

Beneficial effects of a flavanol-enriched cacao beverage on anthropometric and cardiometabolic risk profile in overweight subjects

Efectos beneficiosos de una bebida de cocoa enriquecida con flavanol en el perfil antropométrico y de riesgo cardiometabólico en sujetos con sobrepeso

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ABSTRACT

Overweight and obesity are associated with systemic inflammation and oxidative stress which, in turn, enhance the development of cardiometabolic disruptions. Lifestyle changes and pharmacologic approaches show moderately effective results regarding overall health improvements. Evidence suggests that cacao flavonoids are associated with a reduced cardiometabolic risk, due to the modulation of molecular pathways subjacent to glucose and lipids metabolism. The aim of this study was to assess the effects of cacao flavonoids supplementation on anthropometric and cardiometabolic risk factors in overweight subjects. A double-blind, placebo-controlled, pilot clinical trial was conducted in overweight subjects with borderline criteria of metabolic syndrome. Participants were randomly assigned to either, supplement of cacao flavonoids (80 mg) or placebo, daily, for 4 weeks. Cardiometabolic variables were blood pressure, glycemia and lipid profile. Serum markers of oxidative damage (free protein carbonyls and malondialdehyde) were also analyzed. Anthropometric measurements included body weight, body mass index, waist circumference, and fat and fat-free mass. We found significant reductions in body weight ($p = 0.04$), waist circumference ($p = 0.03$), triacylglycerols ($p < 0.01$), TG/HDL ratio ($p = 0.01$), MDA ($p = 0.02$) and protein carbonyls ($p = 0.01$) in the flavonoid-supplemented group. Results from this study show that cacao flavonoids can effectively modulate anthropometric and cardiometabolic risk factors.

RESUMEN

El sobrepeso y la obesidad están asociados con la inflamación sistémica y el estrés oxidativo, que, a su vez, incrementan el desarrollo de trastornos cardiometabólicos. Cambios en el estilo de vida y tratamientos farmacológicos muestran resultados moderadamente eficaces en relación con la mejora general de la salud. La evidencia sugiere que los flavonoides del cacao se asocian con un riesgo cardiometabólico reducido, debido a la modulación de las vías moleculares subyacentes al metabolismo de la glucosa y de los lípidos. El objetivo de este estudio fue evaluar los efectos de la suplementación de flavonoides del cacao sobre factores de riesgo cardiometabólico y antropométrico en sujetos con sobrepeso. Se llevó a cabo un ensayo clínico piloto, doble ciego y controlado con placebo en sujetos con sobrepeso y criterios limítrofes de síndrome metabólico. Los participantes fueron asignados al azar a cuatro semanas de tratamiento con suplemento oral de flavonoides de cacao (80 mg) diario o placebo. Las variables cardiometabólicas analizadas fueron presión arterial sistémica, glicemia y perfil lipídico. También se analizaron los marcadores séricos de estrés oxidativo (carbonilos proteicos libres y malondialdehído). Las medidas antropométricas incluyeron el peso corporal, índice de masa corporal, circunferencia de la cintura, masa grasa y masa libre de grasa. Se encontró una reducción significativa en el peso corporal ($p = 0.04$), circunferencia de la cintura ($p = 0.03$), triglicéridos ($p < 0.01$), la relación TG/HDL ($p = 0.01$), MDA ($p = 0.02$) y carbonilos ($p = 0.01$) en el grupo con suplemento de flavonoides. Los resultados de este estudio muestran que los flavonoides del cacao pueden modular efectivamente factores de riesgo cardiometabólico y antropométricos.

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INTRODUCTION

Polyphenolic compounds are secondary metabolites of plants, which can be divided into several families, one of which is the flavonoid family.¹ Flavonoids are a diverse class of phenolic substances that share a basic chemical structure constituted by two benzene rings linked through a heterocyclic pyran ring; within this family, there are catechins, procyanidins and (-)-epicatechin, the most abundant flavanol in cacao. The latter has been proposed as the primary constituent of cacao that exerts bioactive actions that yield cacao's well-known health benefits. In fact, there is emerging epidemiologic evidence of their role in the prevention of cardiovascular and metabolic diseases and cancer.²

Overweight and obesity may be the origin of cardiometabolic diseases (e.g. hypertension, type 2 diabetes, and dyslipidemia).³ The therapeutic lifestyle modifications to treat these weight problems including a healthy diet with some caloric restriction and exercise, when it is possible, have no ideal effects, particularly in the long term due mainly to low patient adherence. Strategies focused to increase weight loss and to decrease cardiometabolic risk must be reasonable and attainable, without imposing severe diet restrictions difficult to follow and comply. Cacao derivatives with low caloric content rich in polyphenols could be a good auxiliary measure to attain weight and metabolic control.

Recently, experimental evidence has suggested that cacao polyphenols may influence body weight, body fat mass, serum lipid and lipoprotein levels, and the expression of enzymes regulating lipid metabolism.^{4,5} For many years it was believed that the beneficial effects of flavonoids were exclusively due to their antioxidant capacity.⁶ However, an increasing number of studies suggest that flavonoids do not act as conventional hydrogen-donating antioxidants, but may exert modulatory actions in cells through several signaling pathways⁷ including the phosphorylation status of target molecules and modulating gene expression.⁸⁻¹⁰

Due to the aforementioned effects, it is necessary to implement controlled studies to address the capability of cacao-derived products

to improve the cardiometabolic status in overweight subjects.

In this work we report the effects of a commercially available cacao extract on cardiometabolic endpoints and quality of life perception in overweight subjects.

METHODS

Subjects

Inclusion criteria were: overweight volunteer subjects 20-60 years old with at least two of the following: abdominal (waist) circumference: > 80 cm in women and > 90 cm in men; fasting glycemia 100-126 mg/dL; plasma triacylglycerides 150-200 mg/dL; plasma HDL-C < 50 mg/dL in women or < 40 mg/dL in men; systolic blood pressure (BP) > 120 and < 135 mmHg and/or diastolic BP > 80 and < 90 mmHg.

Exclusion criteria: known chronic diseases that could affect any of the metabolic variables of the study (e.g., cancer, liver disease, CVD, etc.), and/or the use of dietary/pharmacologic agents that could modify the variables of the study (e.g., acarbose, L-carnitine and fiber supplements), and/or pregnancy/lactation. The Institutional Ethics Committee approved the protocol and written informed consent was obtained.

Subjects were randomly allocated in two groups in a 1:2 (placebo:supplement) ratio.

Study design

We conducted a 4 weeks (wk), double blind, placebo-controlled, clinical pilot trial in overweight subjects with borderline criteria of metabolic syndrome. After initial enrollment, study subjects were assessed for dietary and physical activity habits. All participants received general dietary recommendations and the advice of 30 minute of daily vigorous walking. Participants were randomly assigned to either consuming a commercially available cacao bean extract powder, containing flavonoids (80 mg/serving), 8 calories from 0.16 g of total fat, 1.3 g of carbohydrates, and no cholesterol; or placebo powder with similar physical characteristics, sugar-free and

without flavonoids, once a day, for 4 wk. The powders were consumed as a beverage after being dissolved in 200 mL of water. Subjects were instructed to consume the supplement during morning fasting and to return the empty sachets to verify compliance.

Anthropometric and cardiometabolic endpoints

At baseline (wk 0), subjects were cited following an overnight fast (12 h) for blood samples collection and measurement of BP, body weight, height, abdominal circumference, body composition and body mass index (BMI) calculation. Anthropometrics were repeated after 2 and 4 weeks, and blood samples were repeated after 4 weeks. Subjects were also asked to complete the EQ-5D instrument to assess quality of life. All procedures followed ethical standards as stated in the Helsinki Declaration and local legislation. Participants were instructed to inform researchers of any adverse events.

Anthropometric measurements were conducted with patients wearing light clothing and no shoes. Resting seated blood pressure was measured according to the American Heart Association guidelines. Venous blood was collected in fasting conditions and serum was frozen at -80 °C until subsequent analysis. Samples were analyzed for glucose, total cholesterol, triacylglycerols, high density lipoprotein (HDL) using commercially available colorimetric kits (Randox SA, Mexico), low density lipoprotein (LDL) was calculated according to Friedwald formula.

Protein carbonylation

A highly sensitive assay for detection of protein carbonyls involves derivatisation of the carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH), which leads to the formation of a stable 2,4-dinitrophenyl (DNP) hydrazone product which is evaluated spectrophotometrically at 375 nm.¹¹

Lipid oxidation

Malondialdehyde (MDA) a lipid peroxidation product produced during metabolic stress was measured with the 1-methyl-2-phenylindole

colorimetric assay which in presence of MDA produces a blue/purple chromophore spectrophotometrically evaluated at 586 nm.¹²

Statistical analysis

Data are presented as mean + standard deviation, unless otherwise stated. Wilcoxon t-tests were performed for mean comparison between baseline and final measurement of each group for continuous data. Mann-Whitney U test's were used to compare nonparametric differences between groups. A p value < 0.05 was considered as statistically significant. The statistical package herein used was GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Subjects

Fifteen overweight subjects (11 women and 4 men) were included, 2 men and 3 women in the placebo group and 2 men and 8 women in the treatment group. No statistically significant intergroup differences were found at baseline, indicating randomization homogeneity (Table I).

Anthropometric and cardiometabolic variables

After 4-weeks, body weight loss was higher in the subjects assigned to the cacao supplement (2.4 kg) when compared to the placebo (1.7 kg) group (3 versus 2.1%, $p = 0.04$) (Figure 1). Abdominal circumference was also significantly reduced in subjects consuming cacao (3.5 cm) versus placebo (1.8 cm), (3.6 versus 1.8% respectively, $p = 0.03$) (Figure 1).

Glycemia decreased in both groups (12 and 13.9 mg/dL in placebo and supplement groups respectively) however, the decrease was significant only in the supplement group (Table II).

A significant reduction of 66 mg/dL (Table II) in triacylglycerols in the treatment group as compared with a reduction of 27 mg/dL in the placebo groups (23.4 versus 11.1%, $p < 0.01$) was found (Figure 2).

In the cacao group, total cholesterol decreased by 58.5 mg/dL (16.8%, $p < 0.01$),

while in the placebo group only decreased by 37 mg/dL (13.3%, *ns*). Also the LDL-cholesterol decreased by 33 mg/dL (17%, $p < 0.01$), while in the placebo group decreased by 29 mg/dL without significance (16%, *ns*) (Table II).

Blood levels of HDL significantly increased 6.9 mg/dL (Table II) in the treatment group vs. 3.2 mg/dL in the placebo group (17 versus 9%, $p = 0.04$) (Figure 2).

The TG/HDLc ratio has been proposed as a strong and independent surrogate marker of CM diseases since this index relates to insulin resistance.¹³ Interestingly the TG/HDL ratio decreased, almost reaching normal values in the treated group (6.4 to 4.1 a 33.8% change versus 17.9% in the placebo group, $p = 0.01$) (Figure 3).

Oxidative damage markers

Levels of oxidative damage biomarkers were also attenuated, MDA at the end of the study period was significantly lower (17.5 versus 10.3%, $p = 0.01$) (Figure 4) in subjects assigned to the cacao supplement treatment compared with those assigned to the placebo treatment.

Free carbonyls content decrease 8 mmol (16.7%) (Table II) in the treated group versus 2.5 mmol (6.1%) in the placebo group ($p = 0.001$) (Figure 4).

Quality of life

In the subjects assigned to the cacao supplement treatment, the EQ-5D analog visual index

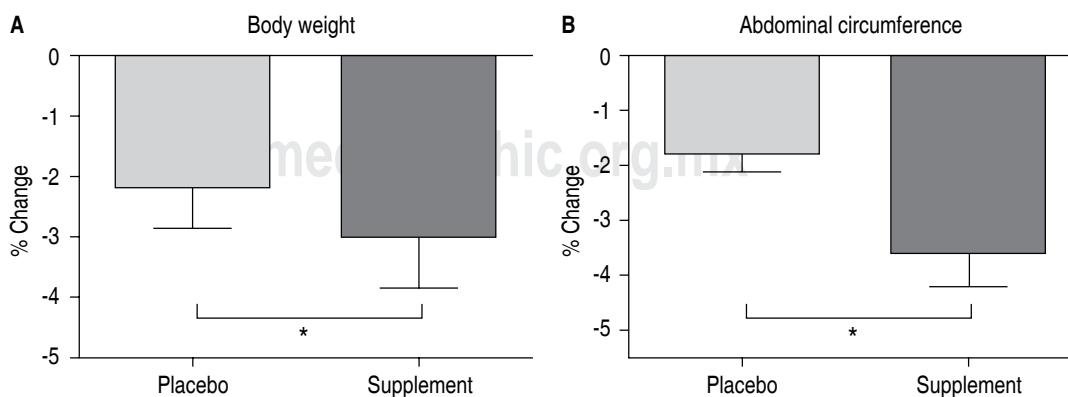
Table I. Changes in anthropometric variables during the study period.

	Placebo			Supplement		
	Week 0	Week 4	p	Week 0	Week 4	p
BW, (kg)	81.5 ± 10.4	79.8 ± 10.5	< 0.05	77.2 ± 8.5	74.8 ± 8.2	< 0.01
% Change		-2.1 ± 0.5			-3 ± 0.7	< 0.05
BMI (kg/m ²)	28.6 ± 0.9	27.9 ± 0.8	<i>ns</i>	28.1 ± 1.2	27.4 ± 1.1	< 0.01
% Change		-2.6 ± 1.1			-2.6 ± 0.7	<i>ns</i>
Waist (cm)	101.2 ± 5.7	99.4 ± 5.5	<i>ns</i>	98 ± 8	94.5 ± 7.4	< 0.01
% Change		-1.8 ± 0.6			-3.6 ± 1.6	< 0.05
SBP (mmHg)	122 ± 3.2	118 ± 4.4	<i>ns</i>	126 ± 5.4	119.5 ± 2.7	< 0.05
DBP (mmHg)	81 ± 1.6	80 ± 1.3	<i>ns</i>	83 ± 4.0	81 ± 1.1	<i>ns</i>

Abbreviations: BW = Body weight; BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure.

Figure 1.

Percent change (from baseline) in body weight and abdominal circumference in supplemented and control groups.



scores improved from an average of 76.5 to 89 (12.5%, $p = 0.002$) (Figure 5) while in the placebo group only a non-significant and slight change was reported (1%, $p = \text{NS}$) (Figure 5).

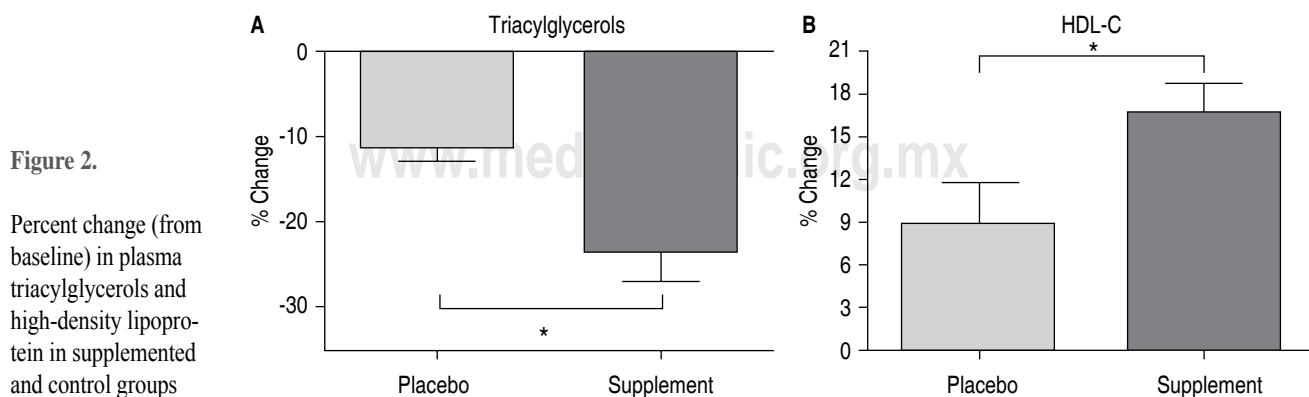
In the digital analysis of EQ-5D, as expected, no change was found in the self-care and pain-discomfort scores. However, a significant 16% ($p = 0.041$) improvement in the motility score

(Figure 6) was found in the treated group while no change was observed in the placebo group. Interestingly, the anxiety/depression score improve a 12% in the treated group almost reaching statistical significance ($p = 0.08$). These improvements were validated by a significant 20% ($p = 0.036$) improvement in the usual activities score with treatment.

Table II. Changes in metabolic variables during the study period.

	Placebo			Supplement		
	Week 0	Week 4	p	Week 0	Week 4	p
Glycemia (mg/dL)	112 \pm 9.6	100 \pm 8.6	ns	108.5 \pm 6.6	94.6 \pm 6	< 0.01
% Change		10.4 \pm 2.1			12.8 \pm 3.6	ns
Total Chol (mg/dL)	272.4 \pm 17.7	235.4 \pm 12	ns	251.5 \pm 63.5	193 \pm 18.7	< 0.01
% Change		13.3 \pm 3.1			16.8 \pm 10.2	ns
LDL Chol (mg/dL)	180 \pm 9.2	151 \pm 5	ns	156 \pm 51.9	123 \pm 30	< 0.01
% Change		16 \pm 3.1			17 \pm 7.8	ns
HDL Chol (mg/dL)	39 \pm 4	42.2 \pm 2.6	ns	41.3 \pm 2.3	48.2 \pm 3.1	< 0.01
% Change		9 \pm 5.7			17 \pm 5.1	< 0.05
Trigs (mg/dL)	247 \pm 36	220 \pm 35	ns	259 \pm 63	193 \pm 49	< 0.01
% Change		11 \pm 3			23.4 \pm 9.5	< 0.01
TG/HDL ratio	6.3 \pm 0.8	5.2 \pm 0.7	ns	6.4 \pm 1.8	4.1 \pm 1.1	< 0.01
% Change		18 \pm 7			33.8 \pm 11	< 0.05
MDA ($\mu\text{mol/mg}$)	1.31 \pm 0.2	1.17 \pm 0.1	ns	1.43 \pm 0.2	1.17 \pm 0.1	< 0.01
% Change		10.3 \pm 1.9			17.5 \pm 6.8	< 0.05
Carbonyls (mmol)	41.3 \pm 6.7	38.8 \pm 6	ns	46 \pm 7	38 \pm 3	< 0.01
% Change		6.1 \pm 3.3			16.7 \pm 6.4	< 0.01

Abbreviations: LDL = Low density lipoprotein; HDL = High density lipoprotein; MDA = Malondialdehyde.



Safety and tolerability

No adverse effects were reported in any of the subjects in the supplement group. All subjects denied symptoms such as light-headedness,

dizziness, fatigue or chest discomfort during the study period.

DISCUSSION

The present study showed that consumption of cacao supplement containing flavonoids at a dosage of 80 mg/day during 4 wk, by overweight subjects with borderline criteria of metabolic syndrome, induced beneficial metabolic changes including decreases in body weight and abdominal circumference, increase in HDL-c and reduction in triacylglycerols levels, with an improvement in the TG/HDL ratio. There was also a reduction in lipid (MDA) and protein oxidative (free carbonyls) markers. Interestingly, subjects under treatment reported an increase in their quality of life. Altogether, these results strongly suggest that the flavonoid-enriched beverage

Figure 3.

Percent change (from baseline) in TG/HDL ratio in supplemented and control groups

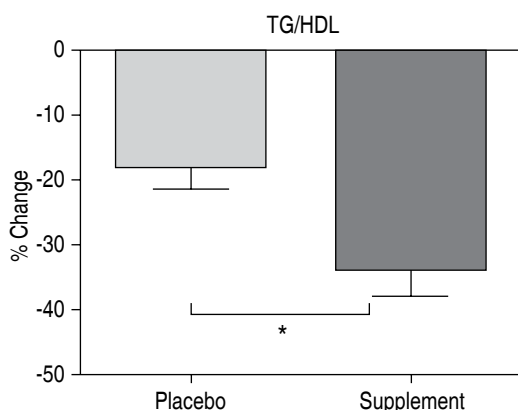


Figure 4.

Percent change (from baseline) in oxidative stress plasmatic markers in supplemented and control groups.

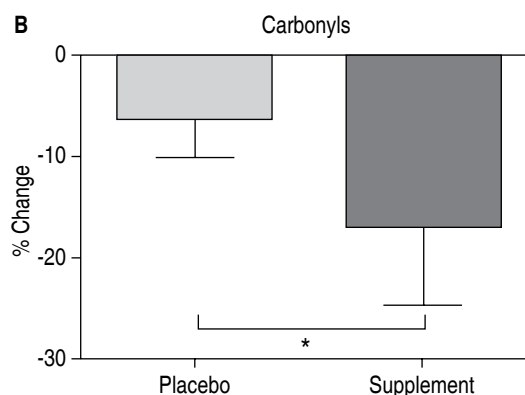
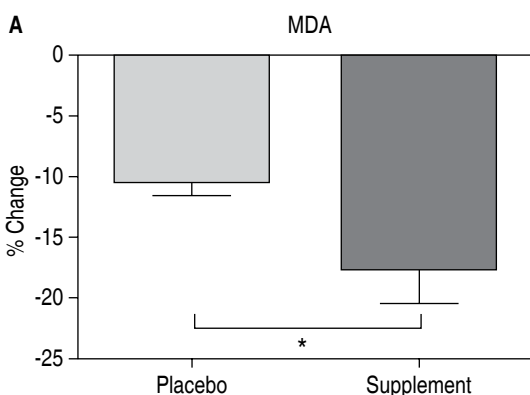
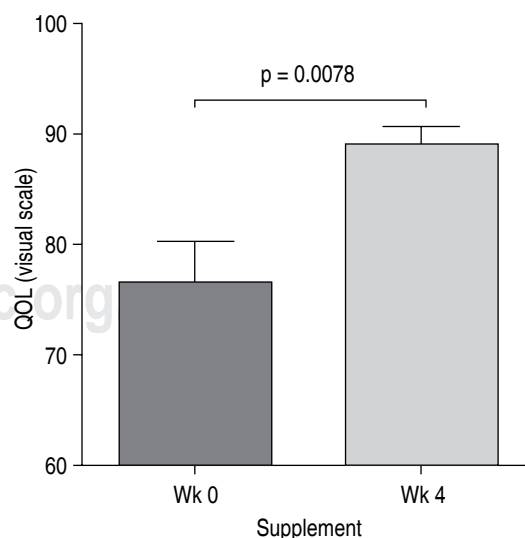
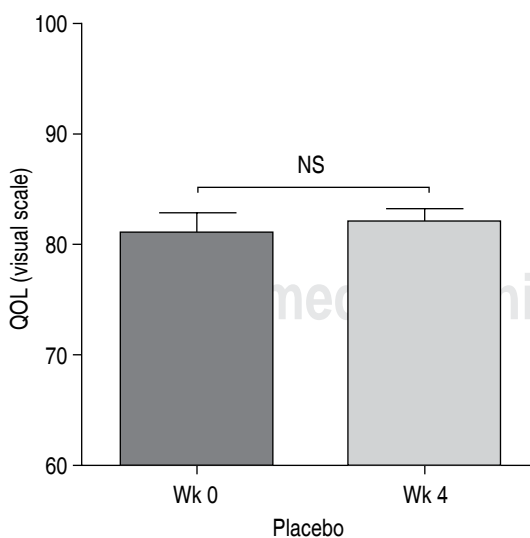


Figure 5.

Baseline and final quality of life visual scores in supplemented and control groups.



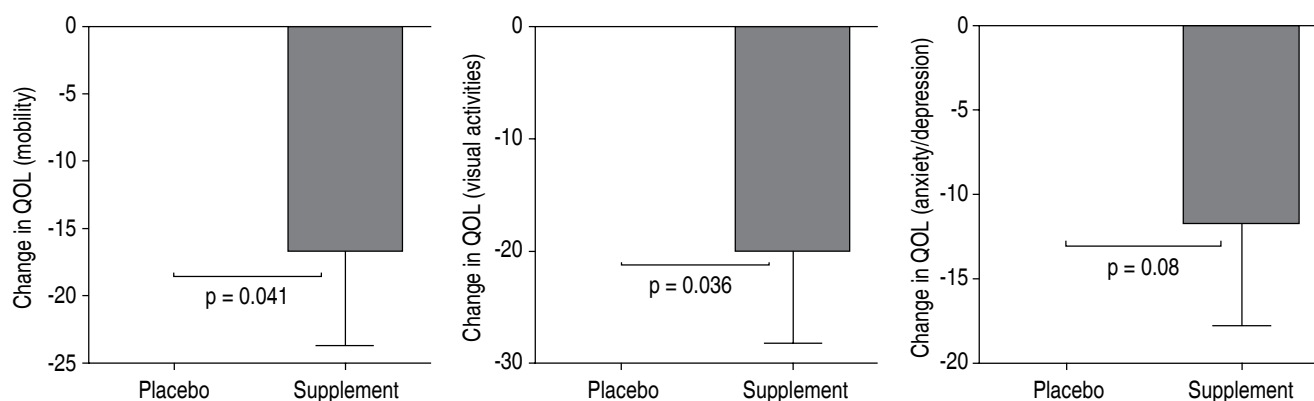


Figure 6. Percent change (from baseline) in quality of life scores in supplemented and control groups.

exerts beneficial cardiometabolic effects, thus decreasing overall health risks and improving the well being of treated subjects.

Our results are in agreement with those of Matsui et al whom demonstrated that cacao intake during three weeks, leads to lower body weight and white adipose tissue weight in male Wistar rats, by suppressing fatty acid synthase and other liver enzymes required for fatty acid synthesis. In addition, fatty acid binding protein and fatty acid synthase were lowered in white adipose tissue of cacao-fed rats, suggesting effects on lipid metabolism with cacao intake.¹⁴ In the present study, cacao flavonoids supplementation reduced body weight by 3.6% compared to 1.8% in the placebo group; abdominal circumference also was reduced by 3% compared to 2.1% of placebo group.

Our results also showed that cacao flavonoids supplementation increase plasma HDL-c significantly by 17%, compared to 9% in the placebo group. In agreement with these results, it has been reported that HDL concentrations increased by 14% after 3-wk intake of dark chocolate enriched with cacao polyphenols.¹⁵ The mechanism through which flavonoids increase plasma HDL-c levels remains unclear. One hypothesis is that the major protein component of HDL, apolipoprotein A1, participates in the induction of those effects.¹⁶ There is also evidence suggesting that some polyphenols (quercetin, isoquercetin) increase the expression of apolipoprotein A1 and that this is probably mediated by the mitogen-activated protein kinase signalling pathway (MAPK).¹⁷ However

we did not evaluate this phenomena and more specific work is necessary in order to define the mechanisms involved.

Our results showed that daily consumption of cacao flavonoids reduced triacylglycerols levels by 23.4% compared to 11.1% in the placebo group. These results are consistent with reports showing that catechins decrease visceral fat deposition and the hepatic concentration of triacylglycerols, due to down regulation of fatty acid synthase.¹⁸

It is noticeable that whereas the pharmacological treatment of isolated hypertriglyceridemia, when blood levels are less than 500 mg/dL is still controversial, it is accepted the benefit of reducing the ratio of TG/HDLc, since it has been proposed that this ratio can be used to predict success in antidiabetic treatment and its reduction can led to significant improvements in cardiovascular risk.¹⁹ Our results showed a significant reduction in this ratio with treatment, suggesting a reduction of cardiovascular risk.

On the other hand, it is well accepted that oxidative stress (ie. free radical-induced damage to carbohydrate, lipids, proteins and DNA) is in close relationship with overweight/obesity induction of endothelial dysfunction, dysglycemia, dyslipidemia, hypertension, inflammation and type 2 DM and in turn, on the increase in cardiometabolic risk.²⁰

On this regard, it has been reported that increases in HDL-c could lead to decreased LDL-c oxidation particularly through increases in the levels and activity of paraoxonase-related HDL-

c subtype 3, this effect can induce: inhibition of monocyte chemotaxis, increase in hydrolysis of lipid peroxides (via paraoxonase activity) and direct inhibition of vascular endothelial activation via apolipoprotein A1.^{21,22}

Lipid oxidation gives rise to a number of secondary products and malondialdehyde is the principal and most studied product of polyunsaturated fatty acid peroxidation. This aldehyde is a highly toxic molecule. Our results showed that 4 weeks of cacao flavonoids supplementation reduced significantly, the plasma malondialdehyde levels by 17.5%, compared to 10.3% in the placebo group.

On the other hand, proteins are main targets of reactive oxygen species (ROS), suffering carbonylation by direct oxidation of lysine, arginine, proline and threonine residues or by interaction with reactive carbonyl species (RCS) produced by carbohydrates and lipid oxidation and by non-oxidative reactions with dicarbonyl compounds.²³ An increase in RCS concentration has been associated with the development of arterial hypertension and elevated plasma concentrations of total cholesterol and LDL-c and reduced HDL-c.^{24,25} In fact, oxidized modified forms of lysine and tyrosine have been detected in human atherosclerotic plaque.²⁶

Free carbonyls, the oxidation products measured in the present study, are stable markers of oxidative damage, and could therefore be useful indicatives for the assessment of oxidative stress that underlies the metabolic syndrome.

Our results showed a significant decrease of 16.7% in the free carbonyl levels in the treatment groups as compared to a 6.1% decrease in the placebo group.

As a whole, our results showed that even when lifestyle changes are beneficial, since they induced body weight loss (placebo group results) as well as convincing modifications in cardiometabolic and oxidative stress markers, the supplementation with flavonoid-enriched cacao beverage bring about even greater effects on those markers, demonstrating clear and significant effects on top of those provoked by lifestyle changes alone.

In addition of the relevance of the results on anthropometric and cardiometabolic risks caused by the administration of the flavonoid-enriched cacao beverage, the results found in

the quality of life analysis are also noteworthy. Assessment of treatment satisfaction and quality of life was performed using the validated 5-Dimension Quality of Life Scale (EQ-5D). The EQ-5D comprises 5 parameters: (1) mobility, (2) self-care, (3) usual activities, (4) pain/discomfort and (5) anxiety/depression, with five possible responses for each one, evaluating levels of discomfort in a scale 1 to 5, where the highest number in the score represents the worst quality of life, and 1 represents the best QoL.²⁷ Our results showed significant decreases in mobility and usual activities scores (improvements) where the anxiety/depression score also decrease almost reaching significance. Changes in these numerical scores demonstrated the positive effects on well-being that is induced by de cacao supplement.

Interestingly, the EQ-5D includes also a visual analogue score (VAS), in this approach any beneficial effect must induce a positive change in the perception of QoL. Our results showed a positive and significant change with the 4 wk treatment with the cacao supplement corroborating the numerical scale.

The increase of 14% in the VAS-Score in the treated group can be interpreted as a consequence of the clinical improvement associated with the anthropometric and metabolic changes.

In the present study, daily consumption of a flavonoid-enriched beverage had no significant influence on blood pressure, glycemia, total cholesterol and LDL-c. There were no adverse effects. These results confirm the findings of previous studies regarding the safety of cacao products.

Finally, the potential clinical relevance and limitations of this study should be considered. The benefits of improving anthropometric and metabolic variables, all critical markers of cardiovascular risk, as well as the quality of life in a short period of time with a product with low or absent side effects is remarkable, and deserve further investigation, particularly in the long term.

The main limitations of this study are the short period of time under treatment and the low number of subjects under study; however the results are unquestionable since they were obtained under a double blind design. Indeed, more work is necessary in order to establish the relevance of these results with a bigger sample with chronic treatment.

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