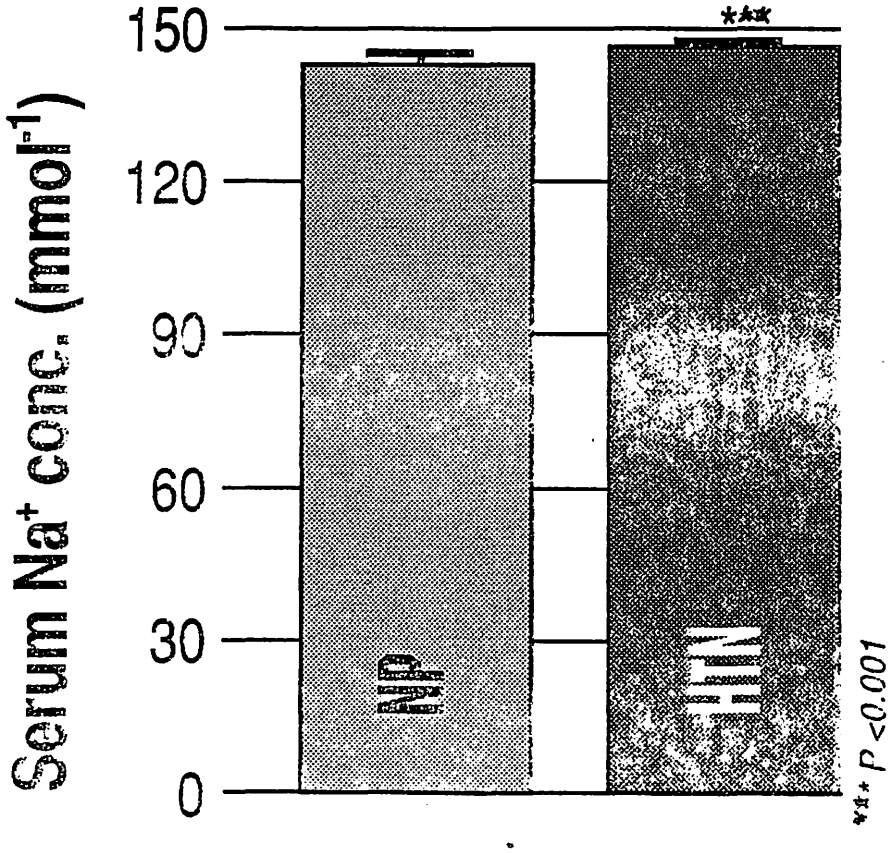


Fig. 6

Serum sodium . concentrations in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

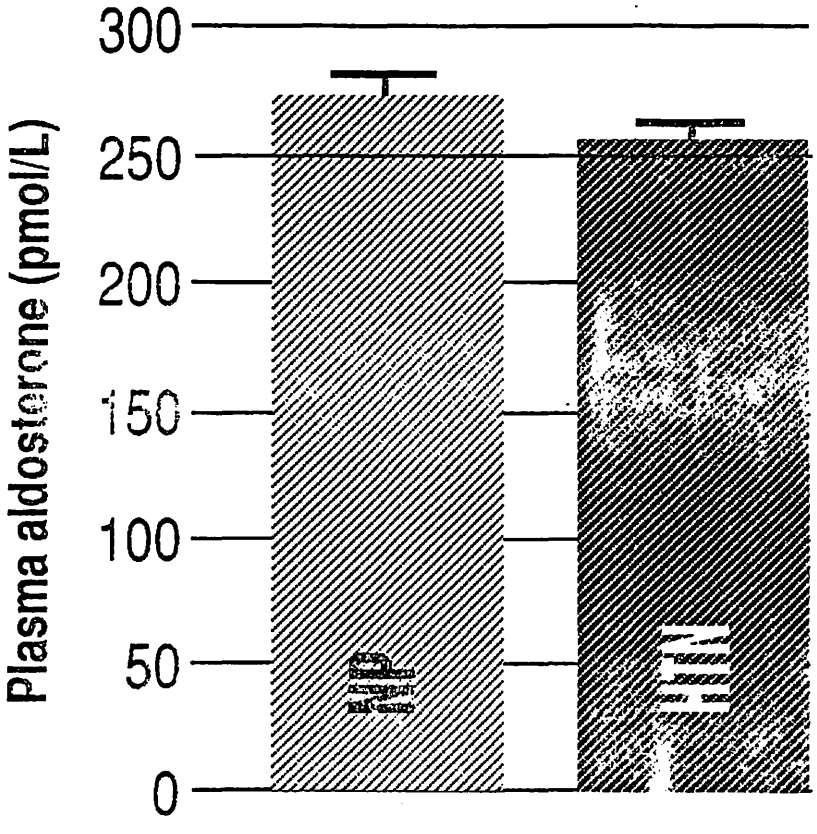
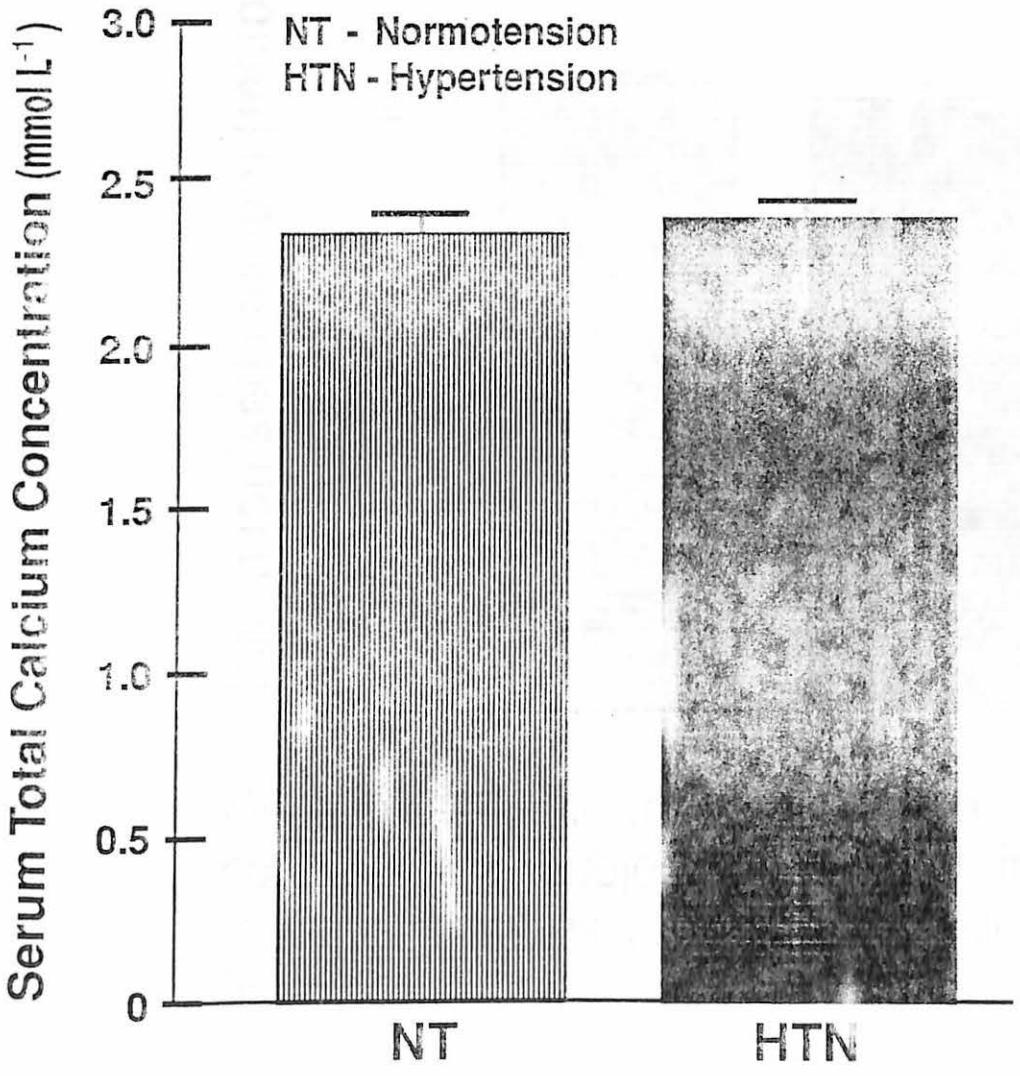


Fig.7

Plasma aldosterone concentrations in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

Fig. 8


NT - Normotension
HTN - Hypertension
Serum Total Calcium Concentration (mmol L-1)
3.0
2.5
2.0
1.5
1.0
0.5
0
NT
HTN

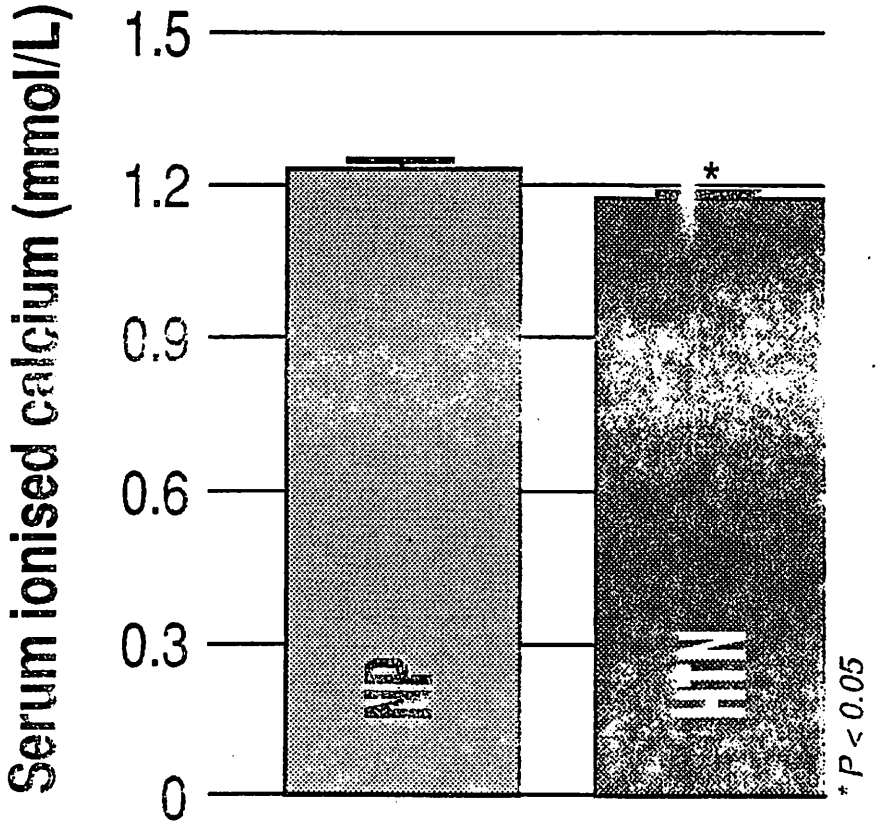


Fig.9

Mean ionised calcium concentrations in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

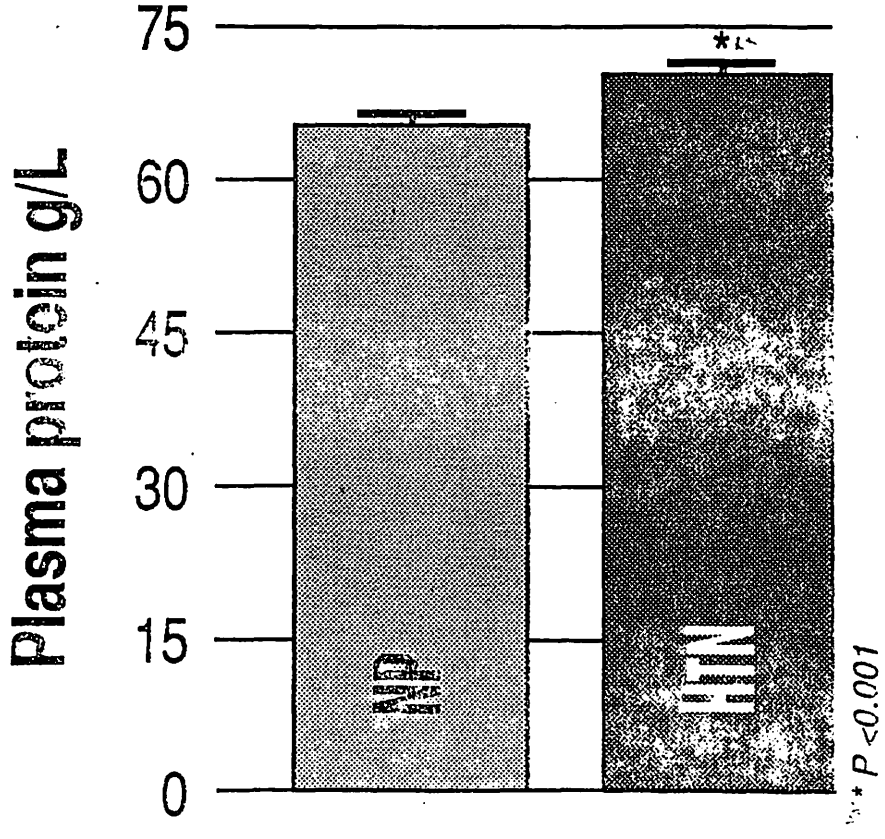


Fig. 10 Total plasma protein in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

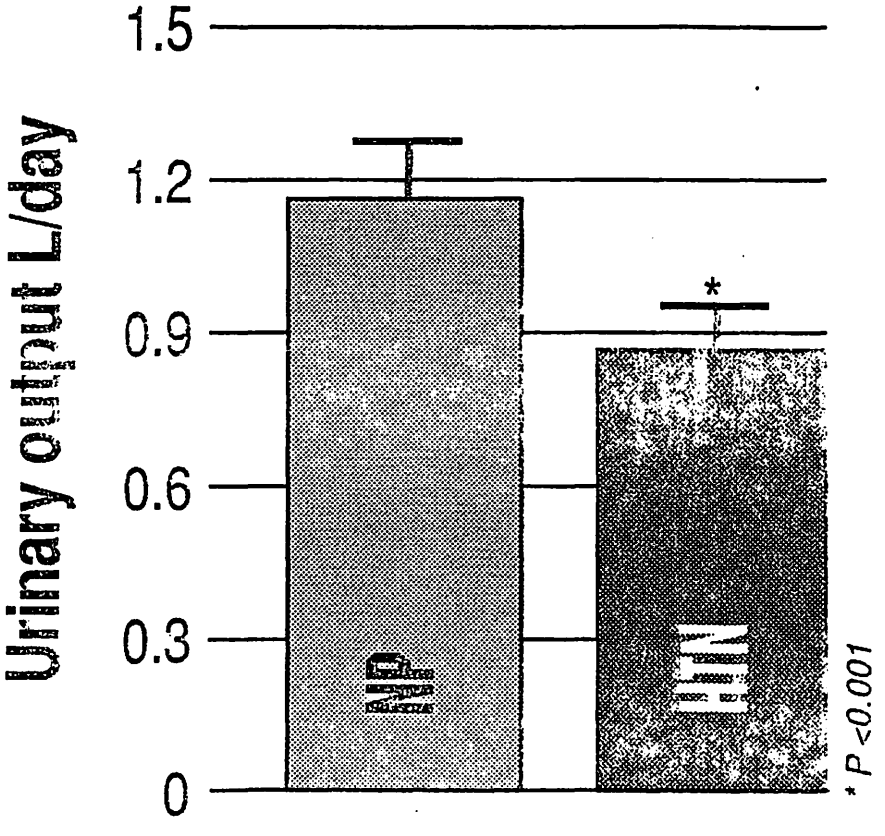


Fig.11

24 hr urinary output in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

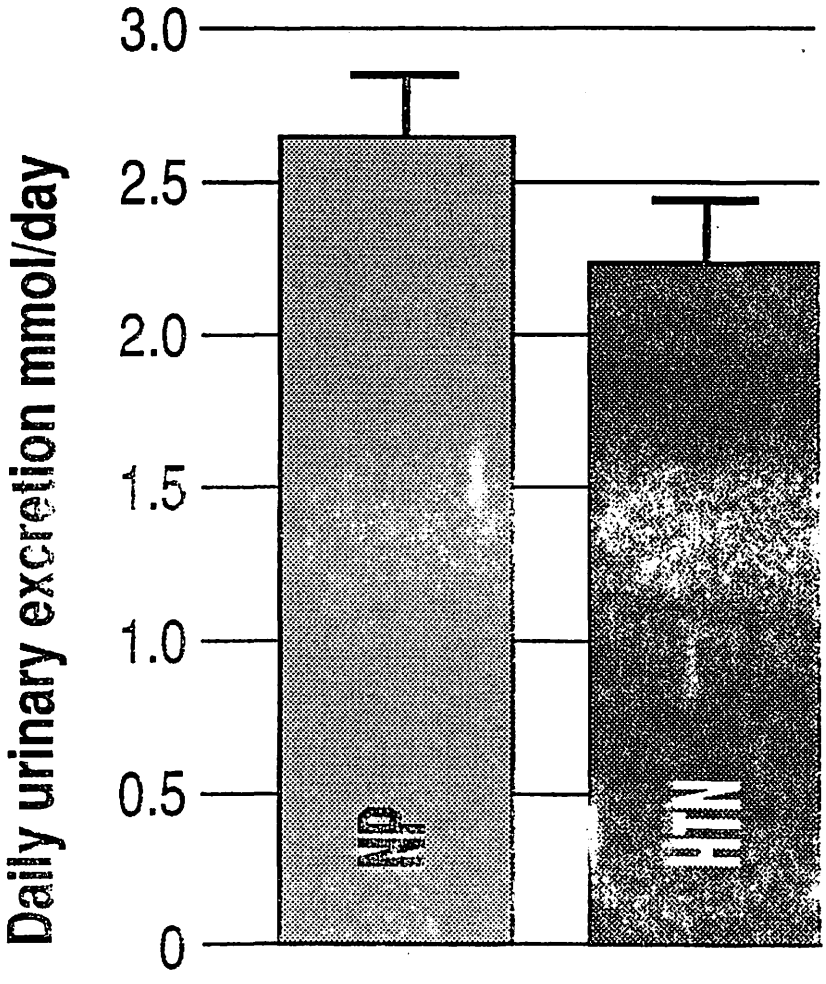


Fig.12 24 hr urinary excretion of magnesium in normotensive subjects (NF) and in subjects with essential hypertension (HTN).

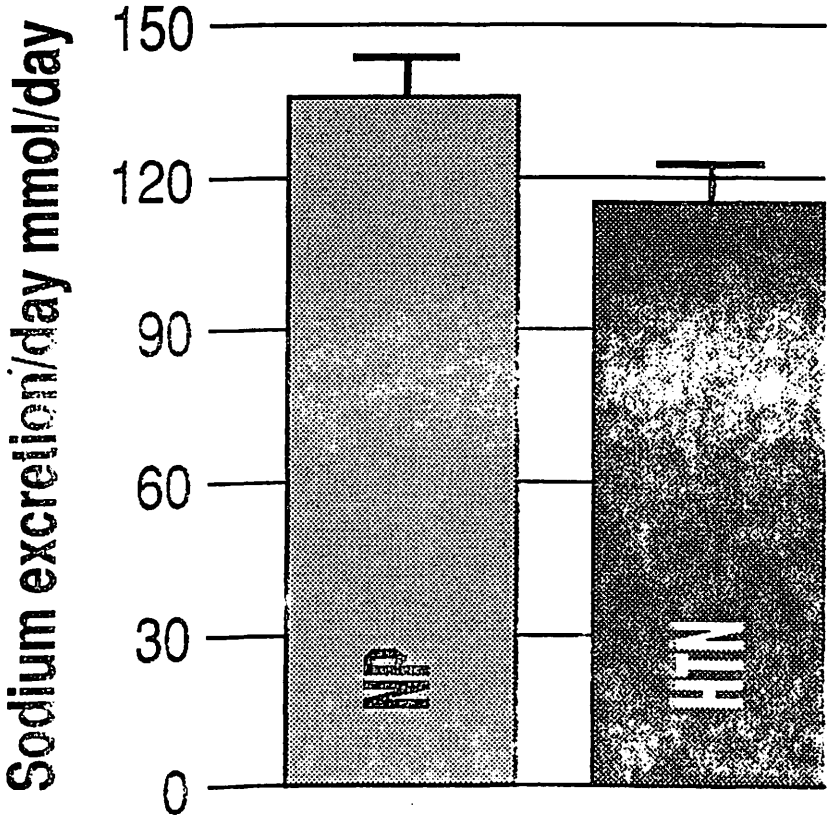


Fig.13

24 hr urinary excretion of sodium in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

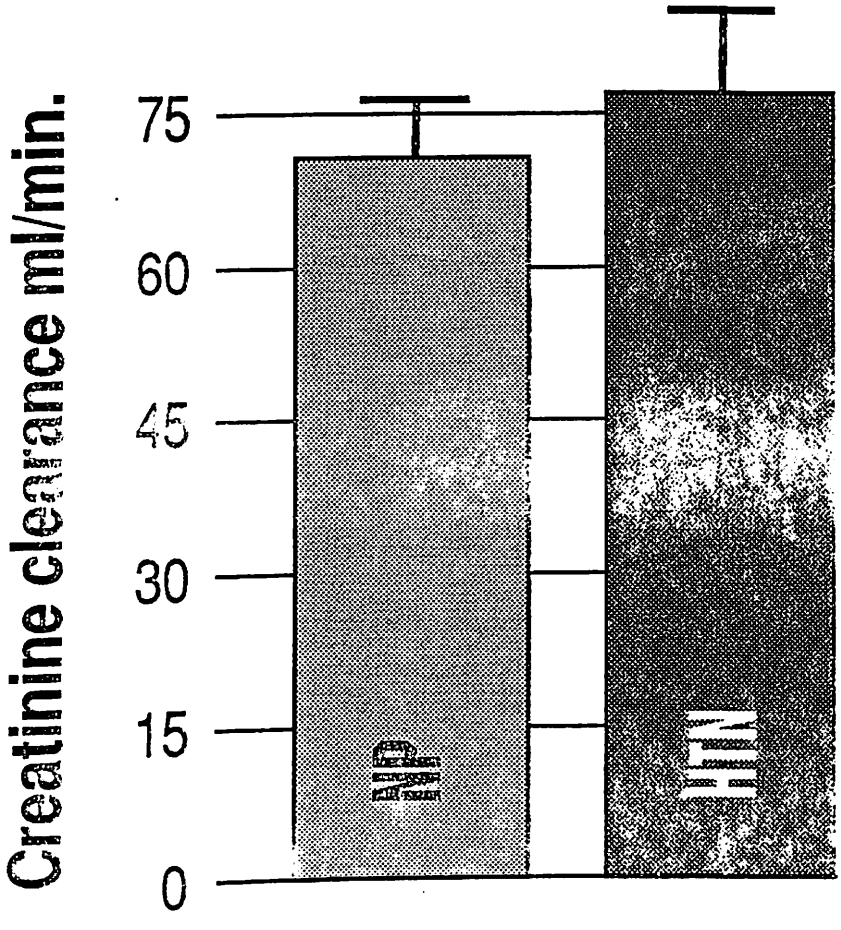


Fig.14

Mean creatinine clearance in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

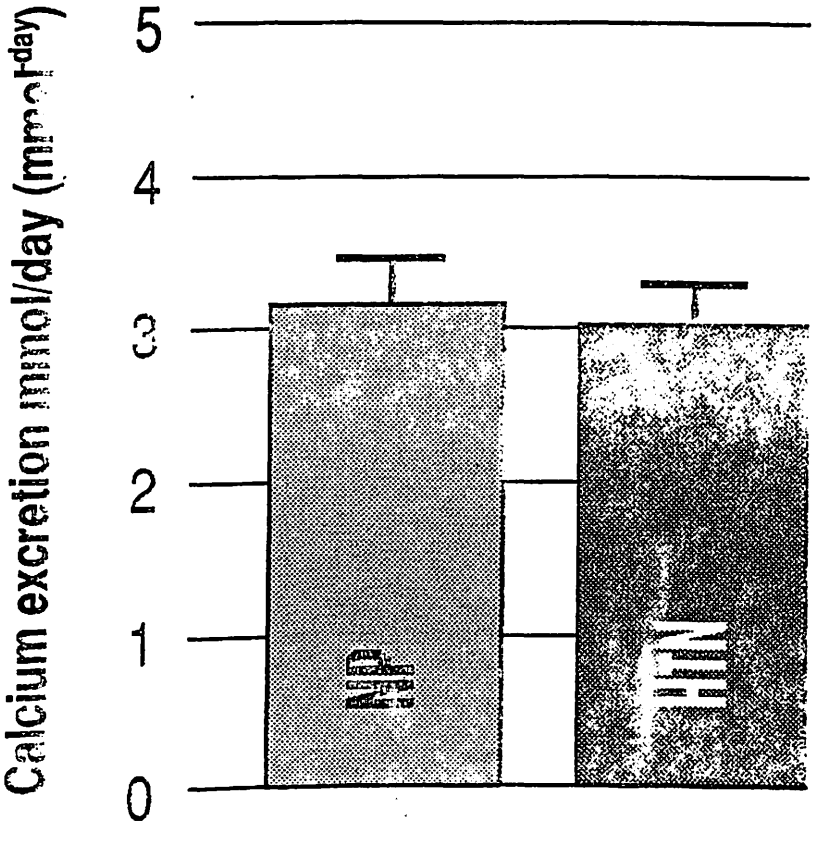


Fig. 15

24 hr urinary excretion of calcium in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

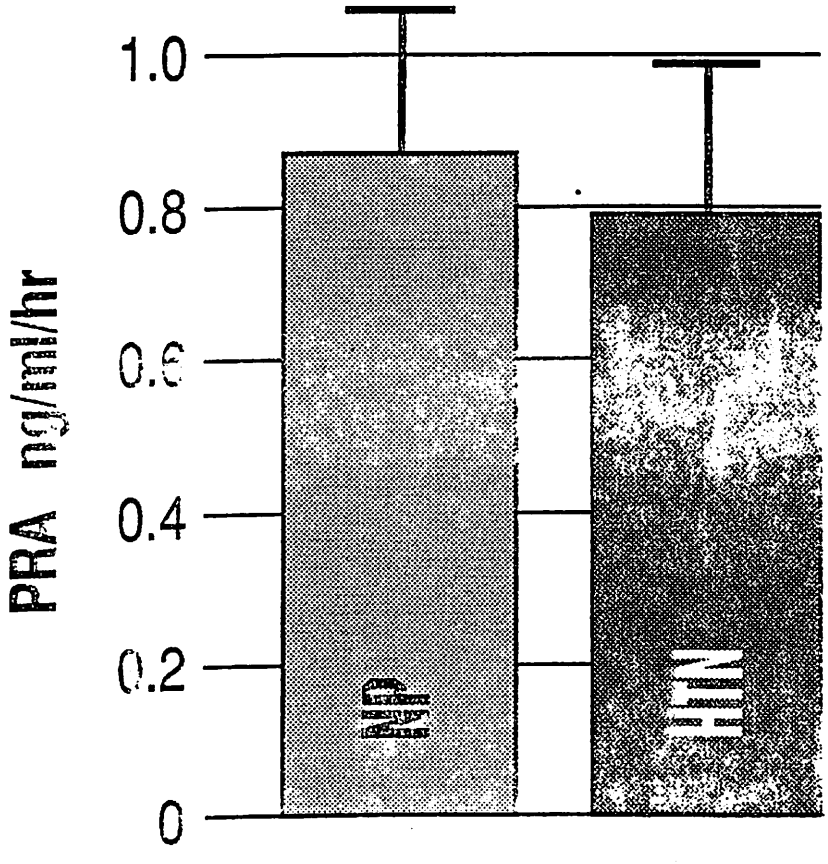


Fig. 16 Mean plasma renin activity (PRA) in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

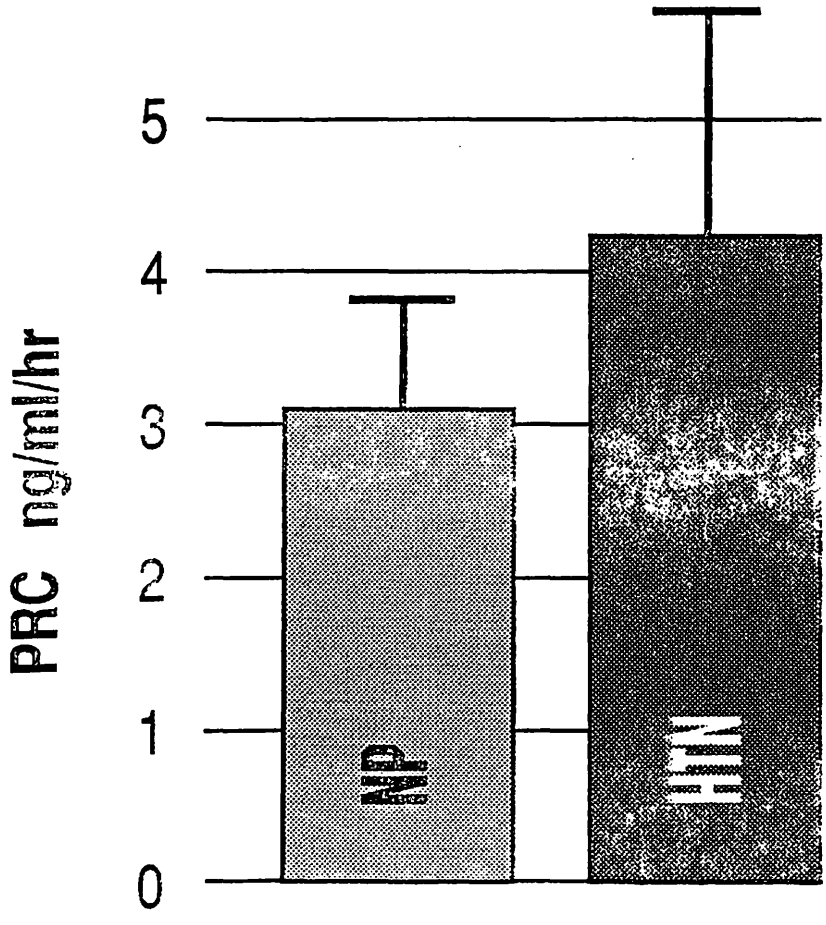


Fig. 17 Mean plasma renin concentration (PRC) in normotensive subjects (NP) and in subjects with essential hypertension (HTN).

SERUM TOTAL AND IONISED CALCIUM IN UNTREATED HYPERTENSIVE WOMEN

MF Karim & HJ Singh. Dept. Physiol. Sch. Med. Sci. USM 16150 Kubang Kerian, Kelantan

Although a number of epidemiological studies have indicated an inverse relationship between dietary calcium intake and high blood pressure, results of direct analysis of serum calcium in humans have been conflicting. We therefore measured serum total calcium, ionised calcium, sodium, potassium and magnesium concentrations in untreated essential hypertensive women (n=12) and normotensive women (n=9). Twenty-four hour urinary excretion of these cations was also determined.

Whilst serum total calcium concentration was not different between the two groups, serum ionised calcium was, however, significantly lower in women with hypertension (1.16 ± 0.02 vs 1.22 ± 0.03 mmol/l ; $p< 0.05$). Serum sodium concentration was significantly higher in women with hypertension (147.7 ± 0.93 vs 142.6 ± 0.56 mmol/l; $p< 0.0001$). The concentrations of potassium and magnesium in serum were not different between the two groups. Twenty-four hour urinary calcium and potassium excretions were not different between the two groups but urinary sodium and magnesium excretions were however significantly lower in women with hypertension (80.9 ± 15.2 vs 145.8 ± 21.9 mmol/day ; $p<0.01$ and 1.72 ± 0.34 vs 3.06 ± 0.41 mmol/day ; $p<0.05$ for sodium and magnesium respectively).

It therefore appears that a disturbance in calcium and sodium metabolism is present in women with essential hypertension. It is however uncertain if this is a consequence of the raised blood pressure or as corroborated by a number of other studies, a cause of this disorder.

Paper presented:

Scientific Meeting
Academy of Medicine of Malaysia
Postgraduate Centre, Hospital Ipoh, Perak
22nd. January 1995

Paper presented:

11th Scientific Meeting of the Malaysian
Society of Pharmacology & Physiology
Delima Resort, Pulau Langkawi
26-28 May, 1995

PLASMA RENIN AND DIVALENT CATIONS IN UNTREATED ESSENTIAL HYPERTENSIVES

HJ Singh, MF Karim, RG Sirisinghe & AR Rashid* *Dept Physiol and Dept Med.*, Sch. Med. Sci., Univ. Sains Malaysia, 16150 Kubang Kerian, Kelantan.*

The role of calcium deficiency in the pathogenesis of hypertension has remained controversial as conflicting reports appear in the literature (1,2). A probable reconciliation was provided by Resnick et al (1) who found clinical evidence of calcium deficiency in only those with a low-renin form of hypertension. Their observations, however, have not been confirmed. We therefore investigated the relationship between plasma renin activity (PRA), plasma renin concentration (PRC) and serum calcium and magnesium concentrations in male and female untreated essential hypertensives (n=31) and age and sex-matched normotensives (n=31)

Approximately 15 ml of venous blood was collected from all subjects who had been seated for about 30 minutes prior to the collection. Plasma renin activity and plasma renin concentration were estimated as angiotensin I generated per hour in the absence and presence of exogenous substrate (nephrectomised sheep plasma) respectively. Angiotensin I was determined by RIA and serum electrolytes were determined spectrophotometrically.

No significant differences were evident in mean PRA and PRC between the two groups. Mean serum ionised calcium concentration, however, was significantly lower in hypertensives (1.18 ± 0.01 *vs* 1.23 ± 0.01 mmol l^{-1} in hypertensives and normotensives respectively). Serum total calcium and magnesium concentrations were not significantly different between the two groups. Correlation of PRA and PRC with serum electrolytes revealed a weak inverse correlation with serum magnesium only ($r = -0.39$; $p<0.05$).

In conclusion, these observations confirm the observed lower serum ionised calcium in some essential hypertensives (3) but they however fail to demonstrate a direct relationship between serum calcium and PRA or between PRA and serum magnesium concentration. Instead, we find a weak negative correlation between PRC and serum magnesium concentration. The reason for this relationship or the lower serum ionised calcium in essential hypertensives in this study is not apparent.

References

1. Resnick LM et al (1983) N Engl. J. Med. 309:888 - 91
2. Kestleloot H & Geboers J (1982) Lancet, 1:813 - 815
3. McCarron DA (1982) N Engl L Med 307:226 - 8

National Conference for Medical Sciences
School of Medical Sciences,
Universiti Sains Malaysia,
Kubang Keria, Kelantan
11 - 12 June 1995.

MAGNESIUM AND PLASMA RENIN IN ESSENTIAL HYPERTENSION

MF Karim[¶], HJ Singh[¶], Abdul Rashid[†] and RG Sirisinghe[¶]

Departments of Physiology[¶] and Pharmacology[†]
School of Medical Sciences, Universiti Sains Malaysia,
16150 Kubang Kerian, Kelantan, Malaysia.

Studies investigating magnesium status in hypertensives have reported conflicting results. Considerable overlap exists between serum magnesium levels among normotensives and hypertensives. Renin profiling, nevertheless, has revealed higher and lower serum magnesium levels in low-renin and high-renin hypertensives respectively. The significance of this seems to be that the influence of magnesium intake on blood pressure may differ according to the underlying state of magnesium metabolism and may only significantly benefit some, but not all, forms of clinical hypertension. These observations remain largely unconfirmed. To investigate this relationship we measured serum and urinary magnesium levels and plasma renin activity (PRA) and plasma renin concentration (PRC) in normotensives (n=34) and age and sex-matched untreated hypertensives (n=34) subjects. Approximately 15 mls of venous blood was collected from all the subjects who had been seated for about 30 minutes prior to the collection. Plasma renin was estimated by RIA for angiotensin I and electrolytes were measured by spectophotometry.

No significant differences were evident in serum and erythrocyte magnesium concentrations between the two groups. Serum sodium concentration was significantly higher in the hypertensives (146.9 ± 0.43 vs 142.5 ± 0.64). Urinary sodium and magnesium were slightly but non-significantly lower in the hypertensives. PRA and PRC were not significantly different between the two groups. In addition we were also unable to demonstrate a significant correlation between PRA and serum magnesium concentration. Interestingly howerver, serum magnesium concentration revealed a weak but significant inverse correlation with PRC ($r = -0.39$; $p<0.05$).

In conclusion, these observations fail to confirm a definite relationship between serum magnesium and systemic blood pressure or between PRA and serum magnesium. The significance of the correlation between PRC and serum magnesium remains unclear.

Paper presented:

National Conference on Medical Sciences
School of Medical Sciences
Universiti Sains Malaysia
Kubang Kerian, Kelantan
11 - 12 June 1995

SERUM CALCIUM AND PLASMA RENIN ACTIVITY IN UNTREATED ESSENTIAL HYPERTENSIVES

HJ Singh, MF Karim, RG Sirirsinghe & Abdul Rashid* *Dept Physiol and Dept Pharm** *Sch Med. Sci. Universiti Sains Malaysia 16150 Kelantan*

Considerable consternation exists regarding the role of calcium in hypertension. Reduced serum calcium has not been consistently demonstrated in all hypertensives. An unconfirmed study (1) reports of lower serum ionised calcium in low-renin form of hypertension. We therefore investigated the relationship between plasma renin activity and (PRA), plasma renin concentration (PRC) and serum total and ionised calcium in normotensives in age and sex-matched hypertensives. In addition 24 hour urinary excretionof calcium was also determined.

Venous blood (15ml) was collected from all subjects who had been seated for about 30 minutes prior to the collection. Plasma renin was determined by RIA for angiotensin I and calcium and sodium were determined spectrophotometrically.

PRA and PRC did not differ significantly between the two groups. Similarly, no significant differences were evident in serum total calcium or sodium between the two groups. Serum ionised calcium however was significantly lower in hypertensives (1.23 ± 0.01 vs 1.18 ± 0.01 mmol l^{-1}; $p < 0.05$). No significant correlation was however observed between PRA or PRC and serum total or ionised calcium concentrations.

The data however confirm the observed lower serum ionised calcium in some essential hypertensives but fails to demonstrate a direct relationship between serum calcium and plasma renin activity.

Reference

(1) Resnick LM et al (1983) *N Engl J Med.* 309:888 - 91

BORANG PERSETUJUAN

Saya _____No K/P_____

dari (alamat)_____

_____

No Tel : _____ memahami bentuk kaedah ujian termasuk sebarang risiko dan kesulitan yang telah dijelaskan kepada saya oleh Dr. _____ .Saya bersetuju untuk melibatkan diri di dalam ujian ini yang dijalankan oleh Jabatan Fisiologi, Pusat Pengajian Sains Perubatan, USM.

Tarikh _____ Jam _____

Tandatangan Subjek : _____

Tandatangan Doktor : _____

Tandatangan Saksi : _____

CONSENT FORM

I _____ No. K/P: _____

of (adress)_ _____

_____No . Tel : _____

understood the nature of the test procedures including any risks and its discomforts which have been explained to me by Dr _____ _____ I therefore, willingly give consent to participate in this study carried out by the Depts. of Physiology, School of Medical Sciences, USM.

Date _____ Time _____

Signature of subject : _____

Signature of Doctor : _____

Signature of Witness : _____

DEPARTMENT OF PHYSIOLOGY
SCHOOL OF MEDICAL SCIENCES
USM

Magnesium and Hypertension

Name : _____

Age : _____ Sex : _____ Race : _____

Adress : _____

_____

Tel no : _____

History of present illness : _____

_____

Past Medical history : _____

_____

Personel and family history : _____

_____

Physical examination :

Build : _____

Skin : _____

Pulse : _____bpm

Blood pressure : _____mmHg _____mmHg _____mmHg

Respiration : _____ rate/min.

PUSAT PENGAJIAN SAINS PERUBATAN
UNIVERSITI SAINS MALAYSIA
KUBANG KERIAN KELANTAN

METHODS FOR COLLECTION A 24 HOUR URINE SAMPLE.

Instruction to subjects.

A 24 hour urine sample is the total amount of urine produced in 24 hours.

1) Start your collection one day before your date of appoinment.

2) You start your urination at 10 am of that day. At this time you go to the toilet and emty your bladder (urinate). Throw this sample away.

3) All urine passed after this must be collected in the container provided. You continue to do this until 10 am the next day.(i.e the date of your appointment).

4) On the date of your appointment present yourself to the clinic at 8.30 am in the morning, together with the urine container.

5) On your arrival about 12 mls of blood will be collected from your arm vein.

6) At 10 am. you once again go to the toilet to complete your collection. The urine that you passed must be retained and put into the container. Return the container, containing the urine to the appropriate staff.

Note : Your 24 hour collection is now complete. Do let the staff know if you have accidently thrown away any of the sample.

PUSAT PENGAJIAN SAINS PERUBATAN
UNIVERSITI SAINS MALAYSIA
KUBANG KERIAN KELANTAN

KAEDAH PEMUNGUTAN SAMPEL URIN 24 JAM

Arahan kepada subjek.

Sampel urin 24 jam adalah jumlah urin yang dihasilkan dalam masa 24 jam.

1) Mulakan pengumpulan urin sehari sebelum tarikh temujanji anda.

2) Pada pukul 10.00 pagi, anda dikehendaki pergi ke tandas dan membuang air kecil. Sampel urin ini perlu dibuangkan.

3) Kesemua urin yang dikeluarkan selepas waktu ini hendaklah dikumpulkan ke dalam bekas yang diberi, sehinggalah pukul 10.00 pagi keesokkannya (hari temujanji).

4) Pada tarikh temujanji, anda dikehendaki menghadirkan diri di klinik KPM pada pukul 8.30 pagi berserta dengan bekas urin.

5) Selepas waktu ini darah vena akan diambil sebanyak 12 ml.

6) Tepat pada pukul 10.00 pagi anda dikehendaki pergi ke tandas untuk memungut sampel urin buat kali terakhir. Kembalikan bekas urin anda kepada kakitangan bertugas.

Nota :

Sekarang anda sudah membuat pemungutan urin selama 24 jam. Sekiranya anda telah terlupa memungut atau terbuang urin, anda hendaklah memberitahu kakitangan berkenaan.