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(54) Titre : UTILISATION DE LA RIBOFLAVINE POUR LE TRAITEMENT DE L'HYPERTENSION
(54) Title: USE OF RIBOFLAVIN IN THE TREATMENT OF HYPERTENSION

(57) Abrégé/Abstract:
The invention provides the use of riboflavin in the manufacture of a medicament for the treatment or prophylaxis of elevated blood pressure in a subject homozygous or heterozygous for the MTHFR C677T polymorphism. The invention also provides a pharmaceutical product for the treatment or prophylaxis of elevated blood pressure in a subject homozygous or heterozygous for the MTHFR C677T polymorphism, comprising a pharmaceutically effective amount of an anti-hypertensive agent and riboflavin, and the invention further provides a method of treatment of such a subject comprising the administration of riboflavin.

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(54) Title: USE OF RIBOFLAVIN IN THE TREATMENT OF HYPERTENSION

(57) Abstract: The invention provides the use of riboflavin in the manufacture of a medicament for the treatment or prophylaxis of elevated blood pressure in a subject homozygous or heterozygous for the MTHFR C677T polymorphism. The invention also provides a pharmaceutical product for the treatment or prophylaxis of elevated blood pressure in a subject homozygous or heterozygous for the MTHFR C677T polymorphism, comprising a pharmaceutically effective amount of an anti-hypertensive agent and riboflavin, and the invention further provides a method of treatment of such a subject comprising the administration of riboflavin.

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Use of Riboflavin in the Treatment of Hypertension

The present invention relates to the use of riboflavin in the treatment of hypertension in a genotype specific population.

Hypertension, commonly referred to as high blood pressure, is a medical condition where the blood pressure is elevated, generally chronically elevated. Hypertension of any aetiology is one of the major risk factors for cardiovascular disease (CVD), which includes heart disease and stroke.

It has been found that there is an increased risk of CVD in individuals homozygous for the commonly occurring C677T polymorphism in the gene coding for the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR). MTHFR is required for the formation of 5-methyltetrahydrofolate which in turn is required to convert homocysteine to methionine. Individuals who are homozygous for the MTHFR C677T polymorphism (TT genotype) are considered to have a significantly higher risk of heart disease and stroke than those with the wild-type gene (CC genotype). People who are heterozygous for the polymorphism (CT genotype) may also have a moderately higher risk of heart disease and stroke. Various anti-hypertensive drugs (e.g. ACE inhibitors, beta blockers and diuretics) are available to lower blood pressure in those deemed clinically to have hypertension. However, these drugs can have undesirable side effects and some subjects may remain hypertensive despite being tried on more than one type of anti-hypertensive drug.

It has now surprisingly been found that riboflavin (vitamin B2) has a significant systolic and diastolic blood pressure lowering effect, specifically in CVD patients homozygous for the MTHFR C677T polymorphism.

According to a first aspect of the present invention, there is provided the use of riboflavin in the manufacture of a medicament for the treatment or prophylaxis of elevated blood pressure in a subject homozygous or heterozygous for the MTHFR C677T polymorphism.

According to a second aspect of the present invention, there is provided a pharmaceutical product for the treatment or prophylaxis of elevated blood pressure in a subject homozygous or heterozygous for the MTHFR C677T polymorphism, comprising a pharmaceutically effective amount of an anti-hypertensive agent and riboflavin, for simultaneous, separate or sequential administration.

Suitable anti-hypertensive agents include ACE inhibitors such as quinapril, captopril, lisinopril, benazepril, perindopril, enalapril maleate, trandolapril, ramipril and cilazapril; beta blockers such as atenolol, tertatolol, metoprolol tartrate, bisoprolol fumarate, nebbivolol, celiprolol and pindolol; Ca^{++} antagonists such as nifedipine, diltiazem, amlodipine, verapamil and felopidine; alpha blockers such as methyldopa, doxazosin, clonidine and prazosin; angiotensin II antagonists such as irbesartan, candesartan cilextil, olmesartan medoxomil, valsartan, losartan, telmisartan and eprosartan mesylate; alpha/beta blockers such as carvedilol and labetalol; and diuretics such as bendroflumethiazide, piretanide, chlorthalidone and hydrochlorothiazide (HCTZ).

Advantages of the invention include the following:

- Riboflavin may be used to reduce blood pressure in the absence of any other anti-hypertensive agents.
- When other anti-hypertensive agents are administered with riboflavin, either simultaneously, separately or sequentially, the amount of the other anti-hypertensive agents necessary to have the required effect may advantageously be reduced, in that lesser amounts of the agents may be required in order to effect the treatment or prophylaxis of elevated blood pressure in a subject, thereby minimising any undesirable side effects of conventional anti-hypertensive agents.

According to a third aspect of the present invention, there is provided a method for the treatment or prophylaxis of elevated blood pressure in a subject homozygous or heterozygous for the MTHFR C677T polymorphism, the method comprising administering riboflavin to the subject.

The medicament or product of the invention is preferably in a form suitable for oral or parenteral administration. Suitable oral dosage forms include tablets, capsules (including

slow release capsules), pills, powders, granules and the like. Parenteral administration includes, for example, intravenous, intramuscular, intraarterial, intraperitoneal, intranasal, intravesical (e. g., to the bladder), intradermal, topical or subcutaneous administration. Suitable parenteral dosage forms include sterile injectable aqueous solutions or dispersions and sterile powders for the preparation of sterile injectable solutions or dispersions. The preferred route of administration is oral.

Riboflavin may be administered together with a pharmaceutically compatible or acceptable carrier suitable for oral or parenteral administration, selected according to the particular type of administration used. For oral administration, riboflavin may be administered with one or more solid inactive ingredients for the preparation of suitable oral dosage forms. For example, riboflavin may be administered with at least one excipient such as fillers, binders, humectants, disintegrating agents, solution retarders, absorption accelerators, wetting agents, absorbents or lubricating agents. For parenteral administration, riboflavin may be administered with a suitable carrier or diluent such as water, ethanol, saline solution, aqueous dextrose (glucose) and related sugar solutions, glycerol, or a glycol such as propylene glycol or polyethylene glycol.

In all cases, the dose of riboflavin will be within dietary reference levels up to and including the maximum level considered to be safe. Although, unlike many other nutrients, no official upper tolerable level has been established for riboflavin, it is generally considered to be safe even at very high doses with evidence showing that levels up to 500mg/day are tolerated with no adverse effects. This compares with recommended dietary levels of riboflavin for adults which range from 1.1 to 1.8mg/day. The medicament or product is most preferably formulated for administration of riboflavin to a subject in an amount of approximately 1.6 mg/day. However, the medicament or product may be formulated for administration of riboflavin to a subject in any other suitable dose greater or less than 1.6 mg/day. For example, a suitable dose may be from about 0.5 mg/day to 50 mg/day, preferably from about 0.75 mg/day to 20 mg/day, more preferably from about 1 mg/day to 10 mg/day, more preferably from about 1.2 mg/day to 5 mg/day, even more preferably from about 1.4 mg/day to about 5 mg/day.

The invention has application in the area of personalised nutrition. There is an increasing interest in gene-nutrient interactions, and with the greater accessibility of genetic profiling, the market for personalised nutrition is emerging in recent years. This approach is based on the view that dietary requirements should consider not only general factors such as age and sex, but also genetic factors which are specific to the subject. For example, a subject could check their MTHFR genotype and then proceed to take low-dose riboflavin, specifically if they are found to have the TT or CT genotype.

The invention also has application in the design of clinical drug trials, e.g. those testing new anti-hypertensive medication drugs. For the successful outcome and correct interpretation of such trials, it will be critical that those with the TT genotype for the MTHFR C677T polymorphism in combination with low riboflavin status are stratified and randomised equally to the different treatment groups.

EXAMPLE 1: CVD PATIENTS

MATERIALS AND METHODS

Subject recruitment

Ethical approval was granted by both the Research Ethical Committee of the University of Ulster and Altnagelvin Hospital Trust, Londonderry, Northern Ireland. Premature CVD patients, males aged 55 years and females aged 65 years and under at time of event were recruited from the cardiac department of Altnagelvin hospital. Patients were indicated by a previous myocardial infarction (MI) or angina diagnosed by ECG changes. Potential participants (n = 404) were screened for MTHFR genotype on the basis of a buccal swab sample. Those with the TT genotype were identified (n = 54); these were age and sex matched with a similar number of patients with the CC or CT genotypes, resulting in a total of 197 patients who were invited to participate in the current intervention study (see Figure 1). Exclusion criteria for all subjects were history of hepatic or renal disease, consumers of B-vitamin supplements, those taking medication known to interfere with folate metabolism, at least 3 months post MI prior to blood sampling. All volunteers provided written informed consent and completed a health and medical/lifestyle

questionnaire which included questions regarding family history of CVD and smoking habits.

Study design

The study was a 16-week placebo controlled intervention trial. Patients were stratified on the basis of their initial screening plasma homocysteine within each genotype group and subsequently randomised within each stratum to receive either riboflavin (1.6mg/day) or placebo, as indicated in Figure 1. In an attempt to maximise compliance, patients were provided with riboflavin every 4 weeks in 7-day pill boxes and asked to return any unused pills which were recorded. Non-fasting blood samples were collected at screening and post intervention either at Altnagelvin hospital, their workplace or their own homes.

Blood pressure and anthropometric measurements

Blood pressure measurements were recorded as the mean of two separate measurements taken 15 minutes apart using an Omcron 705CP electronic blood pressure monitor (Medisave, Dorset, UK). Weight (kg) and height in (m), were used to calculate BMI (weight (kg)/height^2 (m)). Waist circumference (cm) was also measured. All measurements were made using Seca approved equipment (Brosch Direct, Ltd, Peterborough, UK).

Sample collection

One 30ml blood sample was collected from each patient, one 9ml EDTA tube for plasma and washed red cells, one 4ml EDTA tube for the preparation of red blood cell lysates and the measurement of haemoglobin (HB) and packed cell volume (PCV); one 8ml serum separation tube for serum extraction, one 5ml serum separation tube for lipid profile analysis and one 4ml sodium citrate tube for coagulation screening. The 9ml EDTA was immediately wrapped in tin foil and placed on ice. Samples were centrifuged within 2 hours of sampling at 3000rpm for 15 minutes. Plasma was removed and stored at -80^0 C for plasma homocysteine and PLP analysis. The buffy layer was removed for confirmation of MTHFR genotyping and stored at -20^{0}C. The remaining red blood cells were thrice washed with phosphate buffered saline (PBS) with the supernatant and remaining buffy layer being discarded after each wash. The washed red cells were removed and stored at – 80^{0}C for EGRac analysis (a functional indicator of riboflavin status). Serum was removed and stored at -80^{0}C for serum folate.

Biochemical analysis

Plasma homocysteine was measured by immunoassay using the Abbott Imx analyser (Leino A., 1999, "Fully automated measurement of total homocysteine in plasma and serum on the Abbott IMx analyzer." *Clin. Chem,* 45, 569-71). Serum folate and red blood cell folate concentrations were determined by microbiological assay using the cryopreserved, microtitre plate method (Molloy et al, 1997, "Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method." *Methods Enzymol,* 281, 43-53). Plasma PLP was determined by reverse-phase high pressure liquid chromatography (HPLC) with fluorescence detection (Bates et al, 1999, "Plasma pyridoxal phosphate and pyridoxic acid and their relationship to plasma homocysteine in a representative sample of British men and women aged 65 years and over." *British Journal of Nutrition,* 1, 191-201). Identification of the MTHFR C677T genotype was carried out using the polymerase chain reaction (PCR) amplification followed by HinFl (Frosst et al, 1995, "A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase." *Nat Genetc, 10,* 111-113). Riboflavin status was determined on the basis of EGRac, a functional assay whereby the activity of glutathione reductase is measured both with and without added FAD. EGRac is then calculated as a ratio of FAD-stimulated to unstimulated enzyme activity (Powers et al, 1983, "The relative effectiveness of iron and iron with riboflavin in correcting a microcytic anaemia in men and children in rural Gambia." *Hum Nutr Clin Nutr,* 37, 413-425), and values >1.3 are generally considered to reflect sub-optimal riboflavin status. Coagulation screening was measured by ACI Instrumentation Laboratories. HB and PCV were measured using Sysmex® XE-2100 (Sysmex, UK Ltd. Milton Keynes UK) analyser and lipid profiles were measured using Abbott Architect®CI 8200 analyser (Abbott Laboratories, USA). For all assays, samples were analysed blind, in duplicate and within 1 year of collection. Quality control was provided by repeated analysis of stored batches of pooled washed red blood cells (for EGRac), plasma for homocysteine and PLP and serum for folate covering a wide range of values.

Statistical Analysis

All statistical analysis was performed using the SPSS statistical package for the social sciences (version 11.0, SPSS UK Ltd., Chersey, United Kingdom). For normalisation

purposes variables were log-transformed as appropriate prior to statistical analysis. One way ANOVA with Tukey's post hoc test was used to examine the differences in the baseline characteristics among the genotype groups. Categorical data were assessed using chi squared analysis. Correlation analysis was performed using bivariate Pearson correlation coefficients. The effect of riboflavin intervention within each genotype group was examined using paired t-tests. *P* values < 0.05 were considered significant.

RESULTS

Description of Figures:

Figure 1 shows the flow diagram of study design.

Figure 2(i) shows homocysteine concentration (μmol/l) by MTHFR genotype group among healthy controls; Figure 2(ii) shows systolic blood pressure (mmHg) by MTHFR genotype group among healthy controls; and Figure 2(iii) shows diastolic blood pressure (mmHg) by MTHFR genotype group among healthy controls. Values in Figures 2(i), 2(ii) and 2(iii) are mean (standard error bars). Different letters denote statistically significant differences between the MTHFR genotype groups, ANOVA with Tukey post-hoc test, $P < 0.05$ significant. In Figures 2(i)-2(iii), the letter "a" denotes values that do not differ significantly from other "a" values; and the letter "b" denotes values which do not differ significantly from other "b" values, but which do differ from the values denoted as "a".

Figure 3(i) shows homocysteine concentration (μmol/l) by MTHFR genotype group among premature CVD patients; Figure 3(ii) shows systolic blood pressure (mmHg) by MTHFR genotype group among premature CVD patients; and Figure 3(iii) shows diastolic blood pressure (mmHg) by MTHFR genotype group among premature CVD patients. Values in Figures 3(i), 3(ii) and 3(iii) are mean (standard error bars). Different letters denote statistically significant differences between the MTHFR genotype groups, ANOVA with Tukey post-hoc test, $P < 0.05$ significant. In Figures 3(i)-3(iii), the letter "a" denotes values that do not differ significantly from other "a" values; and the letter "b" denotes values which do not differ significantly from other "b" values, but which do differ from the values

denoted as "a". The letters "ab" denote values that do not differ significantly from a value denoted as "a" or "b".

Baseline data in healthy individuals:

Referring to Figure 2, in relation to blood homocysteine levels, the phenotype observed for healthy individuals homozygous for MTHFR C677T polymorphism (i.e. TT genotype) was elevated blood homocysteine concentration, compared to those with either CT or CC genotypes. In relation to corresponding blood pressure levels, it was found that in healthy individuals with the TT genotype there was no elevation in the systolic or diastolic blood pressure compared to values in either CT or CC genotypes, as shown in Figure 2.

Baseline data in patients with premature CVD:

Patients with premature CVD are generally defined as subjects who develop CVD before the age of 55 for men, or before the age of 65 for women. Referring to Figure 3, patients with premature CVD and with the TT genotype had elevated blood homocysteine concentration, compared with both CC and CT patients. Of note, TT patients were also found to have significantly increased systolic and diastolic blood pressure, as shown in Figure 3, compared to CC patients. CT individuals were found to have intermediate levels of diastolic blood pressure, compared to CC and TT patients (as shown in Figure 3). These results are unexpected, as healthy TT subjects showed no elevation in blood pressure compared with the other genotype groups. All patients were taking one or more anti-hypertensive drugs at the time of sampling, as indicated in Table 1 below, but it is clear from Figure 3 that the medication was relatively ineffective in the TT genotype group.

Table 1

Anti-hypertensive medications taken by patients (n=161) at time of sampling

Anti-hypertensive medication	**% of patients**
Betablockers	38
Ace Inhibitors	18
Calcium channel blockers	6
Diuretics	3
Ace inhibitors + Betablockers	18
Ace inhibitors + Diuretics	4
Ace inhibitors + Calcium channel blockers	1
Betablockers + Calcium channel blockers	2
Betablockers + Diuretics	6
Calcium channel blockers + Diuretics	0.6
Ace inhibitors + Calcium channel blockers + Diuretics	0.6
Betablockers + Calcium channel blockers + Diuretics	0.6

The baseline characteristics of premature CVD patients sorted in accordance with their MTHFR genotype (TT, CT, or CC) are shown in Table 2 below. Referring to Table 2, it was observed that TT patients had significantly elevated systolic and diastolic blood pressure, compared to CC patients. CT patients were found to have intermediate values for diastolic blood pressure. Of note, the elevated blood pressure levels found in TT patients were strongly influenced by riboflavin status; patients with the combination of the TT genotype and low riboflavin status were unexpectedly found to have markedly elevated blood pressure.

Table 2 Baseline characteristics of premature cardiovascular disease patients split by MTHFR 677C→T genotype

	MTHFR genotype				
General characteristics	All (n = 197)	CC (n = 67)	CT (n = 76)	TT (n = 54)	P
Age now (y)	53.2 (5.8)	53.4 (6.1)	52.6 (5.0)	54.0 (6.4)	0.382
Age at time of event (y)	46.9 (6.1)	47.1 (5.6)	46.9 (5.4)	47.1(7.5)	0.891
Male (%)	75.1	78.1	69.7	78.2	0.303
Family history of CVD (%)	68.0	68.7	65.8	70.4	0.763
Family history of premature CVD (%)	41.6	40.3	50.0	31.5	0.127
Current smoker (%)	32.0	31.3	27.6	38.9	0.209
Body mass index (kg/m2)	29.1 (4.6)	29.3 (4.8)	28.7 (4.5)	29.3 (4.5)	0.724
Waist circumference (cm)	95.1 (12.1)	96.3(11.9)	94.2 (11.4)	95.0 (13.4)	0.625
Prothrombin time (sec)	13.9 (3.1)	13.7 (2.8)	14.1 (3.4)	13.6 (2.3)	0.700
Fibrinogen concentration (g/l)	3.93 (0.92)	3.93 (0.84)	3.97 (1.05)	3.88 (0.83)	0.853
Total cholesterol (mmol/l)	4.5 (0.9)	4.4 (0.81)	4.5 (0.8)	4.5 (1.0)	0.849
B-vitamin status					
Plasma homocysteine (µmol/l)	10.9 (5.3)	9.8 (3.3)[a]	10.5 (3.9)[ab]	12.8 (8.0)[b]	0.007
EGRac	1.38 (0.20)	1.37 (0.17)	1.39 (0.22)	1.37 (0.19)	0.854
Red cell folate (nmol/l)	959(455)	1030(518)[a]	1011(400)[a]	796(408)[b]	0.009
Pyridoxal phosphate (nmol/l) (B-6)	61.0(37.0)	68.0(35.0)[a]	63.2(40.4)[ab]	49.4(32.0)[b]	0.017
Blood pressure					
Systolic Blood Pressure (mmHg)	135.0 (19.6)	131.1 (18.0)[a]	133.0 (19.7)[a]	142.8 (19.5)[b]	0.002
By riboflavin status:					
Lower riboflavin status	137.1 (20.5)	131.2 (20.4)[a]	135.8 (19.0)[a]	147.4 (19.8)[b]	0.005
Higher riboflavin status	132.5 (18.5)	131.0 (14.8)	129.6 (20.4)	138.6 (19.2)	0.172
Diastolic Blood Pressure(mmHg)	83.0 (12.2)	80.3 (12.5)[a]	83.3 (11.5)[ab]	86.0(12.3)[b]	0.038
By riboflavin status:					
Lower riboflavin status	84.1 (12.8)	80.8 (13.5)	84.6 (11.8)	88.1 (12.7)	0.076
Higher riboflavin status	81.7 (11.4)	79.6 (11.2)	81.9 (11.1)	84.1 (12.2)	0.381

Values are presented as mean (SD). Values among genotypes groups were compared using one way ANOVA with tukey post hoc tests.
Abbreviations: MTHFR, methylenetetrahydrofolate reductase; erythrocyte glutathione reductase activation coefficient (riboflavin status, a higher EGRac value indicates lower vitamin status); 'Higher' and 'lower' riboflavin status was determined using the median EGRac value within each genotype group as a cut-off.

Intervention data in patients with premature CVD

Referring to Table 3 below, in a placebo controlled intervention study in 181 patients with premature CVD, significant lowering of both systolic and diastolic blood pressure in patients with the TT genotype was achieved following 16 weeks of low-dose riboflavin administration. The blood pressure response shown is of both statistical and clinical significance. The magnitude of blood pressure lowering (a decrease of 11 units in systolic and 8 in diastolic blood pressure) observed in response to riboflavin can be estimated from meta-analyses to lower the risk of heart disease by 29% and stroke by 46%. The results show that a significant and clinically important reduction in blood pressure was achieved in response to riboflavin, specifically in patients with the TT genotype. Referring again to Table 3, no significant reduction was observed in blood pressure (either systolic or diastolic) in CC patients following riboflavin administration.

Table 3. Response to riboflavin intervention for 16 weeks in premature CVD patients.

	MTHFR Genotype					
	CC Placebo (n = 32)			**CC Riboflavin (n = 32)**		
	Pre	**Post**	***P***	**Pre**	**Post**	***P***
Homocysteine(μmol/l)	9.5(2.8)	9.3(2.9)	0.474	10.1 (3.9)	9.8(3.6)	0.271
EGRac	1.40(0.19)	1.42(0.20)	0.520	1.34(0.16)	1.24(0.07)	<0.001
Serum folate(μg/l)	12.1(8.5)	14.0(9.6)	0.052	10.5(6.5)	12.0(7.5)	0.094
Red cell folate(nmol/l)	1063(589)	1139(561)	0.245	1021(557)	1075(526)	0.427
Systolic blood pressure (mmHg)	126.6(20.4)	129.0(22.1)	0.421	134.3(14.9)	133.4(15.3)	0.733
Diastolic blood pressure (mmHg)	79.3(14.9)	80.9(15.5)	0.546	80.1(8.9)	80.4(12.0)	0.881
	CT Placebo (n = 33)			**CT Riboflavin (n = 35)**		
	Pre	**Post**	***P***	**Pre**	**Post**	***P***
Homocysteine(μmol/l)	11.2(4.1)	10.8(4.6)	0.327	10.5(3.8)	10.6(6.3)	0.881
EGRac	1.41(0.22)	1.44 (0.29)	0.209	1.38(0.24)	1.25(0.13)	<0.001
Serum folate(μg/l)	7.83(4.34)	8.12(6.44)	0.744	10.41(6.61)	11.80(11.01)	0.342
Red cell folate (nmol/l)	991(388)	991(434)	0.997	1008(400)	1022(460)	0.759
Systolic blood pressure (mmHg)	133.6(21.5)	129.9(21.5)	0.241	136.2(16.9)	135.4(15.4)	0.708
Diastolic blood pressure (mmHg)	82.0(12.1)	81.9(14.2)	0.962	86.0(10.8)	84.2(9.2)	0.336
	TT Placebo (n = 24)			**TT Riboflavin (n = 25)**		
	Pre	**Post**	***P***	**Pre**	**Post**	***P***
Homocysteine(μmol/l)	11.8(6.7)	11.8(7.8)	0.915	11.3(4.4)	10.0(3.1)	0.014
EGRac	1.32(0.12)	1.34(0.11)	0.298	1.41(0.20)	1.27(0.09)	<0.001
Serum folate(μg/l)	7.7(6.6)	8.9(7.5)	0.157	8.1(4.8)	9.6(8.1)	0.244
Red cell folate (nmol/l)	808(488)	903(580)	0.164	845(303)	889(306)	0.305
Systolic blood pressure (mmHg)	143.8(18.1)	141.6(22.5)	0.566	142.8(22.1)	131.6(20.9)	0.005
Diastolic blood pressure (mmHg)	84.5(10.7)	85.4(12.7)	0.739	88.1(14.2)	80.2(14.2)	0.002

Values are presented as mean (SD). Pre and post intervention values within each treatment group were compared using paired t-test.
Abbreviations: MTHFR, methylenetetrahydrofolate reductase; EGRac, erythrocyte glutathione reductase activation coefficient (riboflavin status, higher values indicate lower status).

EXAMPLE 2: HEALTHY CONTROLS

The association between riboflavin and blood pressure in healthy individuals according to MTHFR C677T genotype was investigated. Measurements were taken and recorded according to the procedures described in Example 1 above. The results obtained, namely the baseline characteristics of healthy subjects pre-screened and age-matched for MTHFR genotype (n=124), are shown in Table 4.

Table 4

	***MTHFR* C677T Genotype**			
General characteristics	**CC** (n =46)	**CT** (n =34)	**TT** (n = 44)	***P***
Age (y)	51.4 (4.0)	51.4 (3.0)	50.8 (5.1)	0.718
Male (%)	69.6	85.3	75	0.264
BMI (kg/m²)	28.1 (4.8)	28.0 (4.2)	28.6 (4.6)	0.791
Waist circumference (cm)	90.4 (11.9)	90.6 (12.3)	93.6 (13.5)	0.434
Systolic BP(mmHg)	132.9 (16.5)	134.8 (15.8)	137.4 (19.8)	0.473
Diastolic BP(mmHg)	84.1 (13.2)	86.4 (8.7)	88.1 (13.9)	0.315
Biochemical measurements				
Plasma homocysteine (μmol/l)	9.3 (1.9)a	8.9 (2.1)a	14.1 (8.0)b	<0.001
Riboflavin status (EGRac)	1.34 (0.17)	1.37 (0.15)	1.38 (0.16)	0.492
Red cell folate (nmol/l)	874 (357)	955 (433)	748 (412)	0.071
Vitamin B6 status (PLP; nmol/l)	79.1 (47.4)	79.7 (38.7)	68.3 (35.5)	0.370

Values are mean (SD). ANOVA was used to compare values among the genotype groups; an overall P value < 0.05 indicates a statistically significant difference among the 3 genotype groups. The letters show where the differences are occurring: different letters (a,b) indicate significant differences between any two genotypes, whereas the same letter indicates that values were not significantly different between any two genotypes (Tukey post hoc tests). Thus, the letter "a" denotes values that do not differ significantly from other "a" values; and the letter "b" denotes values which do not differ significantly from other "b" values, but which do differ from the values denoted as "a".

As shown by the results obtained in Example 2 (Table 4), individuals with the TT genotype did not show significantly higher systolic or diastolic blood pressure, compared with the CC or CT genotype groups. However, they did demonstrate the typical phenotype for this TT genotype of significantly elevated plasma homocysteine and a tendency towards lower folate status, though, as shown in Table 4, the difference in folate status did not reach statistical significance (since the P value was not < 0.05).

Intervention data in healthy individuals

A placebo controlled intervention study in 81 healthy individuals was carried out. Blood pressure response to riboflavin intervention (1.6mg/day for 16 weeks) by MTHFR genotype in healthy individuals was investigated, and the results are shown in Table 5.

Referring to the P values, the results of Table 5 show that there was no significant systolic or diastolic blood pressure lowering effect of riboflavin in this healthy cohort with the TT genotype. Therefore, the lowering of systolic and diastolic blood pressure by riboflavin in CVD patients having the TT genotype discussed above in Example 1 was unexpected, and is of statistical and clinical significance.

Referring again to the P values shown in Table 5, the statistical analysis shows that there are no significant increases or decreases in blood pressure in response to riboflavin or placebo across the genotypes, with one exception, namely that diastolic blood pressure did show an increase with riboflavin treatment in the CC group. Whilst this is unexpected, this result may be attributed to chance and the relatively small sample size. Even taking this response to riboflavin in the CC group into account, it is noted that riboflavin was found to increase the diastolic blood pressure in healthy CC individuals, with the result that the lowering of systolic and diastolic blood pressure by riboflavin in CVD patients having the TT genotype discussed above in Example 1 is particularly unexpected.

Table 5. Blood Pressure response to riboflavin intervention (1.6mg/d, 16weeks) by MTHFR genotype in healthy subjects (n=81)

		MTHFR 677C → T Genotype								
		CC (n=25)			CT (n=30)			TT (n=26)		
		Before	After	p-value	Before	After	p-value	Before	After	p-value
Systolic blood pressure (mmHg)										
Placebo	median	136.0(124.7, 140.2)	129.0(120.3, 141.0)	0.663	147.0(136.5, 151.5)	142.0(132.1, 156.1)	0.981	146.0(135.5, 157.8)	150.0(141.6, 157.8)	0.395
Riboflavin treatment	median	137.0(121.9, 148.4)	143.5(129.8, 156.2)	0.077	150.0(135.5, 153.3)	150.0(137.2, 153.4)	0.797	139.5(129.9, 142.5)	145.0(133.3, 151.1)	0.970
Diastolic blood pressure (mmHg)										
Placebo	median	83.0(77.2, 93.0)	83.0(76.6, 85.74)	0.286	87.0(83.7, 92.8)	88.0(81.3, 95.0)	0.970	90.0(82.8, 94.1)	86.0(83.6, 95.5)	0.698
Riboflavin treatment	median	80.5(73.7, 88.3)	86.5(78.7, 91.9)	0.033	87.0(82.7, 94.2)	89.0(83.3, 94.7)	0.767	88.5(81.1, 91.6)	88.5(82.8, 95.1)	0.172

Values are median (95% confidence interval).
Before and After intervention blood pressures were compared using a paired t-test. A P value < 0.05 indicates a statistically significant difference before versus after intervention.

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PCT/EP2008/001437

CLAIMS

1. Use of riboflavin in the manufacture of a medicament for the treatment or prophylaxis of elevated blood pressure in a subject homozygous for the MTHFR C677T polymorphism.

2. Use of riboflavin in the manufacture of a medicament for the treatment or prophylaxis of elevated blood pressure in a subject heterozygous for the MTHFR C677T polymorphism.

3. Use of riboflavin according to claim 1 or claim 2, wherein the medicament is in a form suitable for oral or parenteral administration.

4. A pharmaceutical product for the treatment or prophylaxis of elevated blood pressure in a subject homozygous for the MTHFR C677T polymorphism, comprising a pharmaceutically effective amount of an anti-hypertensive agent and riboflavin, for simultaneous, separate or sequential administration.

5. A pharmaceutical product for the treatment or prophylaxis of elevated blood pressure in a subject heterozygous for the MTHFR C677T polymorphism, comprising riboflavin and a pharmaceutically effective amount of an anti-hypertensive agent, for simultaneous, separate or sequential administration.

6. A pharmaceutical product according to claim 4 or claim 5, wherein the anti-hypertensive agent is selected from ACE inhibitors, beta blockers, Ca++ antagonists, alpha blockers, angiotensin II antagonists, alpha/beta blockers, and diuretics.

7. A pharmaceutical product according to claim 6, wherein the ACE inhibitors are selected from quinapril, captopril, lisinopril, benazepril, perindopril, enalapril maleate, trandolapril, ramipril and cilazapril; the beta blockers are selected from atenolol, tertatolol, metoprolol tartrate, bisoprolol fumarate, nebbivolol, celiprolol and pindolol; the Ca++ antagonists are selected from nifedipine, diltiazem, amlodipine, verapamil and felopidine; the alpha blockers are selected from methyldopa, doxazosin, clonidine and prazosin; the angiotensin II antagonists are selected from irbesartan, candesartan cilextil, olmesartan medoxomil, valsartan, losartan, telmisartan and eprosartan mesylate; the alpha/beta blockers are selected from carvedilol and labetalol; and the diuretics are selected from bendroflumethiazide, piretanide, chlorthalidone and hydrochlorothiazide (HCTZ).

8. A pharmaceutical product according to any one of claims 4 to 7, wherein the pharmaceutical product is in a form suitable for oral or parenteral administration.

9. Use of riboflavin for the treatment or prophylaxis of elevated blood pressure in a subject homozygous for the MTHFR C677T polymorphism.

10. Use of riboflavin for the treatment or prophylaxis of elevated blood pressure in a subject heterozygous for the MTHFR C677T polymorphism.

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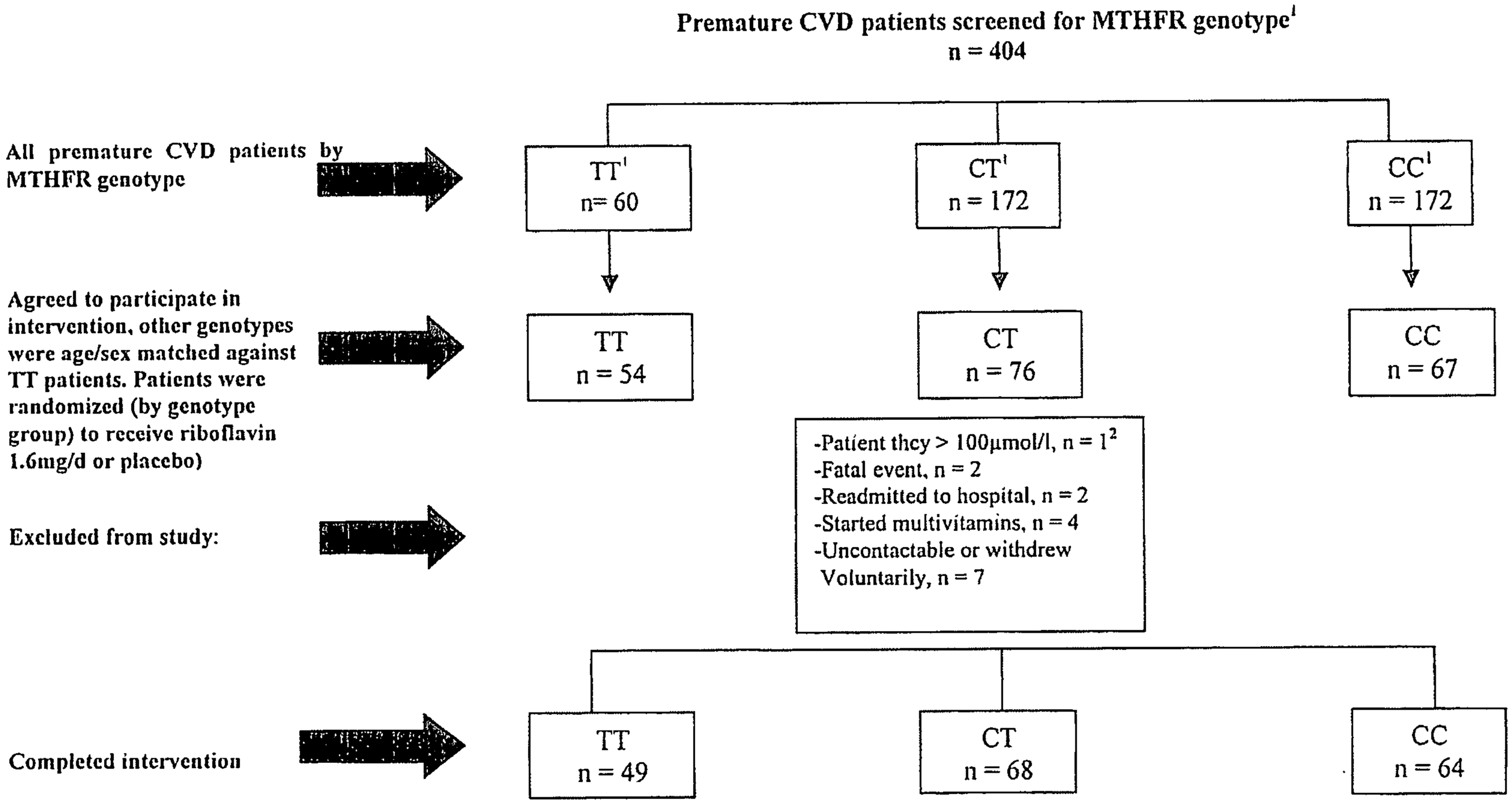

Figure 1: Flow diagram of study design. [1]CC (Wild-type), CT (Heterozygous) and TT (Homozygous) genotypes for the MTHFR 677 C→T polymorphism. [2] One patient had a markedly elevated homocysteine value at baseline (130.4µmol/l) suggesting a metabolic disorder; the patient's physician was notified and the patient was removed from the current investigation.

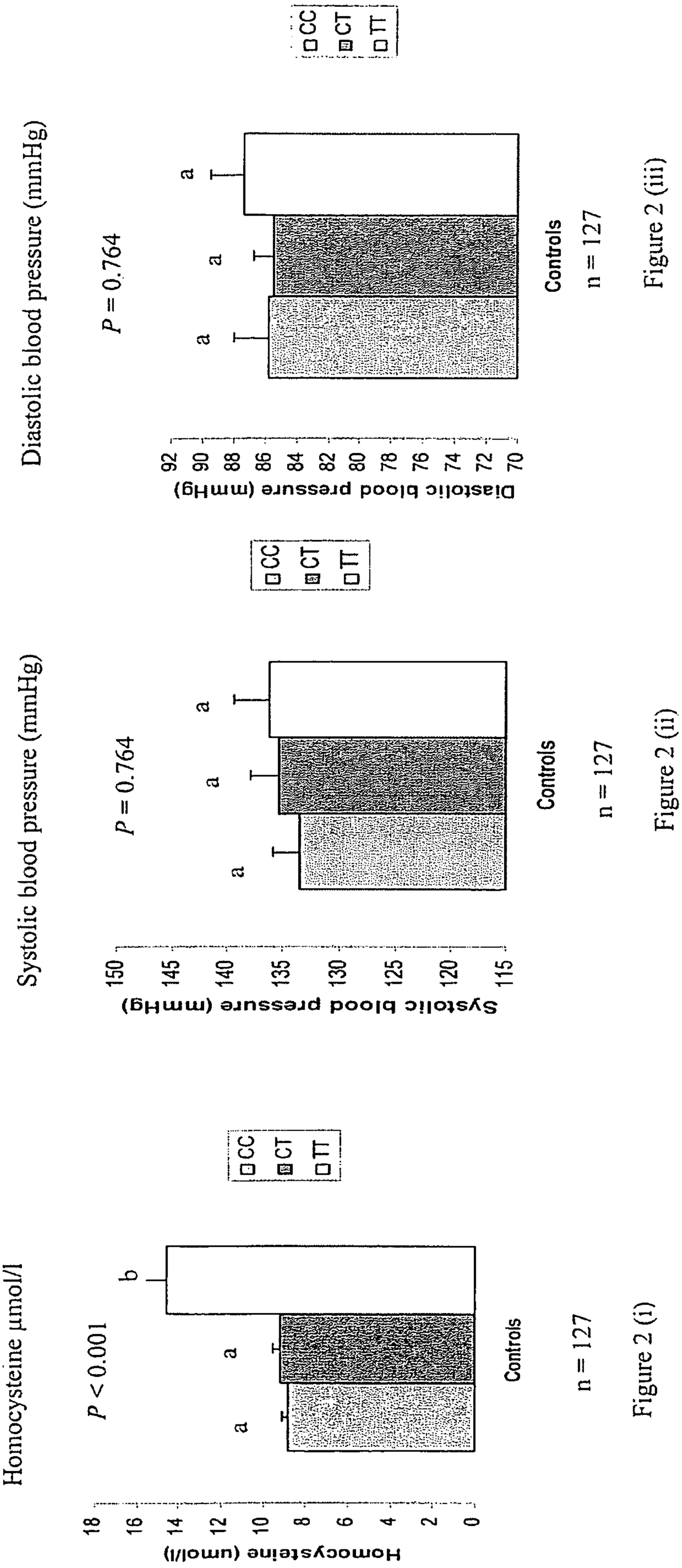

Figure 2 (iii)

Figure 2 (ii)

Figure 2 (i)

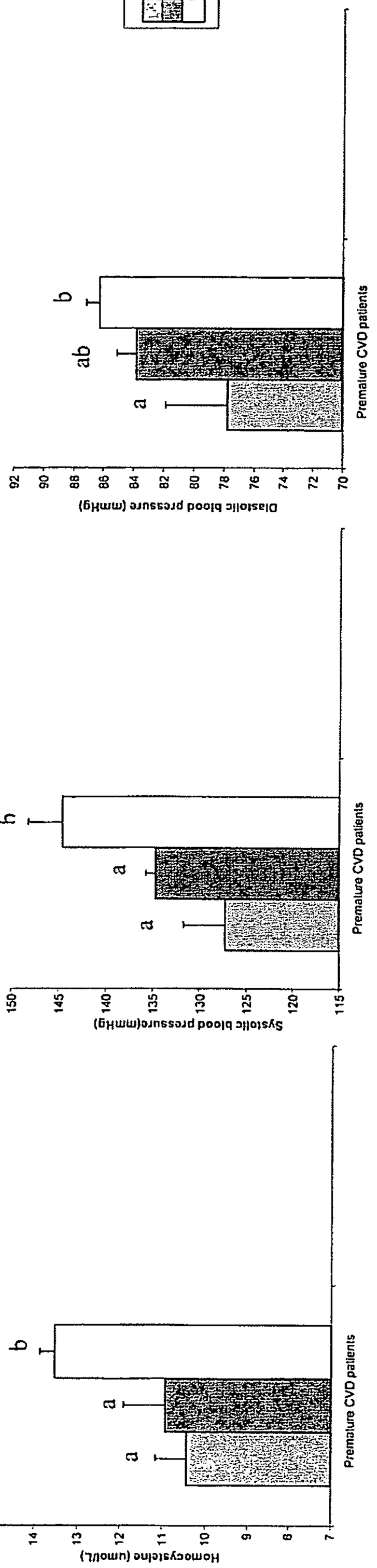

Figure 3 (iii)

(n=161)

Figure 3 (ii)

(n=161)

Figure 3 (i)

(n=161)