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Efeito da modificação do estilo de vida na homeostase pressórica de pacientes com diabetes melito tipo 2 e hipertensão arterial

Tese de doutorado

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ORIENTADORA: Mirela Jobim de Azevedo

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DEDICATÓRIA

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FORMATO DA TESE DE DOUTORADO

A apresentação da presente Tese de Doutorado baseou-se na Resolução Nº 93/2007 da Câmara de Pós-Graduação da UFRGS e orientação do Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, UFRGS, seguindo o formato abaixo descrito:

1. Introdução ao tema ou problema:

Fundamentação Teórica com descrição geral dos objetivos e revisão com Referências Bibliográficas pertinentes, redigidos em Português.

2. Artigo original:

Revisão sistemática com meta-análise de ensaios clínicos randomizados acerca do efeito dos micronutrientes na pressão arterial de pacientes com Diabetes Melito tipo 2, em língua estrangeira apresentado na forma de publicação do periódico científico para onde será enviado o manuscrito.

3. Artigo original:

Ensaio clínico randomizado acerca do efeito de da modificação de estilo de vida na homeostase pressórica de pacientes com diabetes melito tipo 2 e hipertensão arterial, em língua estrangeira apresentado na forma de publicação do periódico científico para onde será enviado o manuscrito.

4. Considerações Finais:

Síntese dos resultados gerais que serviram de base para as conclusões redigidas em Português.

LISTA DE ABREVIATURAS

ABPM = Ambulatory Blood Pressure Monitoring

ADA = American Diabetes Association

BMI = body mass índex

BP = blood pressure

DASH = Dietary Approaches to Stop Hypertension

DBP = diastolic blood pressure

DM = Diabetes Melito

ECR = ensaio clinico randomizado

GLM = generalized linear model

HAS = Hipertensão Arterial Sistêmica

HbA1c = hemoglobina glicada/ glycated hemoglobin

HOT = Hypertension Optimal Treatment

MAPA = Monitorização Ambulatorial da Pressão Arterial

PA = Pressão Arterial

LSD = least significance difference.

PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCT = randomized clinical trial

SD = standard deviation

SBP = systolic blood pressure

UAE = urinary albumin excretion

UFRGS = Universidade Federal do Rio Grande do Sul

UKPDS = *United Kingdom Prospective Diabetes Study*

WHO = World Health Organization

WMD = weighted mean difference

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Importância do problema

O Diabetes Melito (DM) é considerado um problema de saúde pública, cuja prevalência e incidência estão aumentando significativamente, alcançando proporções epidêmicas. De acordo com o Instituto de Diabetes da Austrália, a prevalência mundial de DM entre adultos (20-79 anos) era de 6,4% em 2010, acometendo 285 milhões de pessoas. Estima-se que em 2030, a prevalência de DM aumentará para 7,7%, o que implica em aumento de 69% no número de pessoas com DM nos países em desenvolvimento e de 20% nos países desenvolvidos (1). Em nosso país, dados do estudo VIGITEL apontaram prevalência autorreferida de DM igual a 5,6% (2). Em 2011, o Brasil encontrava-se entre os cinco países com maior prevalência da doença no mundo, com previsão de chegar a 19,6 milhões de pacientes com DM no ano de 2030 (3).

O DM tipo 2 ocorre geralmente na vida adulta e é a forma mais comum de DM, estando associado à obesidade em cerca de 80% dos casos (4). A doença cardiovascular é a principal responsável pela redução da sobrevida de pacientes com DM tipo 2, sendo a causa mais frequente de mortalidade nesse grupo de pacientes (5). A Hipertensão Arterial Sistêmica (HAS) é um importante fator de risco para o desenvolvimento de complicações crônicas micro e macrovasculares em pacientes com DM tipo 2 (6-8). A prevalência de HAS em pacientes com DM tipo 2 atendidos em ambulatórios de hospitais gerais no Rio Grande do Sul foi de 73% (9) e

as médias de pressão arterial (PA) observadas foram 143 ± 24/87 ± 13 mm Hg, valores estes acima das metas recomendadas pela Associação Americana de Diabetes (ADA) (4).

O tratamento da HAS em pacientes com DM tipo 2 está associado à redução de eventos, como foi demonstrado no *United Kingdom Prospective Diabetes Study* (UKPDS). Neste estudo a redução da PA de 180/105 mm Hg para <150/85 mm Hg foi associada a um menor risco de desfechos relacionados ao DM (24%), morte relacionada ao DM (32%), acidente vascular cerebral (44%) e complicações microvasculares (37%) (8). No entanto, apesar das diversas drogas disponíveis para tratamento da hipertensão, muitos pacientes não atingem os alvos preconizados. Estudo avaliando a intensificação do tratamento da hipertensão em pacientes com DM 2 com as medicações disponíveis no Sistema Único de Saúde mostrou que mesmo com visitas realizadas mensalmente, incremento agressivo das doses dos anti-hipertensivos e a associação de fármacos de forma escalonada a cada visita, somente 38,5% dos pacientes o alvo de PA ≤130/80 mm Hg foi atingido (10).

Medidas não farmacológicas como redução de peso são capazes de reduzir a pressão sistólica em até 20 mmHg (11). Outra medida não farmacológica recomendada pelas diretrizes nacionais e internacionais para redução da pressão é a dieta DASH (*Dietary Approaches to Stop Hypertension Study*) (12,13, 14). A dieta DASH consiste no consumo de frutas, vegetais, alimentos integrais, laticínios com baixo teor de gordura, nozes e oleaginosas, e poucas quantidades de carne vermelha e doces. É uma dieta com valores reduzidos de gordura saturada, colesterol e sódio e aumentada em potássio. No estudo original com a dieta DASH, realizados em pacientes sem DM, a adoção da dieta reduziu a PA sistólica em 5.5 mm Hg e a PA diastólica em 3.0 mm Hg quando comparada a uma dieta controle. Este efeito foi ainda mais expressivo

nos pacientes hipertensos (redução de 11,4 mm Hg na PA sistólica e 5,5 mm Hg na PA diastólica) (12).

Contudo, em pacientes com DM tipo 2, o número de estudos abordando DASH e DM é escasso (azakabath). Em estudo transversal realizado pelo nosso grupo com 225 pacientes observou-se que a adoção a uma dieta DASH, com ênfase no consumo de frutas e vegetais, foi capaz de reduzir a pressão (15).Ainda, a dieta DASH mostrou ser altamente efetiva em pacientes sem DM e com HAS resistente ao tratamento, sendo capaz de reduzir em 22,7 mm Hg a PA sistólica e 9,1 mm Hg a PA diastólica (17).

Embora, a adoção de um padrão dietético tipo DASH seja endossado pelas entidades internacionais (ADA 2013, AHA, 2013), a influencia de micronutrientes sobre a pressão arterial em pacientes com DM tipo 2 ainda carece de mais evidência (18 - 24) e não existem recomendações absolutas quanto a suplementação de micronutrientes na dieta de pacientes com DM tipo 2 exceto em casos carenciais. Suplementação de vitamina D em pacientes com hipovitaminose D, reduziu a pressão em pacientes com DM tipo 2 (22,23,24). Meta-analise recente mostrou que o aumento de magnésio na dieta está associado ao aumento da pressão arterial (25) A redução do consumo de sódio faz parte das recomendações internacionais, porém poucos estudos foram conduzidos com pacientes com DM tipo 2 (**27**). Ainda, em pacientes sem DM, a dieta DASH associada à restrição de sódio potencializou os efeitos benéficos na PA na dieta original. Valores reduzidos de sódio na dieta DASH - 3,3 g/dia, 2,3 g/dia e 1,5 g/dia - promoveram uma redução na PA sistólica de 5,9/5,0/2,4 mm Hg e na PA diastólica de 2,9/2.6/1,7 mm Hg, respectivamente, para cada grau de restrição de sódio (13).

Outra modificação no estilo de vida que repercute beneficamente na PA é a prática regular de atividade física. Esta intervenção é capaz de reduzir a PA sistólica em 4 - 6 mm Hg (11,28). A quantidade de passos medida através de pedômetro se associa com melhor perfil de risco cardiovascular em mulheres (29) e em idosos (30). Zucatti e colaboradores encontrou associação inversa entre atividade física usual medida por pedômetro e níveis pressóricos de 24h na MAPA em pacientes com DM tipo 2 (31).

Com base no exposto, a presente Tese de Doutorado foi desenvolvida com o objetivo geral de avaliar o papel de diferentes intervenções não farmacológicas sobre a pressão arterial de pacientes com DM tipo 2, com enfoque na associação de dieta DASH e atividade física e micronutrientes na dieta. Esta tese está divida em uma revisão sistemática e meta-análise do efeito de micronutrientes na pressão arterial e um ensaio clinico randomizado sobre atividade física e dieta DASH, em pacientes com diabetes melito tipo 2.

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Capítulo I

Effect of micronutrients on blood pressure in type 2 diabetes: a systematic review and meta-analysis of randomized clinical trials

(Manuscrito a ser submetido à publicação no periódico Diabetes Care)

Effect of micronutrients on blood pressure in type 2 diabetes:

a systematic review and meta-analysis of randomized clinical trials.

(Nutrients and blood pressure in diabetes)

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OBJECTIVE- Although diet is an important component of hypertension treatment in patients with diabetes, the impact of each dietary micronutrient on blood pressure (BP) levels has not been addressed. Thus, we investigated the effect of micronutrients on BP levels in patients with type 2 diabetes by a systematic review and meta-analysis of randomized clinical trials (RCT).

RESEARCH DESIGN AND METHODS- Medline, Embase, and Cochrane databases were searched for RCT that evaluated the effect of nutrients on BP levels in patients with type 2 diabetes. Data extraction was performed by two independent reviewers. Pooled effect estimates were obtained by using fixed or random-effects meta-analysis.

RESULTS- Seven RCT (nine reports) lasting three to 16 weeks of follow-up were included, providing data for 411 patients. The nutrients evaluated were sodium, magnesium, vitamin C, and vitamin D. Reduction of sodium intake decreased 11.33 mm Hg on systolic BP (95%CI, -19.44 to -3.21 mm Hg). The only identified RCT with supplemental magnesium reduced BP. Vitamin C supplements did not change systolic [-3.93 mm Hg (95%CI, -14.78 to 6.92)] nor diastolic BP [-2.52 mm Hg (95%CI, -5.90 to 0.86). Supplemental vitamin D reduced 6.86 mm Hg (95%CI, -10.36 to -3.36 mm Hg) on systolic and 2.83 mm Hg (95%CI, -4.65 to -1.0) on diastolic BP.

CONCLUSION- Sodium restriction and vitamin D supplementation were associated with significant reduction on BP in patients with type 2 diabetes, suggesting that simple dietary intervention may have benefit for hypertensive patients with type 2 diabetes.

Hypertension is a major risk factor for chronic diabetic complications[1]. Indeed about 67% of patients with type 2 diabetes have hypertension[2] and only 17% of them achieve the optimal levels of blood pressure (BP)[3]. In this context, it has been demonstrated that some dietary interventions can reduce BP levels, prevents or delays the development of hypertension, enhance antihypertensive drug efficacy, and decreases cardiovascular risk [4,5] which highlight a potential therapeutic role of simple dietary interventions.

Dietary recommendations for patients with hypertension include reduction of sodium intake, moderation of alcohol consumption, and adoption of the Dietary Approaches to Stop Hypertension (*DASH)* diet eating plan[6-8]. The American Diabetes Association (ADA) adopts these dietary advices[9] but most of these dietary guidelines were based in studies conducted in non diabetic subjects[4,8, 10, 11]. The positive effects of DASH diet on BP in patients with type 2 diabetes was previously demonstrated in a cross sectional study[12] and in a small clinical trial [13]**.** However, the impact of each dietary micronutrient on BP of patients with diabetes has not been fully addressed[14-20]. In addition, data from these individual studies might not be sufficient to clearly demonstrate the effect of each micronutrient on BP in patients with diabetes. Thus, the aim of the current study is to evaluate the impact of micronutrients on BP of patients with type 2 diabetes by a systematic review and meta-analysis of randomized clinical trials (RCT).

RESEARCH DESIGN AND METHODS

This systematic review was carried out using a predetermined protocol (unpublished) constructed according to the Cochrane Handbook recommendations[22] and reported in accordance

with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[23].

Data sources and search

We searched the electronic databases Medline, Embase, and Cochrane, as well as the Cochrane Controlled Trials Register, and ClinicalTrials.gov registry to identify RCTs that report the effect of nutrients on BP in patients with type 2 diabetes, with or without hypertension, from 1950 to June, 2013. In order to perform the most comprehensive search we included terms related also to macronutrients (protein, lipids, and carbohydrates) in addition to besides terms related to micronutrients. In our search strategy the keywords "Blood Pressure" OR "Hypertension" AND "Diabetes Mellitus" were adopted. The detailed searched medical subject headings (MeSH) was described in the Supplementary Data Online. The reference lists of included articles were also manually searched for further relevant studies. In this initial search, all RCTs were retrieved and all potentially eligible studies were considered for review, regardless of the language.

Study selection

Studies were considered eligible for inclusion if they fulfilled all the following inclusion criteria: presented original data of RCTs assessing the effect of a micronutrient on BP values in patients with type 2 diabetes, examined the effect of a micronutrient on BP after a minimum of two weeks of intervention, and report means (or differences between means) and standard deviations (SD) of BP at baseline and after the intervention. We excluded studies that included children or pregnant women or that evaluated the associated effect of micronutrients with antihypertensive medications, exercise, genetic polymorphisms, or caloric restriction. We have

also excluded studies that evaluated more than one micronutrient intervention at a time or a specific dietary pattern instead of an individual micronutrient. Crossover trials were excluded if BP was not evaluated before and after each diet intervention or if there was not a wash-out period between studied diets.

When studies reported more than one dietary intervention (e.g. different doses of a supplemental micronutrient), each intervention was considered as an individual report.

Data extraction and quality assessment

Two reviewers (T.P. and C.K.K.) independently analyzed the titles and abstracts of every paper retrieved from the literature search to identify potentially eligible studies. All articles that did not meet the selection criteria were excluded. The full text of the remaining papers was obtained for further examination. Then, data were extracted independently by the same two investigators with an agreement value of k = 97%. Disagreements were resolved by a third author (M.J.A. or L.V.V.).

Extracted data, besides assessment of BP changes as mean (SD), included the first author's name, year of publication, trial design and duration of follow-up, assessment of BP change as a primary or secondary outcome, number of participants, gender, age, percentage of patients with hypertension, diabetes duration, BMI, dietary details of intervention and control diets, and medications in use. Information of total energy and macronutrient intakes, as well as evaluation of dietary compliance, was extracted from intervention and control diets descriptions.

In each study, the intervention arm was defined as the diet in which the effect of a specific micronutrient on BP was tested.

The source of bias in included RCTs was assessed by two independent reviewers (T.P.P. and M.J.A.) as proposed by the Cochrane Collaboration[24,25]. Biases were classified into six domains: selection, performance, detection, attrition, reporting, and other bias[25,26]. In the "other bias" domain we included the assessment of dietary compliance. The risk of bias in each individual item was classified as high, low, or unclear. Regarding dietary compliance, the risk of bias was classified as "low" if the study described the method of dietary compliance evaluation. The quality of the body of evidence of our systematic review and meta-analysis was assessed taking into account the GRADE recommendations[25]. This evaluation included factors that may decrease the quality of body of evidence (methodological quality, directness of evidence, heterogeneity, precision of effect estimates, risk of publication bias) and factors that may increase it (large magnitude of effect, all plausible confounding would reduce a demonstrated effect or suggest a spurious effect when results show no effect, dose-response gradient). Each evaluated factor was rated as high, moderate, low, or very low. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration[23].

Data synthesis and analysis

Absolute changes in BP (systolic and diastolic) were reported as differences between arithmetic means at the start and at the end of each study. Changes between baseline and final SD values for BP, for each analyzed micronutrient, were directly extracted from the manuscripts or calculated, assuming a correlation of 0.5 between the baseline and final measures within each group, according to the formula of Follman et al.[27] as proposed by Cochrane Handbook[22.] We assumed equal variance among trials and between intervention and controls.

The Cochran's 2 *x* test (Q test) was used to evaluate heterogeneity between studies and a threshold *p* value 0·1 was considered statistically significant; the I^2 test was also performed to evaluate the magnitude of the heterogeneity between studies[28]. The pooled estimate of the mean differences on blood pressure (mm Hg) between intervention and control groups was calculated using fixed-effect models when there was no heterogeneity in the respective meta-analysis. The random effects model (DerSimonian-Laird method) was used in the presence of a significant heterogeneity between the studies. High heterogeneity in the Q test (I^2 >50%) in each single meta-analysis was investigated by meta-regression and sensitivity (sub-group) analyses when not precluded by the number of included studies.

We assessed the possibility of publication bias visually by funnel plot asymmetry and statistically by Begg's and Egger's tests; a significant publication bias was considered if the P value was <0.10[29].

All statistical analyses were performed using Stata 11.0 software (Stata, College Station, TX, USA). Significance was set at P <0.05 and 95% confidence intervals are quoted throughout.

RESULTS

Literature search

We identified 24,431 studies in the database searches (Figure 1). Of them, 21,790 were excluded based on title and abstract, leaving 2,722 articles for further evaluation. From those, 2,693 studies were excluded because evaluated only macronutrients, their design was not a RCT or did not evaluate the effect of the nutrient on BP changes. Therefore, we selected 29 trials for full text evaluation. From the 29 RCT initially selected, seven studies[14-20] fulfilled the inclusion

criteria. Two studies reported two interventions each one. Then, we included nine reports from seven trials in the final analyses.

Studies characteristics

The characteristics of the included reports on the effects of dietary micronutrients - sodium, magnesium, vitamin C, vitamin D - on BP in patients with type 2 diabetes are summarized in Table 1. BP changes were the primary outcome in all studies but those that evaluated vitamin D supplementation[18-20]. Eight reports were parallel RCTs[14-20] and one had a crossover controlled design[14]. The trials duration ranged from three to 16 weeks. The included studies provide data for 411 patients with type 2 diabetes, 43% to 73% of males, aged 52.5 to 66.0 years. The duration of diabetes varied from 4.6 to 8.6 years, but four reports[14,17-19] did not describe the age. The majority of patients were obese[18, 19] or overweight[15-17]. BMI was not reported in two studies[14,20]. Concerning the description of the main characteristics of intervention and control diets, in most of the reports (8/9) the intervention was a supplement; one sodium study used dietary advice only as the intervention[14]. Medications in use for diabetes treatment did not differ between intervention and control groups, but in two studies[14,18] there was no information about it. Four studies[14,16,17,19] reported the antihypertensive medications in use.

Quality of studies

The quality of the body of evidence in the studies in present systematic reviews is described in the Online-only Supplementary Data. Briefly, all included studies had a low risk of bias for attrition and reporting items since none of them had incomplete outcome data or selective report of results. Also, all studies described assessment of diet or supplement compliance. The two reports of the effect of sodium on BP[14] had low risk of bias in almost all

evaluated items but the method of randomization was not clearly described. Actually, in one of them[14] the blinding of participant and personnel was not applicable since intervention was dietary advice only. The study of magnesium[15] had a low risk of bias except by the absence of description about blinding of outcome assessment. Regarding vitamin C evaluation[16, 17], the methods of randomization were not described and outcome assessments were unclear. Risk of bias was low in most of domains of in the four vitamin D reports[18-20] but there is no description about the blinding of outcome assessment. In those studies, one of them[20] did not describe the allocation method. Detailed information about quality of studies was described in the online-only supplementary data.

Evidence summary

Sodium

The effect of sodium intake on BP in patients with type 2 diabetes and hypertension was evaluated in one study (two reports). In the first part of the study, patients were advised to reduce their daily sodium intake (1st report, n = 34); a reduction of daily salt of ~ 3 g (from 11.6 to 8.2 g/ day) reduced ~ 20 mm Hg on systolic BP. Thereafter, in the second part of the study, a crossover randomized trial (2nd report, n = 13) was conducted only in patients from the intervention arm. No changes on BP values were identified after a daily supplement of 1.84 grams of sodium.

The intervention diet was defined as the diet with the lowest sodium content as compared to the control diet. In the pooled analysis of the two reports[14] the intervention group (lowest sodium intake) had an average decrease of 11.33 mm Hg in systolic BP (95%CI, 19.44 to 3.21 mm Hg) as compared to control group (fixed effects model). There was no heterogeneity between the individual efficacy estimates in the magnitude of BP reduction (I^2 = 0.0%; P

<0.699). The decrease in the diastolic BP was not significant (Figure 2). There was no publication bias as visually evaluated by the funnel plot both for the effect on systolic and diastolic BP (Online-only Supplementary Data). The Begg and Egger tests were not performed due to the number of included studies.

Magnesium

We identify one study[15] including 82 patients with type 2 diabetes that evaluated the effect of oral magnesium supplement (450 mg of magnesium daily) on BP as a primary endpoint in patients with low serum magnesium (≤0.74 mmol/L) without using diuretics. The reduction of BP was significant only in the group which received the supplement. The difference in BP changes between groups was -16.1 mm Hg (95%CI, -27.1 to -5.1) for systolic BP and -7.9 mm Hg (95%CI, -12.6 to -3.1) for diastolic BP.

Vitamin C

Two studies evaluate the effect of Vitamin C supplements (500 mg to 1,500 mg/day)[16, 17] on BP and the results are contradictory. A low dose (500 mg daily) was used in the study in which vitamin C reduced BP but these patients, both in intervention and control groups, had lower values of systolic BP as compared with the other study (P <0.0001). In the pooled analysis of the two reports (65 patients) (Figure 4), no effect of a vitamin C intake was demonstrated in both, systolic [-3.93 mm Hg (95%CI, -14.78 to 6.92); I^2 80.4%; P = 0.024; random effect model] and diastolic [-2.52 mm Hg (95%CI, -5.90 to 0.86); I^2 34.9%; P = 0.215; fixed effect models] BP as compared to control groups. Visually asymmetry was observed in the funnel plots both for systolic and diastolic BP (Online-only Supplementary Data). Begg and Egger tests were not performed because there were only two studies.

Vitamin D

Three studies (four reports) [18-20] including 217 patients with type 2 diabetes evaluated the effects of vitamin D on BP. In three reports the patients received a single dose of vitamin D2[19] (100.000 IU) or vitamin D3[18] (100.000 IU or 200.000 IU). One study evaluated the effect of a D3 fortified yogurt[20] (500 IU D3; 250 ml twice a day for 12 weeks; total dose of 84.000 IU).

Data from these four reports were pooled. Supplementation of vitamin D in the intervention group decreased 6.86 mm Hg in the systolic BP (95%CI, -10.36 to -3.36 mm Hg; fixed effects model) as compared to control group, without significant heterogeneity (I^2 36.3%; P = 0.194). Diastolic BP also decreased after intervention [-2.83 mm Hg (95%CI, -4.65 to -1.0); I^2 49.4%; P = 0.115; fixed effects model] (Figure 4). Funnel plots and the Egger regression tests confirmed the absence of publication bias on the systolic (P = 0.14) and diastolic (P = 0.92) BP analyses (Online-only Supplementary Data).

Patients from the study of Shab-Bidar et al.[20] received the lower vitamin D dose and were younger as compared with patients from the other studies. In addition, this study contributes with the high weight in the pooled analysis. We undertook a meta-regression analysis including age and vitamin D doses (84.000, 100.000, and 200.000 IU) as covariates. Neither age nor doses were associated with BP changes (P = 0.808).

Quality of body evidence

The assessment of the quality of the body of evidence in the present systematic reviews and meta-analyses according to the GRADE approach is described in Table 2. The quality of the body evidence was considered as high for sodium and vitamin D and as low for vitamin C.

CONCLUSIONS

In this systematic review the effect of micronutrients on BP of patients with type 2 diabetes was evaluated through of seven RCTs including four micronutrients. Meta-analyses of sodium and vitamin D demonstrated that these were the micronutrients associated with significant reduction on BP. Sodium restriction caused a decrement of 11 mm Hg on systolic BP while vitamin D supplementation a decrement of 7 mm Hg on systolic and 3 mm Hg on diastolic BP. Pooled data of the effect of vitamin C supplements on BP were not significant. Lastly, in the only one identified trial, magnesium supplement reduced BP values.

Dietary sodium restriction has been documented to lower BP in numerous epidemiologic, clinical, and experimental studies[30] and it is a well-established recommendation in the management of hypertension[4,11]. In fact, abnormalities in the body's handling of sodium can become expressed in an unhealthful diet characterized by an excessive intake of salt (NEJM). The recommendation of sodium restriction is also adopted by the American Diabetes Association[9,21], although most existing data came from studies conducted in subjects without diabetes. A recent systematic review with meta-analysis[31] on the health effect of lower sodium intake demonstrated a reduction of 3.39 mm Hg on systolic and 1.54 mm Hg on diastolic BP in subjects with and without hypertension. This beneficial effect of salt restriction was confirmed even after modest salt reduction (4.4 g/day) as demonstrated by another meta-analysis that included only non-diabetic patients with and without hypertension[32]. In that study, when the subgroup of hypertensive patients was analyzed, the reduction on systolic and diastolic BP was 5.39 and 2.82 mm Hg, respectively. In type 2 diabetic patients included in our review a salt restriction of 3.0 g/day caused an even greater reduction on systolic BP. Interesting, this

reduction occurred even considering that the observed salt intake after intervention (8 g/ day) was higher than the recommended for patients with diabetes (<6.0 g/day)[21].

Observational and experimental data favors the concept that vitamin D is involved in the pathogenesis of arterial hypertension[33, 34]. The proposed mechanism for the link between vitamin D and high BP involves the inhibition of the renin–angiotensin–aldosterone system by vitamin D. An alternative mechanism could be associated with the secondary hyperparathyroidism and hypocalcemia which are commonly seen in patients with hypovitaminosis D[34]. In a meta-analysis that included 11 RCT[35], just one conducted in patients with diabetes, demonstrated that vitamin D supplementation in subjects with vitamin D deficiency causes a modest reduction in diastolic BP (-3.1 mm Hg) only in hypertensive patients[36]. The variability of doses, age of patients (48 to 74 years), duration of interventions (5 weeks to one year), as well as the use of activated (1-25 OHD) or unactivated (vitamin D2, D3, UVB radiation) vitamin D were associated with a high heterogeneity in this study. In the other hand, in our meta-analysis the reduction of BP by supplementary vitamin D2 or D3 had a low heterogeneity. Noticeably, the observed reduction of BP seemed to be independent of vitamin D doses. When the study with the greatest weight contribution (52.72%) in the pooled BP reduction was excluded, the results did not change (data not shown). We believe that these data are the most robust among the BP effects of micronutrients in the current study. However, it is important to note that patients had low baseline values of vitamin D and that this results may not be generalized to vitamin D sufficient patients. Further interventional studies are needed to define the optimum dose and dosing interval to administer for patients with type 2 diabetes in order to reduce BP values.

An inverse association between plasma ascorbate concentration and intake or supplementation of vitamin C with BP was demonstrated in observational studies[37,38]. In a meta-analysis that

evaluated 29 RCT[39] conducted in subjects with and without hypertension, supplementary vitamin C (about 500 mg/day) reduced systolic (3.84 mm Hg) and diastolic (1.48 mm Hg) BP. However, the intervention with vitamin C was combined with other micronutrients in three of the five studies in patients with type 2 diabetes included in this meta-analysis. This meta-analysis had a significant heterogeneity of effects across studies. In our meta-analyses, which included only studies where vitamin C was the only tested micronutrient, we did not demonstrate any effect of vitamin C in BP of patients with type 2 diabetes. Actually, the response of BP was in the opposite direction. In these trials, besides using different doses of vitamin C supplements, the baseline BP values of patients were quite different. We did not find other meta-analyses on the possible effect of vitamin C on BP of patients with diabetes to compare our results.

The association between low dietary magnesium intake with hypertension came from epidemiologic evidences[40,41]. A recent meta-analysis[42] evaluated the effect of magnesium on BP of subjects with and without hypertension using different supplemental magnesium compounds and doses (120 to 973 mg). A small but significant reduction on systolic (3 to 4 mmHg) and diastolic (2 to 3 mm Hg) BP was observed. A sensitivity analysis demonstrated that the higher the magnesium dose the higher the BP reduction. This is in accordance with data from a previous meta-analysis[43] which demonstrated a dose-dependent effect of magnesium with reductions of 4.3 mm Hg on systolic and 2.3 mm Hg on diastolic BP for each supplemental 24.3 mg/day. However the quality of the majority of studies included in these meta-analyses was poor and in both[42,43] there was a high heterogeneity in the effects size across trials. Therefore, the effect of magnesium in BP is still a matter of debate. Especially, in patients with diabetes this possible BP reduction should be confirmed.

In the present meta-analyses, a high level of heterogeneity was detected for the pooled analysis of the effect of vitamin C on BP. Therefore, a random effects model was used instead of a fixed model, since the random effects model involves an assumption that the effects being estimated in different studies are not identical. In fact, the only two included studies had contradictory results and we could not evaluate possible factors associated with this heterogeneity. Regarding the meta-analysis of the effects of vitamin D on BP, even in the absence of a significant heterogeneity, we performed a meta-regression including age and vitamin D doses as covariates since these factors could have influence our results. The inclusion of both covariates did not modify our results.

The present systematic reviews and meta-analyses were conducted in accordance with the Cochrane[22] and PRISMA[23] guidelines. We performed the most comprehensive search as possible and all relevant studies were included, regardless of language. In addition, we excluded studies that evaluated more than one micronutrient intervention together or any combined nutritional intervention. Using the GRADE approach, the quality of the body of evidence in the current review was classified as high for our main result, the effect of vitamin D, as well as for sodium.

A limitation of our systematic reviews and meta-analyses were the inclusion of few reports. In addition, the total number of patients included was relatively small in the sodium and vitamin C meta-analyses. However, because the evaluated data possibly comprises the available literature on this topic, we believe that our meta-analyses provide important information that adds to the current knowledge on the impact of micronutrients on BP levels in diabetes. Another possible limitation is that BP was evaluated at office in all included studies. As such, a more comprehensive evaluation of BP obtained by continuous BP monitoring[44] could have yield more

accurate results. However, we should note that office BP measurement comprises the usual assessment of BP in clinical practice.

In conclusion, we demonstrated by systematic reviews and meta-analyses of RCT that sodium restriction and supplemental vitamin D in the presence of vitamin D deficiency have beneficial effect on BP in patients with type 2 diabetes.

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Table 1. Characteristics of the included studies on the effect of micronutrients on blood pressure in patients with type 2 diabetes.

Author Year	Design Follow-up	Sample description	Diabetes duration (years)	BMI (Kg/m²)	Baseline BP (mm Hg)	Diet Characteristics	BP change (mean, mm Hg);	Medications in use (% of users)
					Sodium			
Dodson 1989	Parallel 3 months	n = 34 67.6% males 61.4 ± 6.9 years old hypertensive	4.6 ± 4.3	NA	SBP I. 179.7 ± 18.2 C.173.8 ± 20.3 DBP I. 91.4 ± 11.1 C. 92.4 ± 10.9	Intervention Diet with sodium restriction advice to reduce daily sodium intake Baseline: 11.6 g NaCl* End-of-study: 8.16 g NaCl* Change: -3.44 Control Usual diet for diabetes (ADA) Baseline: 10.8 g NaCl* End-of-study: 10.6 g NaCl* Change: -0.2 * 24-h urinary sodium	Intervention SBP supine: -19.2 DBP supine: -3.8 Control SBP supine: -6.2 DBP supine: -2.0	Oral hypoglicemics: 18% Atenolol: 12%

Dodson 1989	Crossover 1 month, (wash-out =1 month)	n = 13 67.3% males 62.5 ± 6.5 years old hypertensive	NA	NA	NA	Intervention Usual diet for diabetes (ADA) Baseline: 10.8 g NaCl* End-of-study: 7.2 g NaCl* Change: -3.36 Control Daily oral supplement of 1.84 g sodium Baseline: 8.32 g NaCl* End-of-study: 11.7 g NaCl* Change: +3.38 * 24-h urinary sodium	Intervention SBP supine: +1.8 DBP supine: -0.5 Control SBP supine: +11.5 DBP supine: +4.6	NA
Magnesium								
Guerrero Romero 2009	Parallel 3 weeks	n = 82 48.1% males 59.5 ± 8.9 years old with low serum magnesium without	8.6 ± 0.9	29.1 ± 1.3	SBP I. 161 ± 26 C. 154.5 ± 21.2	Intervention Daily oral supplement: 2.5 MgCl2 (= 0.45 g Mg) Baseline: 0.62 ± 0.10 mmol/l† End-of-study: 0.81 ±0.11mmol/l†	Intervention SBP: -20.4 DBP: -8.7	Glibenclamide: 100%

		use of diuretics				Change: +0.19 Control Baseline: 0.61 ± 0.10 mmol/l† End-of-study: 0.68 ±0.11mmol/l† Change: +0.07 † 24-h urinary magnesium		
					DBP I. 88.4 ± 14.5 C. 84.9 ± 12.4		Control SBP: -4.7 DBP: -1.1	
				Vitamin C				
Darko 2002	Parallel 3 weeks	n = 35 66% males 56.1 ± 1.5 years old	8.6 ± 0.9	29.1 ± 1.3	SBP I. 141.0 ± 5.0 C. 138.0 ± 4.0	Intervention Daily oral supplement: 1.5g ascorbic acid Plasma ascorbate Baseline: 58 ± 6 µmmol End-of-study: 122 ± 10 µmmol Change: +64	Intervention SBP: 0 DBP: +1.0	Diuretics = 9% ACE inhibitors = 11% Sulphonylureas = 23% Metformin = 37%
					DBP	Control (placebo) Plasma ascorbate	Control	

					I. 80.0 ± 2.0 C. 76.0 ± 3.0	Baseline: 51 ± 5 µmmol End-of-study: 53 ± 5 µmmol Change: +2	SBP: +2.0 DBP: +1.0	
Mullan 2002	Parallel Double blind 4 weeks	n = 30 73% males 59.5 ± 6.5 years old	NA	28.6 ± 4.3	SBP I. 130.1 ± 12.4 C. 129.7 ± 11.7 DBP I. 80.5 ± 6.2 C. 85.1 ± 6.4	Intervention Daily oral supplement: 500 mg ascorbic acid Plasma ascorbic acid Baseline: 43.3 ± 19.3 µmmol/l End-of-study:78.1 ± 19.5µmmol/l Change: + 34.8 Control (placebo) Plasma ascorbic acid: NA	Intervention SBP: -9.8 DBP: -4.4 Control: SBP: -1.0 DBP: +0.6	NA
					Vitamin D			
Witham	Parallel	n = 41	NA	32.2 ± 6.9	SBP	Intervention Single dose supplement: 100.000 IU	Intervention	NA

2010	16 Weeks	68% males 66.0 ± 10.4 years old			I. 149.6 ± 24.8 C. 143.9 ± 24.4	D3 Serum 25 OHD: Baseline: 41 ± 14 nmol/l (End-of-study: 63 ± 20 nmol/l Change: +22	SBP: -8.2 DBP: -2.5	
						Control		
					DBP I. 80.7 ± 14.3 C. 80.3 ± 9.7	Single dose placebo Miglyol® oil Serum 25OHD: Baseline: 45 ± 17 nmol/l End-of-study: 54 ± 20 nmol/l Change: +9	Control SBP: +2.5 DBP: -1.4	
Witham 2010	Parallel 16 weeks	n = 42 59% males 65.0 ± 9.6 years old	NA	31.5 ± 5.7	SBP I. 145.1 ± 25.0 C. 143.9 ± 24.4	Intervention Single dose supplement: 200.000 IU D3 Serum 25 OHD: Baseline: 48 ± 21 nmol/l End-of-study: 79 ± 31 nmol/l Change: + 31	Intervention SBP: -9.3 DBP: -3.5	NA

					DBP I. 80.7 ± 14.3 C. 80.3 ± 9.7	Control Single dose placebo Miglyol® oil Serum 25 OHD: Baseline: 45.0 ± 17 nmol/l End-of-study: 54 ± 20 nmol/l Change: +9	Control SBP: +2,.5 DBP: -1.4	
Sugden 2007	Parallel double blind 8 weeks	n = 34 53% males 64.2 ± 9.9 years old plasma vitamin D < 50 mmol/l	NA	31.7 ± 5.4	SBP I. 145 ± 9.2 C. 137 ± 14.1	Intervention Single dose supplement: 100.000 IU D2 Serum 25 OHD: Baseline: 40.2 ± 10.3 nmol/l Change: 22.9 ± 16.6 nmol/l	Intervention SBP: -7.3 DBP: -2.2	ACE inhibitor or angiotensin blocker = 62% Metformin = 53% Insulin = 18%
					DBP I. 82 ± 10.5 C. 79 ± 6.0	Control Single dose placebo Miglyol® oil Serum 25OHD: Baseline: 36.4 ± 8.5 nmol/l Change: 7.6 ± 10.5 nmol/l	Control SBP: +6.6 DPB: +2.3	
Shab-Bidar	Parallel	n = 100	I. 8.3 ± 4.6	NA	SBP	Intervention Vitamin D3 fortified yogurt: 170 mg	Intervention	Oral antihyperglycemic = 100% (metformin,

2011	double blind 12 weeks	43% males 52.5 ± 7.4 years old	C. 7.0 ± 5.2	I. 125.7 ± 14.4 C. 128.2 ± 16.6 DBP I. 78.5 ± 10.3 C. 77.8 ± 10.8	calcium and 500 IU D3/250 ml, twice/day (total dose 84.000 IU) Serum 25OHD: Baseline: 38.5 ± 20.2 nmol/l End-of-study: 72 ± 23.5 nmol/l Change: +33.5 Control Plain yogurt: 170 mg calcium without vitamin D3/250ml Serum 25OHD: Baseline: 38.5 ± 22.8 nmol/l End-of-study: 33.4 ± 22.8 nmol/l Change: -5.1	SBP: -7.3 DBP: -3.5 Control SBP: -2.5 DBP: -0.6	glibenclamide, glitazones)

Abbreviatures: ADA = American Diabetes Association; BP = blood pressure; C = control group; DBP = diastolic blood pressure; DM = diabetes mellitus; I = intervention group; NA = not available; SBP = systolic blood pressure. * = NaCl estimate by 24-h urinary sodium, † = intake of magnesium based on 24-h urinary magnesium (mmol/l), ‡ = intake of protein based on urinary 24-h urea (g/kg)

Table 2. Assessment of the quality of the body of evidence in the present systematic review.

Factor	Nutrient	Quality	Support for judgment
Within-study risk of bias (methodological quality)	Sodium	Moderate	Methods of randomization and allocation were not described. The two included reports had low risk of bias regarding performance, detection, attrition, reporting, and assessment of diet/supplement compliance.
	Magnesium	High	In almost all evaluated items (selection, performance, detection, attrition, reporting, and assessment of diet/supplement compliance) the included study had low risk of bias.
	Vitamin C	Moderate	Both participants and personnel were blinded. The risk of bias regarding the attrition and reporting domains, as well as assessment of diet/supplement compliance were low. Methods of randomization and allocation were not described.
	Vitamin D	High	Only one report did not describe the randomization method. Risk of bias in attrition, reporting, and assessment of diet/supplement compliance were low.
Directness of evidence	All studied nutrients	High	Direct comparisons of an intervention with a control were performed in all RCTs.
Heterogeneity *	Sodium	High	A low heterogeneity was observed in the meta-analysis.
	Vitamin C	Moderate	A high heterogeneity was observed in the systolic BP meta-analysis; the diastolic BP meta-analysis had low heterogeneity.
	Vitamin D	High	A low heterogeneity for systolic BP and diastolic BP was observed in the meta-analyses.
Precision of results *, †	Vitamin C	Low	High heterogeneity between the included studies could be related to different doses of vitamin C.

Risk of publication bias*	Sodium	High	No asymmetry was demonstrated by the funnel plot.
	Vitamin C	High	No asymmetry was demonstrated by the funnel plot
	Vitamin D	High	No asymmetry was demonstrated by the funnel plot and Begg's and Egger's test.
Large magnitude effect *	Sodium	High	The reduction on BP was clinically relevant.
	Vitamin D	High	The reduction on BP was clinically relevant
No demonstrable effect *	Vitamin C	Low	No effect on BP was demonstrated probably due to only two studies were included and their effect was opposite
Dose- response gradient *, ‡	Vitamin D	High	There was no dose response effect of vitamin D on BP changes.

* This factor was analyzed only when a meta-analysis was performed.

† Precision of results was described only for meta-analyses in which it was demonstrated a high heterogeneity.

‡ This factor was analyzed only when a meta-regression analysis was performed (vitamin D).

Figure 1. Flow diagram of literature search to identify randomized clinical trials evaluating the effect of micronutrients on blood pressure in patients with type 2 diabetes.

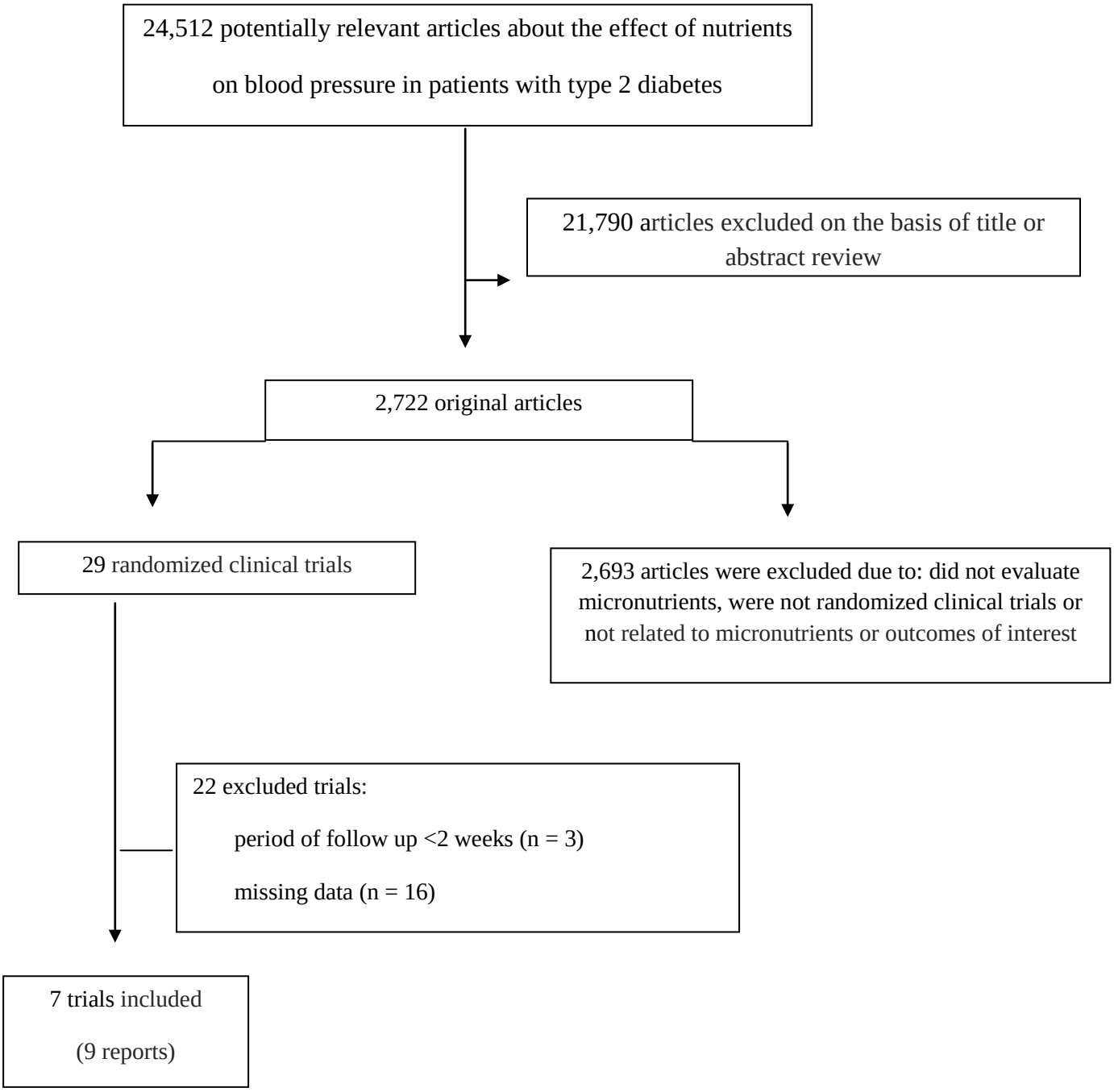
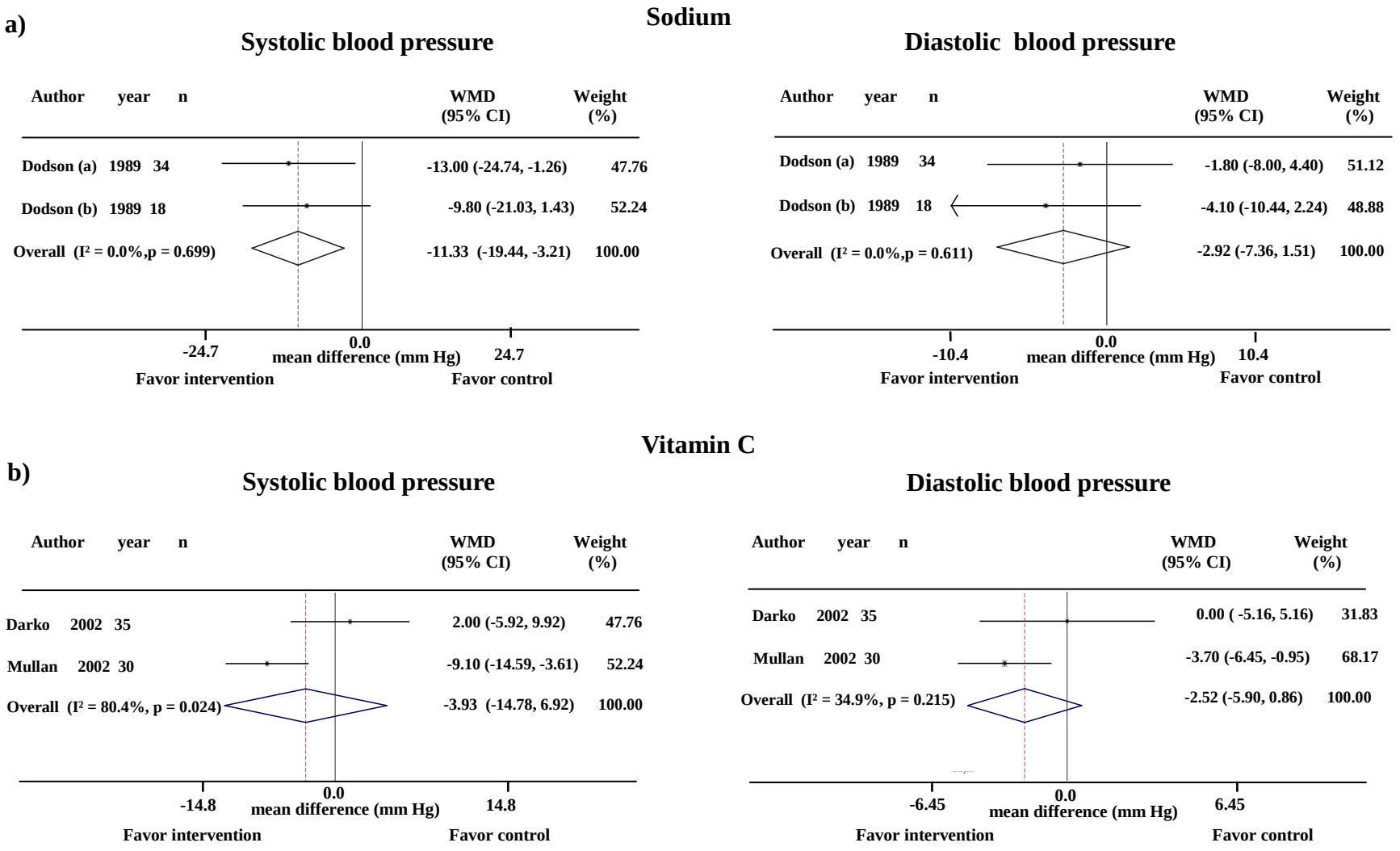


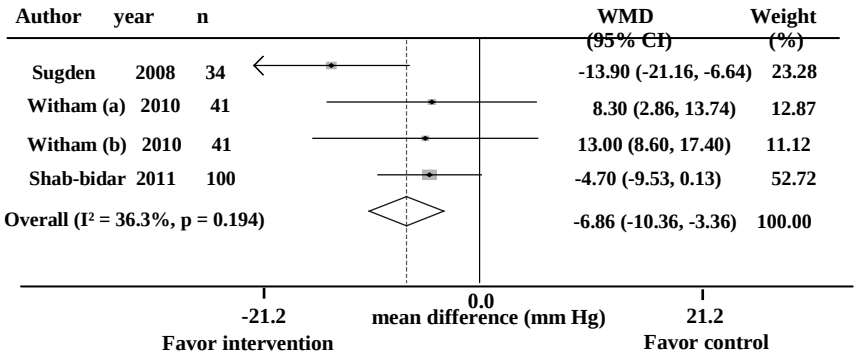
Figure 2. Forest plots of the effects of micronutrients on blood pressure of patients with type 2 diabetes: a) sodium restriction, b) vitamin C, and c) vitamin D.



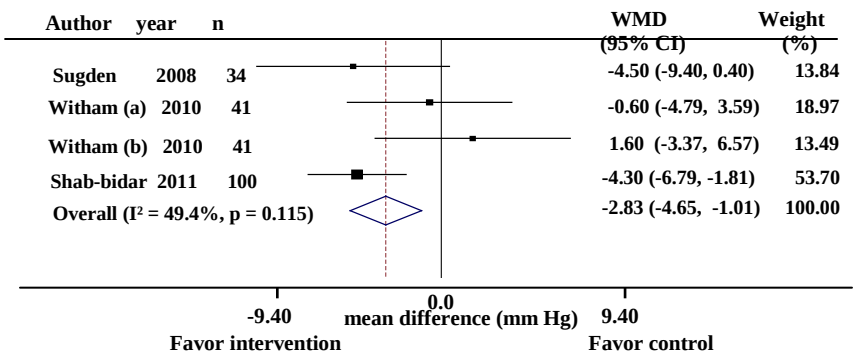
c)

Vitamin D

Systolic blood pressure



Diastolic blood pressure



SUPPLEMENTAL DATA.

Online-only **Supplementary** Medline search strategy:

("Micronutrients"[Mesh]) OR ("Phosphorus"[Mesh] OR "Phosphorus, Dietary"[Mesh])) OR ("Sodium"[Mesh] OR "Sodium, Dietary"[Mesh])) OR "Chromium"[Mesh]) OR "Calcium"[Mesh]) OR ("Iron"[Mesh] OR "Iron, Dietary"[Mesh])) OR ("Magnesium"[Mesh] OR "Magnesium Chloride"[Mesh])) OR ("Potassium"[Mesh] OR "Potassium, Dietary"[Mesh])) OR ("Vitamins"[Mesh] OR "Vitamin B Complex"[Mesh])) OR "Vitamin A"[Mesh]) OR "Ascorbic Acid"[Mesh]) OR "Vitamin B 6"[Mesh]) OR "Vitamin B 12"[Mesh]) OR "Vitamin D"[Mesh]) OR "Vitamin E"[Mesh]) OR "Zinc"[Mesh]) OR "Carbohydrates"[Mesh]) OR "Proteins"[Mesh]) OR "Lipids"[Mesh]) OR "Dietary Fiber"[Mesh]) OR "Ethanol"[Mesh]) OR "Alcohols"[Mesh]) OR "Alcohol Drinking"[Mesh]) OR ("Cholesterol"[Mesh] OR "Cholesterol, Dietary"[Mesh])) OR Fish oil OR Fatty acids OR omega-3 OR omega 6 OR Tea OR cocoa OR chocolate AND "Blood Pressure"[Mesh]) OR "Hypertension"[Mesh]) AND "Diabetes Mellitus"[Mesh].

Supplementary Table: Assessment of the quality of included studies: summary of risk of bias.

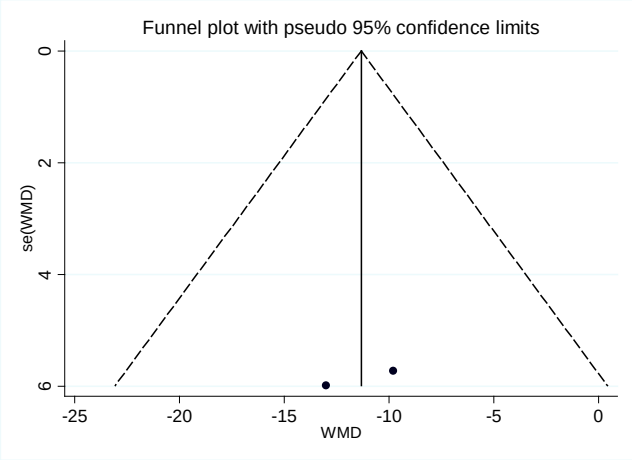
	Selection Bias		Performance Bias	Detection Bias	Attrition Bias	Reporting Bias	Other Bias
	Random sequence generation	Allocation Concealment	Blinding of participant and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Diet/supplement compliance assessment
			Sodium				
Dodson[1989 (14)]	unclear	unclear	low *	low	low	low	low
Dodson [1989(14)]	unclear	unclear	low	low	low	low	low
			Magnesium				
Guerrero-Romero[2009(15)]	low	unclear	low	unclear	low	low	low
			Vitamin C				
Darko [2002(16)]	unclear	unclear	low	unclear	low	low	low
Mullan [2002(17)]	unclear	unclear	low	unclear	low	low	low
			Vitamin D				
Witham [2010(18)]	low	low	low	unclear	low	low	low
Witham [2010(18)]	low	low	low	unclear	low	low	low
Sugden [2007(19)]	low	low	low	unclear	low	low	low
Shab-Bidar [2011(20)]	unclear	unclear	low	unclear	low	low	low

* blinding of participant and personnel was not applicable since intervention was dietary advice only

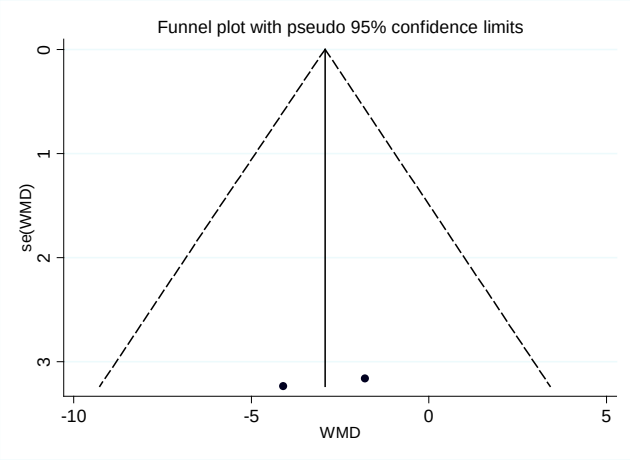
Supplementary Figure: Funnel plots diagram of publication bias of the meta-analyses of the effects of micronutrients on blood pressure of patients with type 2 diabetes: a) sodium restriction, b) vitamin C, and c) vitamin D.

a)

Sodium restriction: systolic BP

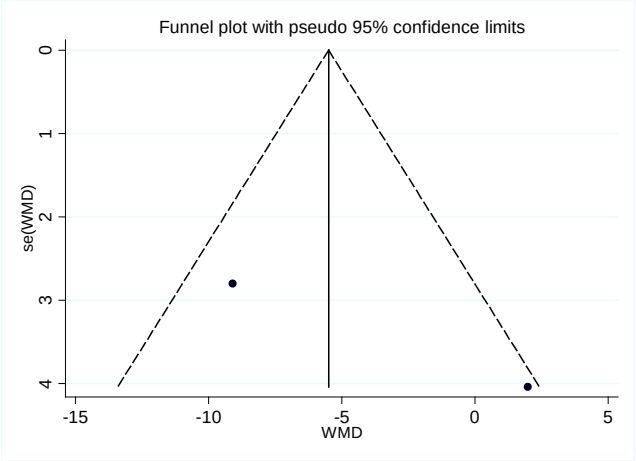


Sodium restriction : diastolic BP

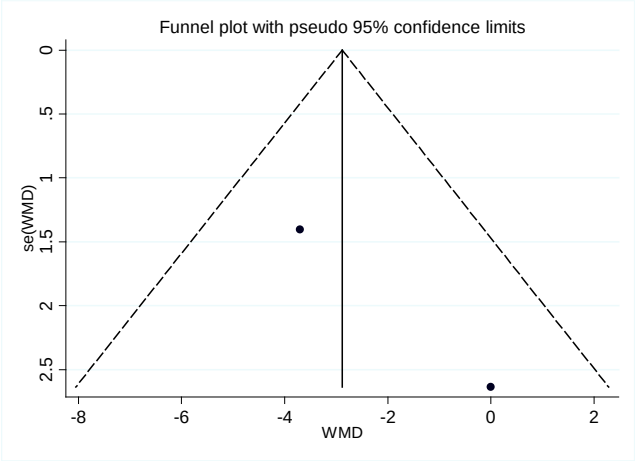


b)

Vitamin C: systolic BP

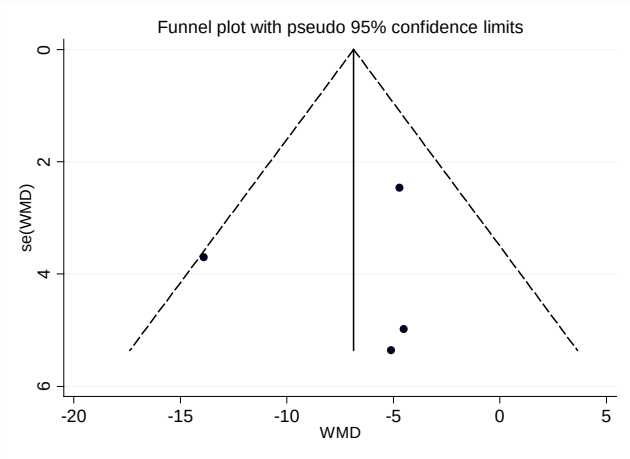


Vitamin C: diastolic BP

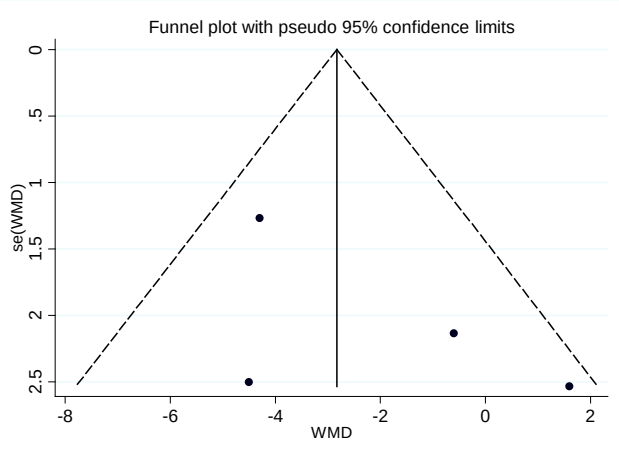


c)

Vitamin D: systolic BP



Vitamin D: diastolic BP



Capítulo II

Effect of lifestyle intervention on blood pressure homeostasis in patients with type 2 diabetes and hypertension

(Manuscrito a ser submetido à publicação no periódico Diabetes Care)

Effect of lifestyle intervention on blood pressure homeostasis in patients with type 2 diabetes and hypertension

Running title: lifestyle and blood pressure in type 2 diabetes

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OBJECTIVE- The aim of the present study was to evaluate the effects of a lifestyle intervention on blood pressure (BP) homeostasis in patients with type 2 diabetes and hypertension.

RESEARCH DESIGN AND METHODS- A parallel randomized clinical trial was conducted in 40 patients with type 2 diabetes with uncontrolled BP at office (≥140/90 mm Hg) and at ambulatory blood pressure monitoring (ABPM) (daytime ≥135/85 mm Hg). Patients were randomly assigned to follow a DASH diet associated with walking (*Intervention Group*) or a usual diet (*Control Group*) during four weeks.

RESULTS- There was no difference in office BP between groups. Daytime ABPM (mm Hg) was reduced only in *Intervention Group* (systolic BP: 151.3±10.7 to 134.0±9.40; diastolic BP: 86.0±8.3 to 77.7±7.1) as compared to *Control Group* (systolic BP: 148.3±14.3 to 146.1±15.6; diastolic BP: 84.1±9.6 to 81.7±6.3) ($P<0.01$ for all). The reduction of 24-h systolic and diastolic and night-time systolic ABPM was greater in *Intervention Group* than *Control Group* ($P <0.05$ for all). Night-time diastolic ABPM did not differ between groups. Compliance was confirmed by a significant increase in the number of steps, decrease in 24-h urinary sodium, and increase in 24-h urinary aldosterone and potassium at the end-of-study only in the *Intervention Group* ($P <0.05$ for all).

CONCLUSION- A DASH diet associated with walking significantly reduced BP as evaluated by ABPM in hypertensive patients with type 2 diabetes.

Hypertension is a major risk factor for the development and progression of microvascular and macrovascular chronic diabetic complications [1]. Clinical trials have also demonstrated that lowering blood pressure (BP) reduced ischemic cardiac events, stroke, and nephropathy in patients with diabetes [2] and maintenance of good BP control must be continued [3]. Generally, hypertensive diabetic patients are in use of three or more antihypertensive drugs and most of them are out of BP guidelines goals [4]. In these sense, lifestyle modifications such as diet and physical activity are key factors in the management of BP in patients with diabetes [5,6]. Dietary recommendation for patients with hypertension includes reduction of sodium intake, moderation of alcohol consumption, and the adoption of the Dietary Approaches to Stop Hypertension (DASH) diet eating plan[5, 7-10]. Most of these dietary guidelines are based in studies conducted in non diabetic subjects [7,11,12]. In patients with type 2 diabetes, the beneficial association of the components of DASH diet with BP was previously demonstrated by us in a cross sectional study [13]. The reduction of BP due to the adoption of DASH diet was confirmed in a clinical trial in patients with type 2 diabetes [14].

The American Diabetes Association (ADA) recommends 150 min/week of moderate-intensity aerobic physical activity (50–70% of maximum heart rate). Resistance training should also be encouraged at least twice a week[5]. In fact, all types of structured exercise improve glucose control in patients with type 2 diabetes, as demonstrated by us, in a recent meta-analysis which included 8538 patients from 47 RCTs [6]. Another meta-analysis demonstrated, besides the beneficial effect on HbA1c values, that aerobic exercise, alone or combined, reduced systolic (-6.08 mm Hg) and diastolic BP (-3.59 mm Hg) in patients with type 2 diabetes[15]. The beneficial health effects of walking, a simple, non-expensive, and easily accessible means of increase physical activity, has been demonstrated in patients with type 2 diabetes[15]. In these sense, the

ADA and American College of Sports Medicine Exercise Joint Position Statement recommends over 10,000 steps/day for patients with type 2 diabetes[16]. Actually, the number of steps walked per day has been associated with improvement on cardiovascular risk profile in women [17] and older adults [18]. This walking activity can be accurately quantified by the use of pedometers (step counters) [19].

Therefore, the aim of the present study was to evaluate the effect a DASH diet associated with walking on BP homeostasis in patients with type 2 diabetes and hypertension.

RESEARCH DESIGN AND METHODS

Patients

We recruited outpatients with type 2 diabetes who attended the Endocrine Division of the Hospital de Clínicas and the Diabetes Research Center (CPD), Porto Alegre, Brazil. Subjects were also recruited through newspaper and radio announcements. Patients who were regularly using anti-hypertensive medications were selected based on the following criteria: uncontrolled BP at office BP ≥140/90 mm Hg and at ambulatory BP monitoring (ABPM) (daytime ≥135/85 mm Hg); BMI ≤40 kg/m^2; and serum creatinine <176 mmol/L. Exclusion criteria were night shift work or physical disability associated with abnormal gait.

Type 2 diabetes was defined based on diabetes onset at age >30 years, the absence of previous episodes of ketoacidosis or documented ketonuria, and the start of treatment with insulin not before 5 years following diagnosis. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki[20] and was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre, Rio Grande do Sul, Brazil. Written informed consent was obtained from all patients. The present trial was registered at clinicaltrials.gov as NCT 01461330.

Study protocol

At least two weeks before the randomization, potentially eligible patients undertook a 24-h ambulatory blood pressure monitoring (ABPM) examination and their usual walking habits was assessed by using a pedometer during one week. A total of 373 patients were initially screened but the adopted criteria for BP were not confirmed in 188 patients by ABPM criteria and in 138 patients by office BP measurements. From the 47 eligible patients, two patients refused to participate and five patients were not included due to difficulties to follow the initial protocol. Thus, 40 patients were included in the study.

Patients were randomly assigned to the *Intervention Group* or the *Control Group*. Randomization was performed according to computer generated allocation using the site http://www.randomization.com.

Clinical, nutritional, physical activity and laboratory evaluations were performed at baseline and at end-of-study. Both *Intervention* and *Control Group*s were advised to do not change any usual medication during the study. The duration of the follow-up was four weeks.

Intervention Group

This group was assigned to follow a DASH diet and to use a pedometer everyday during the study. The DASH diet eating plan [11,12] was adapted to local dietary habits and was individually prescribed: total energy 25-30 kcal body weight, 55% of energy as carbohydrates, 18% as proteins, and 27% as total fat. The consumption of fruits, vegetables, low-fat dairy foods, whole grains, lean meat, nuts, seeds, and beans were stimulated. The intake of salt, fats, and sweets were discouraged. Whole bread and soya oil were provided biweekly to the *Intervention Group* during the study. An example of a dietary prescription is described in Online-only Supplementary Data. A pedometer (Digiwalker CW200, Yamax, Japan) was also provided to

the patients to be used every day during the four-week intervention period. Patients were asked to walk at least 15-20 min per day (each 10 min period of walking corresponds to approximately 1,000 steps) five days per week, in addition their baseline physical activity. During the study, twice a week, the same research (T.P.P) sent SMS or made phone calls in order to stimulate the compliance with the general protocol recommendations.

Control Group

Patients received dietary recommendations according to ADA dietary guidelines[5,10] and were asked to follow their usual physical activity during the study. Their diet was individually prescribed: total energy, 25-30 kcal body weight; 50 to 60% of energy as carbohydrates; 10% to 20% as proteins; and 25% to 30% as total fat. During the study the *Control Group* biweekly received soya oil and was advised to maintain their usual physical activity during the study. A prescription of an individual patient diet is described in Online-only Supplementary Data. A pedometer (Digiwalker CW200, Yamax, Japan) was also provided to the patients to be used every day in the first and last week of the study. No increase in physical activity was encouraged.

Clinical evaluation

BP was measured twice, after a 10 min rest, by a standard digital sphygmomanometer (Omron HEM-705CP; Omron Healthcare, Inc.) using an appropriated cuff size, in a sitting position, after a 5-min rest. The mean of two measurements taken 1 min apart was used for the analysis. Office hypertension was defined as BP ≥140/90 mm Hg, measured in two occasions, or the use of antihypertensive drugs[7]. The ABPM was evaluated by oscillometry with a device (Spacelabs 90207) with a validated certificate[21], with 15-minutes intervals during the day and 20 min intervals overnight, on an ordinary working day, starting around 8:00 h. Patients were advised to maintain their usual daily activities during the ABPM. Sleep time was recorded as the

period between the time when the patient went to bed and the time when the patient woke up in the next morning. The mean BP values of 24-h, daytime and night-time systolic and diastolic during the ABPM were recorded.

Positive alcohol intake was considered in patients who mentioned the current intake of any amount of alcoholic beverage. Patients were classified as current smokers or not and were self-identified as white or non-white. 24-h urinary albumin excretion (UAE) were classified as normal UAE (<30 mg) or with increased UAE (>30 mg)[5,22] . Increased UAE was always confirmed. Glomerular filtration rate (GFR) was estimated by GFR EPIC[23].

Physical activity

Physical activity was assessed by a standardized questionnaire[24] adapted to local habits. This questionnaire graded physical activity in four levels, according to activities during a typical day, from a sedentary lifestyle to high physical activity. Physical activity was also assessed by a pedometer (Digiwalker CW200, Yamax, Japan). During the use of pedometer patients registered their daily physical activity and the pedometer readings (number of steps/day) in a folder.

Nutritional assessment

The body weight and height of patients (light clothes without shoes or coats) were obtained using an anthropometric scale, with measurements recorded to the nearest 100 g for weight and to the nearest 0.1cm for height. BMI was calculated [weight (kg)/height^2 (m)]. Waist circumference was measured midway between the lowest rib margin and the iliac crest, near the umbilicus. Flexible, non-stretch fiberglass tape was used for measurements. The percentage of fat was calculated using data from a bioimpedance (InBody 230, DSM-BIA).

The diet was assessed by 24-h dietary records. Nutrients were analyzed using Nutribase 2007 Clinical Nutritional Manager software version 9 (Cybersoft, Phoenix, AZ, USA).

Laboratory measurements

Blood samples were obtained after a 12-h fast. Plasma glucose was determined by a glucose oxidase method, creatinine values by Jaffe's reaction, HbA1c by an ion-exchange HPLC procedure (reference range 4.8-6.0 %), total cholesterol and triglycerides by an enzymatic colorimetric methods, and HDL by an homogeneous direct method. LDL-cholesterol was calculated using Friedewald's formula[25] in patients with serum triglycerides <400 mg/dl. UAE was measured by immunoturbidimetry (Ames-Bayer, Tarrytown, NY, USA). Serum BNP was measured in batches using a quimioluminiscency technique (Centaur XP). PRA was determined by radiomunoassay (Genesys LTi 1001).

Urinary aldosterone was determined by radiomunoassay (Genesys LTi 1001). Potassium was measured by colorimetric method, calcium by o-cresoftalein colorimetric technique, and sodium by selective ion eletrod (ISE – Advia 1800).

Statistics and data analyses

Sample size was calculated based on a reduction of 22 mm Hg on systolic BP [26]. It was estimated that 20 participants would be required in each group to achieve a power of 80% and an alpha of 0.05.

Student's *t* test, Mann-Whitney *U* test, and Pearson Chi Square were used as appropriate. Changes in variables during the study were analyzed by the general linear model (GLM) for repeated measures. Measurements at different times were considered the within-subject factor and the variable that classifies patients as belonging to intervention or *Control Group* was specified as the between-subjects factor. Results were expressed as mean (± SD), median (P^{25}-P^{75}), or number of patients with the characteristic (%). In the GLM models, variables with non-Gaussian distribution were log transformed before analyses (BNP, renin, total cholesterol, HDL-

cholesterol, aldosterone). P values <0·05 were considered as statistically significant. SPSS 18.0 statistical software (SPSS, Chicago, IL, USA) was used for analyses.

RESULTS

Baseline patients characteristics

The baseline features of patients with type 2 diabetes are shown in **Table 1**. There were no differences between the *Intervention* and *Control Groups* regarding demographic features, anthropometric indices, lifestyle characteristics, BP values, proportion of patients with diabetic retinopathy and increased UAE. Also, estimated GFR was not different. Regarding anti-hypertensive medications, the proportion of patients using angiotensin receptor blockers was higher in the *Control Group* than in the *Intervention Group*.

There was no spontaneous complains during the study regarding any aspect of the protocol both in patients from the *Intervention* and *Control Groups*.

Blood pressure measurements during the study

Table 2 describes the changes on BP during the study in *Intervention* and *Control Groups*. Changes in office BP did not differ between the two groups both for systolic (P = 0.053) and diastolic BP (P = 0.060). The reduction in 24-h systolic ABPM occurred only in the *Intervention Group* as compared with the *Control Group* (P = 0.000). The 24-h diastolic ABPM reduction was greater in the *Intervention Group* than in *Control Group* (P = 0.018). Daytime systolic and diastolic ABPM was reduced at the end of study only in patients from the *Intervention Group* (P = 0.000). Night-time systolic ABPM decrease was greater in the *Intervention Group* than in *Control Group* (P = 0.047) and the reduction of night-time diastolic ABPM did not differ between groups (P = 0.129).

The greatest magnitude of reduction was observed in daytime BP values: systolic ABPM was -15 mm Hg (-24.5 to -9.8) in the *Intervention Group* as compared to -3.0 mm Hg (-10.8 to 4.5) in the *Control Group* (P = 0.000) and diastolic changes were -9.0 mm Hg (-11.8 to -4.3) in the *Intervention Group* and -1.5 mm Hg (-5.5 to 1.5) in the *Control Group* (P = 0.003). Values for changes of all evaluated BP parameters (office BP and ABPM) are detailed in **Table 1** in the Online-only Supplementary Data.

At the end-of-study, 55% of patients in the *Intervention Group* reached daytime BP values <135/85 mm Hg as compared to 15% in the *Control Group* (P = 0.008).

Other measurements at baseline and at the end-of-study

Nutritional, 24-h urinary, blood, and pedometer measurements in patients with type 2 diabetes during the study are described in the **Table 3**; values of all indices evaluated at baseline did not differ between the *Intervention* and *Control Group*s (P>0.05; data not shown).

Nutritional indices

During the study there was no difference between *Intervention* and *Control Groups* regarding total energy and the intake of protein, carbohydrates, and lipids (P >0.05 for all). Total fiber intake was reduced in the *Control Group* and, as expected, increased in the *Intervention Group* (P = 0.000). Soluble fiber consumption was reduced only in the *Intervention Group*, and this was different from the *Control Group.* (P = 0.005). Insoluble fiber intake changes did not differ between groups (P = 0.121). Waist circumference, BMI, and fat mass did not differ between patients from *Intervention* and *Control Groups* (P >0.05 for all).

24-h urinary measurements

Urinary potassium excretion was increased at the end-of-study in the *Intervention Group* as compared with the *Control Group* (P = 0.012). Urinary sodium decreased only in patients

from the *Intervention Group* (P = 0.017) and urinary calcium did not change during the study (P = 0.707). There was no difference in 24-h urinary UAE between the groups during the study (P = 0.602). Urinary aldosterone increased only in patients from the *Intervention Group* as compared to the *Control Group* (P = 0.024).

Blood measurements

Serum BNP was reduced during the study only in the *Intervention Group* as compared with the *Control Group* (P = 0.001). No significant changes in PRA occurred (P = 0.517). Regarding glycemic control during the study, HbA1c was reduced in both groups but without difference between them (P = 0.944). Also, fasting plasma glucose changes did not differ between *Intervention* and *Control Groups* (P = 0.238). None of the changes in the indices of serum lipid profile differed between the groups during the study (P >0.05 for all).

Pedometer measurements

The number of steps per day raised in the *Intervention Group* from baseline to the end-of-study as compared to the *Control Group* (P = 0.000).

CONCLUSIONS

The present study demonstrated that patients with type 2 diabetes with uncontrolled hypertension who followed a DASH diet associated with daily walking, during a four-week period, the BP was importantly reduced as demonstrated by ABPM. The greatest BP reduction due to this lifestyle intervention was observed at the daytime: 17 mm Hg in systolic BP and 9 mm Hg in diastolic BP.

The relevance of our data is reinforced by have using ABPM. This method fully evaluated BP homeostasis, has been considered the reference standard for the diagnosis of hypertension, and is a better predictor of future cardiovascular events as compared with

conventional office-based BP measurements[27]. Furthermore, in the present study, reduction on systolic BP, our most important result, occurred both during the day and during the night. Indeed, we have previously demonstrated in a cross-section study that systolic BP was the most important ABPM parameter associated with echocardiographic left ventricle structural abnormalities and UAE in patients with type 2 diabetes[28].

The magnitude of BP reduction due to DASH diet and walking in our study seems to be clinically relevant. It was already demonstrated in patients with type 2 diabetes using antihypertensive medications that each 10 mm Hg reduction in systolic BP was associated with 13% decrease in microvascular chronic diabetic complications and 12% in fatal and non fatal myocardial infarction[1]. We should also take into account that in our trial the adopted lifestyle intervention was not associated with common side effects of antihypertensive drugs[7] and more than a half of the patients in the *Intervention Group* reached the current recommended goals for daytime BP [5, 29,30].

The BP effect induced by our intervention in hypertensive patients with type 2 diabetes is greater than the reduction observed in hypertensive subjects without diabetes who were included in the original DASH diet trial (-11.0 mm Hg on systolic BP and -5.5 mmHg on diastolic BP)[11]. Possible the association of physical activity in our *Intervention Group* could explain the superior BP response. Alternatively, patients with diabetes can be more sensitive to DASH diet interventions. As far as we know this is the first trial that evaluates the combination of the DASH diet with walking in patients with type 2 diabetes. The number of daily steps during the study increased 33% in the *Intervention Group.* Although at the end-of-study the daily number of steps was lower (8,389 steps) than the current recommended 10.000 steps day[16], we believe that the number of reached steps was effective and can be applicable in clinical practice. In fact, it

was already demonstrated that patients with diabetes usually walks about 6,000 steps/day[31] and that an increment of 1,562 steps/day results in a reduction in office BP of about 6 mm Hg in patients with type 2 diabetes [32].

A scarce number of studies were conducted in patients with type 2 diabetes to assess the effect of diet[14] or physical activity[33] on BP. An 8-week cross-over clinical trial including 31 Iranian patients with type 2 diabetes [14] evaluating the effect of DASH diet demonstrated a reduction on office BP. Comparison with our data is not allowed since there was no reference to medications in use or the presence of hypertension. Furthermore, the calorie density of foods in the DASH diet was lower than in the control diet and body weight was reduced only in DASH diet group. So, it was not possible to separate the BP effect of weigh reduction from the dietary intervention. Regarding the effects of physical activity on BP of patients with type 2 diabetes, a cross-sectional study demonstrated an inverse association of walking with office BP only in females; for each 1,000 steps/day increment there was a decrease of 2.6 mm Hg in systolic and 1.4 mm Hg in diastolic BP[33].

In our study the compliance with the intervention was confirmed by an increase intake of fiber at the end-of study, as demonstrate by dietary records, and by an increase in the number of steps during the study, from 6,294 to 8,389 steps. Moreover, dietary biomarkers confirmed the adherence of patients to the DASH diet: an increase in urinary potassium and a decrease in urinary sodium associated with an increase of urinary aldosterone. The increase in urinary potassium occurred due to an increase in the intake of fruits and vegetables that are foods which have high potassium content[34,35]. This increase in the potassium consumption possible contributed to observed BP reduction associated with DASH diet. Evidences from recent meta-analysis of RCT and cohort studies showed that increase potassium intake reduces BP in people

with hypertension. In our study the reduction of daily urinary sodium excretion at the end-of-study confirmed that patients reduced their salt intake, from approximately 10.0 g to 7.0 g as well as by the BNP reduction only in the *Intervention Group* [36,37]. In fact, decreased urinary sodium, an indicator of dietary sodium intake, has been connected with BP reduction in free living population[38] . A recent systematic review with meta-analysis[39] on the health effect of lower salt intake (<5.0 g/day vs. ≥5.0 g/day) demonstrated a reduction of 3.39 mm Hg in systolic BP and of 1.54 mm Hg in diastolic BP in subjects with and without hypertension. Furthermore, low sodium intake was also associated with a reduced risk of stroke and fatal coronary heart disease [40]. As a final point, the simultaneous decrease in sodium excretion and increase in urinary aldosterone in our patients reinforces the compliance with the salt restriction during the intervention. This result is expected since physiological increase in aldosterone concentrations is associated with dietary salt reduction[40,41].

On possible limitation of the current study would be related to its short-term follow-up. This relatively short period could have explained why we could not identify some expected changes in evaluated biomarkers. Even so, no other trial evaluated the association of DASH diet with physical activity and we think that this study is a proof of the efficacy of this lifestyle intervention. There is no reason to suppose that the observed beneficial effect on BP would be transient; however the long-term compliance with this intervention should be tested. Another possible limitation would be related to an intrinsic characteristic of the device used to evaluate usual physical activity. Pedometers capture only movements of the lower body in the vertical plane, and are unable to count other activities such as exercise involving upper limbs. Nonetheless this aspect probably did not influence our results since the majority of our patients in both *Intervention* and *Control* groups were sedentary.

In conclusion, the results of the present study demonstrated that a DASH diet combined with walking expressively reduced BP in patients with type 2 diabetes and hypertension. The beneficial BP effects of this lifestyle intervention should also be assessed in long-term clinical trials in order to evaluate its influence on hard cardiovascular outcomes.

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Table 1. Baseline characteristics of patients with type 2 diabetes

	Control Group	***Intervention Group***	P
Sex (M,F)	6M, 14F	12M, 8F	0.057 †
Age (years)	62.5 ± 8.8	61.8 ± 8.1	0.810 *
White ethnicity	18.0 (90.0%)	16.0 (80.0%)	0.376 †
Diabetes duration (years)	16.1 ± 7.4	16.9 ± 7.9	0.778 *
Duration of hypertension	18.4 ± 7.8	16.7 ± 9.5	0.531 *
BMI (kg/m²)	31 ± 4.9	28.6 ± 2.6	0.060 *
Waist circumference (cm)	103.7 ± 11.6	99.4 ± 9.6	0.215 *
Fat mass (%)	34.9 ± 7.8	31.5 ± 5.9	0.120 †
Sedentary	15 (75%)	15 (75%)	1.000 †
Current alcohol intake	9 (45%)	9 (45%)	1.000 †
Current smoking	1 (5%)	3 (15%)	0.292 †
Office systolic BP (mm Hg)	158.0 ± 21.5	162.7 ± 10.8	0.260 *
Office diastolic BP (mm Hg)	79.4 ± 8.1	82.8 ± 11.5	0.206 *
24-h systolic BP (mm Hg)	147.4 ± 15.4	148.1 ± 12.2	0.887 *
24-h diastolic (mm Hg)	82.2 ± 8.9	82.4 ± 8.4	0.948 *
Use of ACE inhibitors	11 (55%)	16 (80%)	0.091 †
Use of diuretics	15 (75%)	15 (75%)	1.000 †
Use of ARB	9 (45%)	2 (10%)	0.013 †
Use of beta blockers	8 (40%)	10 (50%)	0.525 †
Use of aspirin	11 (55%)	11 (55%)	1.000 †
Proliferative diabetic retinopathy	2 (10%)	1 (5%)	0.480 †
UAE > 30 mg/24-h	12 (60%)	12 (60%)	1.000 †
Estimated GFR (ml/min/1.73m^2)	80.3 ± 14.4	84.6 ± 19.3	0.425 *

ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; UAE = urinary albumin excretion

* = *T* test. †= Pearson's x^2

Data are mean (SD), median (P^{25}- P^{75}), number of patients with the characteristic (%)

Table 2. Blood pressure measurements in patients with type 2 diabetes during the study.

	Control Group			*Intervention Group*			
	Baseline	**End-of-study**	P*	**Baseline**	**End-of-study**	P*	P†
Office systolic BP (mm Hg)	158.0 ± 21.5	151.7 ± 16.6	0.246	162.7 ± 10.8	145.1 ± 13.0	0.000	0.053
Office diastolic BP (mm Hg)	79.4 ± 8.1	80.7 ± 8.5	0.589	82.8 ± 11.5	76.8 ± 10.3	0.045	0.060
24-h systolic ABPM (mm Hg)	146.9 ± 14.9	144.3 ± 14.6	0.215	147.9 ± 11.8	132.6 ± 11.3	0.000	0.000
24-h diastolic ABPM (mm Hg)	81.5 ± 8.9	78.3 ± 6.8	0.013	82.7 ± 8.0	75.2 ± 7.0	0.000	0.018
Daytime systolic ABPM (mm Hg)	148.3 ± 14.3	146.1 ± 15.6	0.343	151.3 ± 10.7	134.0 ± 9.4	0.000	0.000
Daytime diastolic ABPM (mm Hg)	84.1 ± 9.6	81.7 ± 6.3	0.055	86.0 ± 8.3	77.7 ± 7.1	0.000	0.001
Night-time systolic ABPM (mm Hg)	142.6 ± 17.7	135.9 ± 16.0	0.004	141.7 ± 16.0	128.5 ± 14.3	0.000	0.047
Night-time diastolic ABPM (mm Hg)	75.2 ± 8.6	72.6 ± 9.3	0.048	76.3 ± 7.8	70.8 ± 7.8	0.000	0.129

BP = blood pressure; ABPM = ambulatory blood pressure measurement

GLM for repeated measurements: P* = difference within group; P†= difference between groups

Data are expressed as mean (SD)

Table 3. Nutritional, urinary, blood, and pedometer measurements in patients with type 2 diabetes during the study.

	Control Group			*Intervention Group*			
	Baseline	**End-of-study**	P*	**Baseline**	**End-of-study**	**P***	P†
Nutritional indices							
Total energy (kcal/day)	1742.3 ± 550.9	1752.2 ± 298.5	0.045	1811.2 ± 515.2	1585.3 ± 320.8	0.009	0.634
Carbohydrate (% total en)	41.3 ± 11.1	39.3 ± 9.9	0.096	47.6 ± 7.1	47.1 ± 7.3	0.642	0.387
Protein (% total en)	20.1 ± 5.0	23.0 ± 3.8	0.014	21.4 ± 5.6	23.5 ± 6.7	0.068	0.626
Lipids* (% total en)	38.2 ± 9.0	36.8 ± 8.0	0.449	33.3 ± 9.3	29.4 ± 5.8	0.031	0.304
Total Fiber (g/day)	16.5 ± 6.1	14.1 ± 4.8	0.007	14.8 ± 4.1	20.1 ± 4.3	0.000	0.000
Soluble fiber (g/day)	5.2 ± 2.3	4.7 ± 2.1	0.291	4.8 ± 1.8	6.1 ± 2.1	0.003	0.005
Insoluble fiber (g/day)	10.4 ± 5.1	11.0 ± 5.3	0.542	9.8 ± 3.6	12.9 ± 2.9	0.007	0.121
BMI (kg/m²)	31.1 ± 4.9	30.9 ± 5.0	0.148	28.6 ± 29.6	28.2 ± 2.7	0.001	0.162
Waist circumference (cm)	103.7 ± 11.6	102.8 ± 10.9	0.033	99.4 ± 9.6	97.6 ± 8.8	0.003	0.152
Fat mass (%)	34.9 ± 7.8	31.5 ± 5.8	0.821	34.9 ± 8.6	31.3 ± 5.8	0.690	0.902
24-h urinary measurements							
Sodium (mEq)	186.6 ± 77.6	174.4 ± 71.1	0.400	190.2 ± 96.8	127.2 ± 75.3	0.000	0.017
Potassium (mEq)	56.6 ± 22.5	58.4 ± 17.6	0.619	55.5 ± 14.2	70.4 ± 20.6	0.000	0.012
Calcium (mEq)	92.0(49.5-177.0)	94.0(58.3-186.5)	0.840	73.0(48.0-97.5)	63.0(46.5-119.8)	0.464	0.707
UAE (mg)	43.5(18.5-194.4)	33.4(11.2-119.6)	0.079	41.6(22.1-185.8)	31.8(10.2-132.7)	0.015	0.602
Aldosterone unidade	7.0(3.2-9.7)	5.9(2.8-8.9)	0.535	5.7(4.0-7.5)	9.4(6.8-14.7)	0.010	0.024
Blood measurements							
BNP (pg/ml)	15.8(12.6-18.5)	19.6(13.9-35.5)	0.100	24.2(14.2-58.2)	12.6(7.6-21.3)	0.001	0.001
PRA (ng/ml/h) median^{25}-^{75}	3.8(0.6-6.0)	3.3(0.3-5.4)	0.737	4.9(2.7-8.2)	4.4(0.9-10.0)	0.214	0.517

HbA1c (%)	8.6 ± 1.6	8.1 ± 1.2	0.002	8.8 ± 1.9	8.2 ± 1.8	0.001	0.944
Fasting plasma glucose (mmol/L)	170.2 ± 62.3	153.2 ± 56.2	0.840	171.7 ± 87.3	124 ± 42,9	0.012	0.238
Total cholesterol (mmol/L)	4.5 ± 0.8	4.3 ± 0.9	0.410	4.8 ± 1.0	4.5 ± 0.9	0.107	0.566
HDL-cholesterol (mmol/L)	1.1 ± 0.4	1.1 ± 0.4	0.739	1.2 ± 0.4	1.2 ± 0.4	0.831	0.910
Triglycerides (mmol/L)	1.8 (1.2-2.5)	1.7(1.2-2.4)	0.950	1.8 (1.1-2.6)	1.5(1.0-1.9)	0.013	0.081
LDL-cholesterol (mmol/L)	94.3 ± 24.8	92.6 ± 22.3	0.746	112.0 ± 34.9	99.6 ± 31.3	0.020	0.144
Pedometer measurements							
Number of daily steps (3 days)	5848 ± 2827	5708 ± 2277	0.552	6294 ± 2544	8389 ± 2680	0.000	0.000

GLM for repeated measurements: P* = difference within group; P†= difference between groups

PRA = plasma renin activity; BNP= brain natriuretic peptide

Urinary calcium, aldosterone and UAE, PRA, BNP, and triglycerides were log transformed before analyses

Data are expressed as mean (SD) and median (P^{25}- P^{75})

Supplementary Data

Figure 1: Daily foods consumed according to each diet*.

Intervention Group		***Control Group***	
Breakfast			
Whole bread	(2 slices - 50 g)	Bread	(2 slices - 50 g)
Mozzarella cheese	(1 slice - 15 g)	Mozzarella cheese	(1 slice - 15 g)
Lean ham	(1 slice - 15 g)	Lean ham	(1 slice - 15 g)
Skimmed milk	(1 cup - 150 ml)	Semi- skimmed milk	(1 cup - 150 ml)
Coffee	(50 ml)	Coffee	(50 ml)
Snack 1			
1 fruit (100g)		1 fruit (100g)	
Lunch			
Vegetable A	freely	Vegetable A	freely
Vegetable B	(3 tablespoons - 60 g)	Vegetable B	(3 tablespoons - 60 g)
Rice or other	(3tablespoons - 60 g)	Rice or other	(3tablespoons - 60 g)
Beans	(1 small scoop - 60 g)	Beans	(1 small scoop - 60 g)
Lean meat	(1 medium piece - 90 g)	Meat	(1 medium piece - 90 g)
Fruit	(1 porcion - 100 g)	Fruit	(1 porcion - 100 g)
Snack 2			
Skimmed Yogurt	(1 cup - 120 g)	Bread	(2 slices - 50 g)
Fruit	(1 porcion - 100 g)	Margarine	(2 g)
		Semi- skimmed milk	(1 cup - 150 ml)
		Coffee	(50 ml)
Dinner			
Vegetable A	freely	Vegetable A	freely
Vegetable B	(3 tablespoons - 60 g)	Vegetable B	(3 tablespoons - 60 g)
Rice or other	(3tablespoons - 60 g)	Rice or other	(3tablespoons - 60 g)
Lean meat	(1 medium piece - 90 g)	Beans	(1 small scoop - 60 g)
Fruit	(1 porcion - 100 g)	Meat	(1 medium piece - 90 g)
		Fruit	(1 porcion - 100 g)

* Diets prescriptions for a patient with body weight equal to 70kg.

Intervention Group = DASH DIET; Control Group = ADA recommmendations

Supplementary data

Table 1: ABPM changes during the study in patients with type 2 diabetes

	Control	Intervention	P*
24-h systolic ABPM (mm Hg)	-1.5 (-5.8 ; 2.5)	-12.5 (-18.0 ;-10.3)	0.000
24-h diastolic ABPM (mm Hg)	-2.0 (-8.0 ; 0.0)	-7.0 (-11.5 ; -3.5)	0.013
Daytime systolic ABPM (mm Hg)	-3.0 (-10.8 ; 4.5)	-15.0 (-24,5 ; -9,8)	0.000
Daytime diastolic ABPM (mm Hg)	-1.5 (-5.5 ; 1.5)	-9.0 (-11,8 ; -4,3)	0.003
Night-time systolic ABPM (mm Hg)	-3.5 (-10.5 ; -2.0)	-10.5 (-18.0 , -4.0)	0.041
Night-time diastolic ABPM (mm Hg)	-3.0 (-5.8 ; 0.8)	-6.0 (-9.8 ; 0.0)	0.207

BP = blood pressure; ABPM = ambulatory blood pressure measurement

* =Mann Whitney Test.

Data are expressed as median (^{25} - ^{75})

CONSIDERAÇÕES FINAIS

Através de revisão sistemática com meta-análise de ensaios clínicos randomizados com pelo menos duas semanas de duração, comprovamos que a restrição de sódio e a suplementação de vitamina D em pacientes com insuficiência desta vitamina, tem efeitos benéficos na pressão arterial de pacientes com diabetes melito tipo 2. Estas intervenções não farmacológicas são capazes de reduzir a pressão arterial sistólica em cerca de 6 mm Hg (restrição de 3,0 g de sal na dieta usual) a 7 mm Hg (suplementação de vitamina D ; ~800 a 1700 UI/dia)

Ainda, demonstramos, através de um ensaio clínico randomizado, que em pacientes com diabetes melito tipo 2 e hipertensão não controlada em uso de múltiplos medicamentos anti-hipertensivos, a adoção de uma dieta tipo DASH associada a caminhadas reduziu a pressão arterial. Este efeito benéfico foi demonstrado utilizando a monitorização de pressão arterial em 24 horas sendo a magnitude desta intervenção relevante do ponto de vista clínico. Como exemplo, citamos a redução durante o dia de 17 mm Hg na pressão arterial sistólica e de 9 mm Hg na diastólica.

Os dados originais apresentados na presente Tese de Doutorado confirmam a importância da orientação nutricional de pacientes com diabetes tipo 2. Na presença de hipertensão deve ser priorizado o consumo de alimentos preconizados pela dieta DASH, com recomendação de um aumento no consumo de frutas, vegetais, alimentos integrais e laticínios com baixo teor de gordura e redução do consumo de sódio. Ainda, pacientes com diabetes melito tipo 2 e hipertensão arterial deveriam sempre realizar uma medida plasmática da vitamina D (25OH) independente de terem fatores de risco associados à deficiência desta vitamina. Além do benefício conhecido desta vitamina sobre o metabolismo do cálcio, as evidências apresentadas

sugerem que a administração de vitamina D reduza os valores de pressão arterial. Associado a estas intervenções, uma orientação simples e factível, o estímulo a caminhadas diárias. Recomenda-se que o paciente acrescente às suas atividades usuais, 15 a 20 minutos de caminhadas, com o objetivo de, junto com a dieta DASH, reduzir os valores de pressão arterial.

Os dados desta Tese de Doutorado demonstram claramente a importância de modificação de estilo de vida como parte do arsenal terapêutico do tratamento da hipertensão arterial nos pacientes com diabetes. Intervenções simples, sem custo excessivo, e sem efeitos colaterais devem ser prioritariamente adotadas, associadas ao acompanhamento médico regular do paciente. Como perspectiva futura, novos ensaios clínicos devem comprovar a adesão em longo prazo a estas medidas de intervenção de estilo de vida e avaliar desfechos duros, como por exemplo, eventos cardiovasculares, mortalidade geral e cardiovascular. Importante é também a comprovação dos efeitos benéficos da vitamina D sobre a pressão arterial em estudos delieados para tal fim. Nestes, a homeostase pressórica deve ser mais bem avaliada por monitorização de pressão em 24 horas e possíveis fatores de confusão como dieta, peso e atividade física, entre outros, devem ser fatores em estudo.