



## Trabajo Original

Epidemiología y dietética

### A randomized control trial for reduction of caloric and non-caloric sweetened beverages in young adults: effects in weight, body composition and blood pressure *Ensayo clínico controlado para evaluar el efecto de la reducción de bebidas dulces calóricas y no calóricas sobre peso, composición corporal y presión arterial*

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#### Abstract

**Introduction:** Recently has been documented that the consumption of sweetened non-caloric beverages has increased as an option to weight control, however randomized control trials have demonstrated a modest weight loss.

**Objective:** To evaluate the effect of reducing consumption of beverage with caloric and non-caloric sweeteners on weight, body composition and blood pressure in young Mexican adults.

**Methods:** In an experimental study 148 nursing students were randomly assigned to one of 3 groups: 1) no sweetened beverages were permitted, only plain water, tea or coffee without sugar; 2) consumption of beverages with non-caloric sweeteners was allowed; and 3) no restriction of sweetened beverages was imposed. All groups were given individualized isocaloric diets monitored by a 24-hour record of consumption and food frequency questionnaire and blood pressure, weight, waist circumference and body composition by tetrapolar bioelectric impedance were taken at the beginning of the study and three and six months later.

**Results:** Differences between groups were found in body mass index at 3 months that decrease in group 1 and 2 and increase in group 3 (-1.75 vs. -0.61 vs. 0.54% of change,  $p < 0.001$ ). At six months there were also statistical differences in waist circumference (-4.07 vs. -1.23 vs. 0.62% of change,  $p < 0.001$ ) and sugar consumption (-62.0 vs. -54.61 vs. 11.08% of change,  $p < 0.001$ ) in groups 1, 2 and 3 respectively.

**Conclusions:** The reduction in consumption of both caloric and non-caloric sweetened beverages contributes to significant body mass index loss and waist circumference.

#### Key words:

Sweetened beverages. Caloric and non-caloric sweeteners. Body weight. Body composition. Blood pressure.

#### Resumen

**Introducción:** recientemente se ha documentado que el consumo de bebidas dulces calóricas y no calóricas ha incrementado como una opción para el control de peso. Sin embargo, algunos ensayos clínicos han demostrado solo pérdidas de peso modestas.

**Objetivo:** evaluar el efecto de la reducción del consumo de bebidas con endulzantes calóricos y no calóricos en el peso, composición corporal y presión arterial en adultos jóvenes mexicanos.

**Métodos:** en un ensayo clínico controlado fueron asignados al azar 148 estudiantes de enfermería a 3 grupos: 1) no se permitió consumo de bebidas endulzadas, solo agua simple, café o infusiones sin azúcar; 2) consumo de bebidas con endulzantes no calóricos; y 3) ninguna restricción en el consumo de bebidas. A todos los grupos se les proporcionó una dieta individualizada isocalórica que fue monitoreada mediante un recordatorio de 24 horas y un cuestionario de frecuencia consumo de alimentos. Al inicio del estudio, tres y seis meses después se tomó la presión arterial, peso, circunferencia de cintura y composición corporal mediante impedancia bioeléctrica tetrapolar.

**Resultados:** se encontraron diferencias estadísticamente significativas en el cambio del índice de masa corporal a los tres meses, el cual disminuyó en los grupos 1 y 2 y aumentó en el grupo 3 (-1,75 vs. -0,61 vs. 0,54% de cambio,  $p < 0,001$ ). A los 6 meses se encontraron diferencias en el cambio de la circunferencia de cintura (-4,07 vs. -1,23 vs. 0,62% de cambio,  $p < 0,001$ ) y en el consumo de azúcar (-62,0 vs. -54,61 vs. 11,08% de cambio,  $p < 0,001$ ) en los grupo 1, 2 y 3 respectivamente.

**Conclusiones:** la reducción del consumo de bebidas endulzadas calóricas y no calóricas contribuye a una reducción significativa del índice de masa corporal y la circunferencia de cintura.

#### Palabras clave:

Bebidas endulzadas. Endulzantes calóricos y no calóricos. Peso. Composición corporal. Presión arterial.

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## INTRODUCTION

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The World Health Organization estimates that there are 1.4 billion overweight and obese people in the world (1); in Mexico alone 70% of the adult population suffers from overweight and obesity, and 34.4% of school age children are above their normal weight (2). At the same time, arterial hypertension affects more than a third of adults throughout the world and contributes to 9.4 million deaths a year from heart disease (3). In Mexico there are 22.4 million adults with hypertension, only 5.7 of whom are controlled (4).

Obesity and hypertension are associated with life style both in the world and in Mexico, where changes in eating habits with increased fat in the diet, overconsumption of soft drinks and refined sugars and decrease in physical activity have led to numerous diseases (4). Mexico is the Latin American country with the highest consumption of carbonated beverages (5), averaging 384 soft drinks/person/year (6). It has been proposed that the shift from the consumption of plain water to sugar-sweetened beverages is associated with the increase of obesity frequency (7). Because the diminished sensation of satiety and the high ingestion of sugar (8) facilitate the formation of triglycerides stored in fatty tissue and overweight and obesity (9,10).

Recent studies have examined the relationship between sweetened beverages and weight, albeit with controversial results. The majority of the them have focused on increased consumption of sweetened drinks and theirs effects on weight over short periods of time and in almost all the population are adults or children (11,12). Meanwhile, consumption of low-calorie beverages has increased, although there is no consensus about the evidence of substituting caloric sweeteners with non-nutritive sweeteners to achieve better weight control (13,14). Inasmuch as obesity is a worldwide health issue, it is important to establish whether beverages, as the principal source of artificial sweeteners (15) can aid in regulating weight or is better to drink minimal amounts of non-sweetened beverages (14). Also, recent studies have demonstrated an association between sugar-sweetened beverages and blood pressure, but the has been observational (16) or have not analyzed all types of sweetened beverages (17,18).

Because of these issues, we aimed to evaluate the effect of reducing consumption of beverage with caloric and non-caloric sweeteners on weight, body composition and blood pressure in young Mexican adults on nursing students. The working hypothesis was that nursing students from the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán who substituted their intake of caloric and non-caloric sweetened drinks with plain water would have greater weight, body fat and blood pressure loss than students who did not diminish their intake of sweetened drinks.

## METHODS

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### DESIGN AND STUDY POPULATION

This was a randomized controlled clinical study with the participation of nursing students with mean age of  $21.99 \pm 0.25$

years, body mass index (BMI)  $26.24 \pm 0.36$  and 81% were female from the María Elena Maza Brito School of Nursing of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City from August of 2012 to May of 2014.

### SELECTION OF PARTICIPANTS

Subjects who fulfilled the following inclusion criteria were selected: nursing students from the School of Nursing of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, male and female gender, more than 18 years of age and consumed at least 12 ounces of sweetened beverages per day (based on values from the Mexican Ministry of Health). Fifty-two subjects were excluded because: special diet, arterial hypertension diagnosed with or without use of antihypertensive medications, hyperthyroidism or hypothyroidism, use of weight loss medications, diabetes mellitus, cardiovascular or psychiatric diseases or hormonal therapy, participated in vigorous physical activity for more than one hour/day. The study was approved by the Ethics an Investigation in Humans Committee of the Instituto Nacional de Ciencias Medicas y Nutrición Salvador Zubirán (Mexico).

### ASSIGNMENT OF PARTICIPANTS TO INTERVENTION STUDY GROUP

The 148 participants were asked to sign an informed consent form and one of the researchers of the present study that did not had contact with participants randomly assigned using a sequential series of numbers computer-generated to one of the 3 treatment groups. *Group 1*: no sweetened beverages were permitted, only plain water, lemon and hibiscus flavored water, coffee and tea without sugar were permitted; *group 2*: only allowing beverages with non-caloric sweeteners, plain water, lemon and hibiscus flavored water, coffee and tea without sugar were permitted; *group 3*: had no modification in consumption of beverages, and only given general recommendations about beverages. All groups were given individualized isocaloric diets monitored by a 24-hour record of consumption and frequency of meals. The participants and the nutritionist, who prescribed the diet, were not masked to group allocation; however, the cardiologist and nutritionist who performed the clinical evaluations were blinded to the selection process.

### MEASUREMENTS

The primary objective was to compare weight, percent fat and blood pressure among the three groups at the beginning and after three and six months of follow-up.

Body composition (*i.e.*, resistance/height and phase angle) was evaluated by bioelectric impedance (BIA) analysis, using tetrapolar and multiple frequency equipment (Bodystat QuadScan

4000, Bodystat Ltd.; Isle of Man, United Kingdom). Weight and height were measured according to the anthropometric standardization reference manual (19); all subjects wore lightweight clothing and were barefoot and were obtained body mass index (BMI). Blood pressure was measured according to the American Heart Association with a pedestal aneroid sphygmomanometer (ADC®, model EM-023) with subjects in a seated position and after resting 5 minutes without prior consumption of coffee or cigarettes. Two readings of systolic and diastolic pressure were taken with an interval of two minutes. When the difference in readings was greater than 5 mmHg a third measurement was taken. Ingestion of food and adherence to the diet was evaluated by a record of consumption over 24 hours using ESHA Food software with an SQL processor (version 7.9, ESHA Research, Salem, OR, 2001). Waist, hip and arm circumferences were also evaluated according to the anthropometric reference manual (SECA201 brand, Germany). Grip strength was measured using a Smedley dynamometer (Stoelting, Wood Dale, United Kingdom). Subjects were instructed to apply as much pressure as possible on the grip with right and left hands. Measurements were taken twice for each side, and the highest value in kilograms was recorded. Members of the three groups were given isocaloric diets based on age, ideal weight and height with a distribution of 50% carbohydrates, 20% protein and 30% lipids. The program continued for 6 months with an evaluation at 3 months.

## ADHERENCE

In order to evaluate adherence to the reduction of sweetened beverages, an internet questionnaire of one 24 hour record once a week for six months was used for all groups. Each student received an e-mail, a reminder about healthy eating (emphasizing drinks) at the beginning and at three and six months of the program. On a visit each month the students were only weighed.

## SAMPLE SIZE

Sample size was calculated using a table value of sample sizes required under a parallel-group design with  $\alpha$  adjustment for multiple comparisons (three arms) significance level of 0.05 and power of 0.80 (20). We used the percentage of change in the BMI between the three studies groups obtained in a pilot test that we performed (group 1: -3.2; group 2: -1.6; group 3: 0.02 with standard deviation of 0.8) (21). A sample size of 31, which was increased by 20 % lost to follow up, gave a total of 37 patients in each group.

## STATISTICAL ANALYSIS

The data were assembled on an Excel 2007 program calculation sheet and analyzed using the statistical program SPSS ver-

sion 17 and STATA VERSION (419.12.0.870). The results were expressed as mean  $\pm$  standard deviation for continuous variables and as frequencies and percentages for categorical variables. In the baseline comparison among study groups a one-way analysis of variance (ANOVA) was used when the variables were continuous and Chi square when the variables were categorical. When the groups were compared after intervention percentages of change were obtained, and a one-way ANOVA was also used. Multiple linear regression analysis was performed to analyze those dietary and anthropometric variables that best explained weight and blood pressure reduction.

## RESULTS

The baseline characteristics of the entire population and differences between the study groups appear in table I. The majority of the population was female ( $n = 120$ , 81.1%) with a mean age of  $21.99 \pm 0.25$  years. On the basis of BMI, 60.1% of the population was overweight or obese with a global mean BMI of  $26.24 \pm 0.36$ . The group 1 had higher BMI and waist circumference compared with the other two groups. In the total population we found that 37% of the total calories came from beverages and the principals were soft drinks, industrialized juice, coffee and tea with sugar. No statistically significant differences at baseline were found among the groups.

In figure 1 is shown the number of participants for each group according to randomization. After the introduction of the isocaloric diet and reduction of sweetened beverages, 98.64% of the participants completed the study, while 1.35% did not return for subsequent measurements.

Table II shows the mean change in anthropometry, blood pressure and body composition in the intermediate 3-month interval and at the end of the study at six months in each group. In comparison with the other groups group 1 had significantly greater reductions in body mass index, waist and hip circumferences, and resistance/height both in the intermediate and final measurements as well as increased phase angle. The group that did not drink sweetened beverages had a statistically significant weight loss, from  $69.1 \pm 14.9$  to  $67.9 \pm$  at 3 months and to 66.8 kg at six months. In the others groups there were not statistical significant changes.

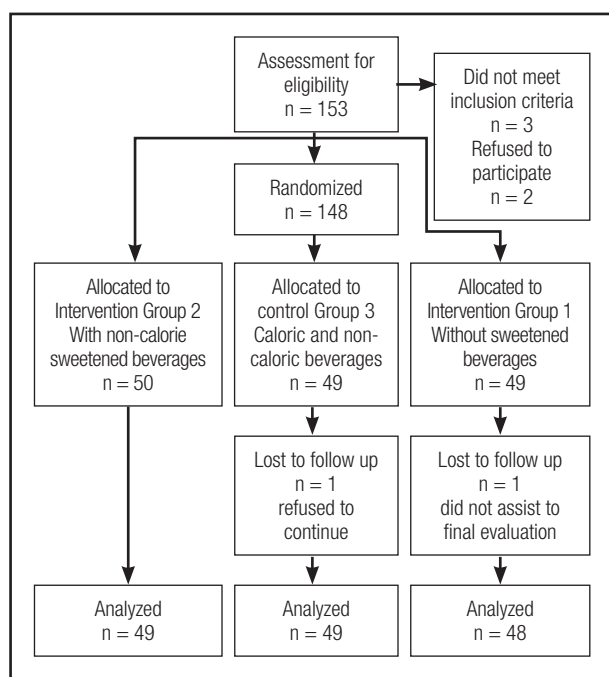
The mean changes in dietary ingestion by study groups appear in table III. Statistically significant changes among the groups were observed; Group 1 had a greater reduction in calories (from  $2021 \pm 671$  to  $1635 \pm 514$  at six months), lipids and carbohydrates, most notably in sucrose and fructose than the other groups. Sodium consumption also decrease in groups 1 and 2. No adverse effects occurred with the nutritional treatment given.

With respect to the change in beverage consumption, in group 1, 80% of the subjects ceased to drink soft drinks and industrialized juice and 90% ceased to drink whole milk; all the subjects increased the consumption of plain water. Similar results were observed in group 2, in addition to an increase in beverages with non-caloric sweeteners.

**Table I.** Baseline characteristics of the total population and study groups

Variables	Group 1 Without sweetened beverages n = 49	Group 2 With non-calorie sweetened beverages n = 50	Group 3 Caloric and non-caloric beverages n = 49	Total	p
Gender M/W n (%)	10 (20.4)/39 (79.6)	10 (20)/40 (80)	8 (16.3)/41 (83.7)	28 (18.9)/120 (81.1)	0.85
Age (years)	22.55 ± 0.51	21.46 ± 0.31	21.98 ± 0.45	21.99 ± 0.25	0.21
Weight (kg)	69.13 ± 2.11	65.48 ± 1.92	65.75 ± 1.67	66.78 ± 1.10	0.33
Height (cm)	159.01 ± 1.25	159.97 ± 1.04	158.90 ± 1.17	159.30 ± 0.66	0.77
Body mass index (kg/cm <sup>2</sup> )	27.27 ± 0.70	25.48 ± 0.62	26.00 ± 0.55	26.24 ± 0.36	0.12
Waist circumference (cm)	87.62 ± 1.69	84.53 ± 1.60	85.12 ± 1.66	85.75 ± 0.95	0.38
Hip circumference (cm)	102.37 ± 1.30	99.31 ± 1.22	99.42 ± 1.26	100.36 ± 0.73	0.15
R/H (ohms/m)	363.77 ± 54.24	367.35 ± 52.75	377 ± 41.02	369.59 ± 49.7	0.36
Phase angle (°)	6.86 ± 0.8	6.57 ± 0.9	6.78 ± 0.8	6.74 ± 0.9	0.25
Overweight n (%)	20 (40.8)	22 (44)	24 (49.0)	66 (44.6)	0.24
Obesity n (%)	13 (26.5)	4 (8.0)	6 (12.2)	23 (15.5)	0.36
SBP (mmHg)	110.53 ± 1.78	107.56 ± 1.59	110.43 ± 1.44	109.49 ± 0.93	0.33
DBP (mmHg)	71.45 ± 1.22	71.30 ± 1.26	73.00 ± 1.20	71.91 ± 0.71	0.56

R/H: resistance/height; SBP: systolic blood pressure; DBP: diastolic blood pressure. Data are represented as mean ± standard deviation (SD) or number of subjects and percentage.



**Figure 1.** Flowchart of subjects included in the study.

## DISCUSSION

In the present study we found that a reduction in the consumption of sweetened beverages (caloric and non-caloric) and

replacing it with plain water, in addition to an isocaloric diet, could contribute to a better body mass index at 6 months, compared with subjects who did not change their consumption of sweetened beverages. This agrees with the conclusion of the systematic review of Muckelbauer et al. where stated that studies of individuals dieting for maintenance or weight loss found a weight-reducing effect of increased water consumption and can be explained because drinking water instead of sugar-containing beverages was shown to reduce the total energy intake with the subsequent meal in adults and short-term effects of water consumption include suppression of hunger and increased satiety (7).

Also, our study demonstrated that subjects who eliminated both caloric and non-calorie beverages (group 1) had greater reduction of weight, hip circumference, resistance/height, and sodium consumption at six months of follow-up. In contrast, group 2, which drank artificially sweetened drinks (non-calorie), lost less body weight and resistance/height compared to group 1. That is, the subjects lost weight in the short term (3 months), but over a longer period of time (6 months), weight no longer reached a statistical difference, and the consumption of carbohydrates tended to increase. So the consumption of low-calorie sweetened beverages can be only a transition option in a weight control plan.

Recent studies have examined the association of sweetened beverages and weight, albeit with contradictory results. The majority has focused on increasing consumption of sweetened beverages and seeing their effect on weight over the short term (22,23). Our experimental design involved an analysis of decreased consumption of soft drinks and its effect on weight and blood pressure over a period of 6 months. The group that did not drink sweetened beverages had a statistically significant weight

**Table II.** Comparison of percent of changes in anthropometric variables and body composition at 3 months and 6 months between study groups

Variables	Group 1 Without sweetened beverages n = 49		Group 2 With non-calorie sweetened beverages n = 49		Group 3 Caloric and non-caloric beverages n = 48		p between groups at 6 months
	3 months	6 months	3 months	6 months	3 months	6 months	
BMI (% of change)	-1.75 ± 0.6	-3.34 ± 0.75	-0.61 ± 0.08	0.11 ± 0.06	0.54 ± 0.06	0.57 ± 0.07	< 0.001 <sup>A&amp;B***</sup>
Waist circumference (% of change)	-2.45 ± 0.44	-4.07 ± 0.54	-1.56 ± 0.46	-1.23 ± 0.58	0.53 ± 0.16	0.62 ± 0.60	< 0.001 <sup>A&amp;B***</sup>
Hip circumference (% of change)	-1.63 ± 0.30	-3.00 ± 0.44	-0.71 ± 0.35	-0.35 ± 0.46	0.33 ± 0.27	0.51 ± 0.31	<0.001 <sup>B***</sup>
SBP (% of change)	-4.35 ± 1.46	-4.83 ± 1.26	-2.29 ± 1.42	-2.21 ± 1.40	-1.64 ± 2.93	-1.38 ± 0.77	0.10
DBP (% of change)	-4.92 ± 1.57	-2.68 ± 1.57	-2.78 ± 1.66	-0.46 ± 1.44	-2.38 ± 1.85	-1.60 ± 1.87	0.53
R/H (% of change)	-1.92 ± 1.61	-2.12 ± 0.95	-0.85 ± 1.49	2.00 ± 1.38	-0.43 ± 1.83	0.34 ± 0.62	0.02 <sup>A*</sup>
Phase angle (% of change)	4.88 ± 0.76	8.40 ± 0.85	5.57 ± 1.20	4.92 ± 1.28	3.02 ± 0.70	3.58 ± 0.96	0.005 <sup>B**</sup>

Data are expressed as % change from the baseline value at intermediate follow-up (3 months) and final (6 months), mean ± standard deviation (SD), BMI: body mass index. R/H: resistance/height; SBP: systolic blood pressure; DBP: diastolic blood pressure. \*Statistically significant difference  $p < 0.05$ . A: difference between group 1 and group 2. \*\*Statistically significant difference  $p < 0.01$ . B: difference between group 1 and group 3. \*\*\*Statistically significant difference  $p < 0.001$ .

**Table III.** Comparison of percent of changes in dietary components at 6 months between study groups

Variables	Group 1 Without sweetened beverages n = 49	Group 2 With non- calorie sweetened beverages n = 49	Group 3 Caloric and non-caloric beverages n = 48	p
Kilocalories (% of change)	-16.88 ± 2068	-6.92 ± 3.46	-4.93 ± 2.85	0.01 <sup>B*</sup>
Protein as % of total kcal (% of change)	29.77 ± 5.89	24.34 ± 6.94	19.79 ± 7.58	0.58
Carbohydrates as % of total kcal (% of change)	-3.21 ± 3.56	-0.02 ± 3.60	1.56 ± 3.41	0.36
Carbohydrates (% of change)	-2.16 ± 25.36	4.16 ± 34.49	2.40 ± 38.15	0.81
Lipids as % of total kcal (% of change)	-10.30 ± 6.35	-17.28 ± 5.52	7.22 ± 4.73	0.007 <sup>B**</sup>
Glucose (% of change)	-37.23 ± 6.97	-23.18 ± 13.86	13.92 ± 25.87	0.09
Fructose (% of change)	-41.75 ± 1.56	52.24 ± 24.58	83.80 ± 31.34	< 0.001 <sup>A***&amp;B*</sup>
Sucrose (% of change)	-60.78 ± 6.33	-46.57 ± 16.96	27.48 ± 16.46	< 0.001 <sup>A***&amp;B***</sup>
Sodium (% of change)	-27.26 ± 28.59	-13.00 ± 25.35	8.74 ± 18.23	0.13
Potassium (% of change)	13.49 ± 36.33	14.56 ± 29.40	18.92 ± 47.74	0.92

Data are expressed as % change according to the 2 records obtained at baseline and after 6 months as mean ± standard deviation (SD). A: difference between group 1 and group 2. B: difference between group 1 and group 3. \*Statistically significant difference  $p < 0.05$ . \*\*Statistically significant difference  $p < 0.01$ . \*\*\*Statistically significant difference  $p < 0.001$ .

loss (1.75 kg) at 3 months that had doubled by six months (3.34 kg). This finding was similar to PREMIER (24) results, which found a 3.90 kg loss at six months.

The energy provided by sweetened beverages could be influenced by the caloric value and the amount of sugar in the drink (25). As we know, there are a wide variety of beverages with and without nutritional value as well as low calorie and high calorie drinks. At present there has been an increase in consumption of

low calorie drinks (13), even though no consensus exists about the utility of substituting sucrose for artificial sweeteners to achieve better weight control. In view of the increased worldwide prevalence of obesity, it is important to clarify whether artificially sweetened soft drinks can aid in regulating body weight. The majority of published studies concerning artificial sweeteners have been carried out over short periods of time. The present study showed that by six months, those subjects who drank artificially sweetened



beverages lost 0.61 kg. It is thought that because these drinks were low calorie the subjects did not gain weight. However, after six months this group had a weight increase of 0.11 kg. It would be interesting to analyze the long-term effect of soft drinks on weight; since a moderately positive correlation was found between increased consumption of soft drinks and body weight could progressively increase. Current knowledge at this moment is modest and does not yet permit an informed view of how the ingestion of energy-containing sugars and low and no-calorie sweeteners affects overall mechanisms of energy balance and thus influences body weight (26).

Another interesting result was a higher increase in phase angle in group 1, and this could be explained because the decrease in sodium and sucrose intake, that are associated with enhanced sympathetic nervous system activity and sodium and water retention (24), changing the ratio between extracellular and intracellular water and soft tissue hydration (27).

With respect with the effect of reduced soft drink consumption on blood pressure, since soft drinks contain sodium few studies have evaluated this association (16-18,28,29). The 18 month long PREMIER study of hypertensive subjects found that systolic blood pressure diminished 1.8 mmHg and diastolic blood pressure 1.1 mmHg when subjects' limited beverages sweetened with sugar (17). Unlike the PREMIER study, our population was normotensive, so one would assume that their blood pressure would be unchanged. However, in those subjects who reduced consumption of both naturally sweetened and artificially sweetened drinks systolic blood pressure decreased 4.83 mmHg and diastolic blood pressure 2.68 mmHg. This may be associated in part to lose weight and has important implications since even small reductions in blood pressure have important health benefits to the population level, and sodium intake also diminished.

In another study Brown evaluated the impact of sugar-sweetened beverages on blood pressure on normotensive adults and found a significant increase in systolic blood pressure of 1.6 mmHg and diastolic blood pressure of 0.8 mmHg (18). Our findings indicate that when consumption of all sweetened beverages is reduced the negative effect on blood pressure is more important than the increase observed when soft drink consumption was increased. In our study consumption of low calorie drinks diminished both systolic and diastolic blood pressure, as Brown reported, with an inverse relationship between consumption of low calorie drinks and blood pressure (18). This indicates that low calorie beverages can contribute to lower blood pressure, however when both normal calorie and low calorie drinks are reduced, systolic and diastolic blood pressure decrease even more.

The sample size was small but enough to find differences between groups with statistical significance was a single center study so the external validity is lacking and longer follow-up might show more conclusive results. The decreased in other dietary variables other than fructose or sucrose like lipids couldn't be explained by beverage intake reduction, is another limitation of this study.

In conclusion the reduction in consumption of beverage with caloric and non-caloric sweeteners and substituted with plain

water, along with a change in eating habits with a isocaloric dietary plan to reduce sugars and sodium, significantly decrease body mass index and waist circumference at 3 and 6 months. So, the prescription to avoid overall sweetened beverages intake should be emphasized for weight reduction or maintenance.

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MVD concept, design, definition of intellectual content, literature search, data acquisition, statistical analysis, manuscript preparation; LCM: concept, design, critical input and revision of the manuscript, AOT, CKD and LTO: manuscript editing and manuscript review; MACG and MFBC: recruitment and assessment of patients, data acquisition. All authors read and approved the final manuscript

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