1 Regularly consuming a green/roasted coffee blend reduces the risk of metabolic syndrome

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Abstract

Purpose: Preventive health effects of coffee could have a widespread impact on public health. Green coffee has more phenols than roasted and thus is healthier, although less acceptable organoleptic properties. Therefore, the effects of regularly consuming a green/roasted coffee blend (35/65) on the main components of MetS in humans were evaluated.

Methods: A crossover, randomized, controlled study was performed in 25 normocholesterolaemic and 27 hypercholesterolaemic men and women, aged 18-45y with BMI 18-25kg/m². Three servings/day of the blend, providing 510.6mg hydroxycinnamic acids and 121.2mg caffeine/day, were consumed *versus* a control drink, during 8 weeks each. Polyphenol and methylxanthine-rich foods were restricted along the study. At the beginning (baseline) and end of the control and coffee interventions, blood samples were collected and glucose, HDL-Cholesterol, triglycerides, insulin, leptin, plasminogen activator inhibitor-1 (PAI-1), resistin and visfatin were analysed; waist circumference, %body fat, blood pressure were measured and dietary records and physical activity questionnaires completed.

Results: Systolic and diastolic blood pressure decreased ($p=0.001$ and $p<0.001$, respectively) in both groups as well as %body fat ($p=0.001$) which may be related to the lower leptin ($p=0.001$), PAI-1 ($p<0.001$) and resistin ($p=0.034$) levels in the two groups after coffee consumption. Glucose concentration ($p=0.030$) and insulin resistance ($p=0.011$; HOMA-IR), also decreased, as well as triglyceride levels ($p=0.017$) the reduction was much greater in the hypercholesterolaemics (group effect, $p=0.027$).

Conclusion: Regular consumption of the green/roasted coffee blend may be recommended to healthy and hypercholesterolaemic subjects to prevent MetS, as it produces positive effects on blood pressure, glucose and triglycerides levels.

Key words: Coffee, hydroxycinnamic acids, diabetes, hypertension, weight loss, metabolic syndrome

Abbreviations

BMI: body mass index; BP: blood pressure; CGA: chlorogenic acid; FA: fatty acids; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance index; MetS: metabolic syndrome; PAI-1: plasminogen activator inhibitor-1; TC: total cholesterol.

Introduction

The prevalence of metabolic syndrome (MetS) has risen during the last decade due to unhealthy lifestyle and dietary habits [1]. MetS is characterized as a combination of underlying risk factors that when occurring together culminate in adverse outcomes, including type 2 diabetes mellitus, cardiovascular disease, and an approximately 1.6-fold increase in mortality [2]. A single precise definition and the contributions of the underlying components of MetS have been of much debate. According to the National Heart, Lung and Blood Institute/American Heart Association, MetS is diagnosed if any three of the five following criteria is met: central obesity, raised triglycerides, reduced high-density lipoprotein cholesterol (HDL-C), raised blood pressure and elevated fasting plasma glucose [3,4]. The complex interactions between these risk factors contribute to chronic organ damage. Obesity, hypertension and dyslipidaemia lead to the remodelling of the heart and blood vessels, producing chronic cardiovascular complications in which a variety of bioactive substances secreted by the adipose tissue, known as adipokines, are involved [5]. Adipokines play a key role in the development of metabolic syndrome.

Health protective effects of coffee could have a widespread impact on the population health, considering the high intake of this beverage, particularly in industrialized countries where it is the largest source of dietary antioxidants [6]. The potential impact of coffee on health has been recently reviewed in Ludwing et al [7]. Most recent studies observed a 30-60% reduction of diabetes risk, whereas coffee seems to have a mix of beneficial and harmful cardiovascular effects, as the undesirable influence of diterpenes and caffeine may be compensated by the beneficial effects of the phenolic components, mainly chlorogenic acid (CGA). CGA, which also shows positive effects on glucose metabolism [8], may reduce fat accumulation in numerous tissues via inhibition of enzyme regulators involved in lipogenesis [9], although recently such effect was not observed in mice fed a high fat diet [10]. On the other hand, caffeine, with lipolytic and thermogenic activities, increases metabolic rate, energy expenditure, and lipid oxidation, and so it has potential as an aid in weight loss and reducing the overall risk for developing MetS [11]. Balancing all these effects, a positive beneficial relationship between coffee consumption and MetS could be expected, attributed mainly to the bioactive compounds polyphenols and caffeine.

However studies aimed at evaluating the association between long term coffee consumption and the components of MetS are scarce and have not reached a consensus. Studies carried out in a Japanese population demonstrated a significant inverse correlation between coffee intake

and MetS [12-14]. Other studies conducted in a Dutch population described that after adjustment for physical activity, energy intake, smoking behavior and alcohol consumption the relationship between coffee consumption and MetS or its components was not significant [15-16]. In contrast, a study conducted in a Mediterranean population described a positive and inverse association [1]. The differences in the outcome of the coffee-MetS association may be attributed, to a certain extent, to the overall diet quality of the population studied. In this sense, in Japan and Mediterranean countries a healthier diet is consumed than in Central Europe. In fact, subjects more adherent to the Mediterranean diet are less likely to suffer MetS or its components [17].

In view of the foregoing, this work looks into the preventive effects of regularly consuming three cups of a green/roasted coffee blend per day, within a Mediterranean diet, on MetS components and certain serum adipokine levels in healthy and cardiovascular risk subjects.

Subjects

The present study was approved by the Clinical Research Ethics Committee of Hospital Puerta de Hierro (Madrid, Spain) and therefore has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Volunteers' recruitment was carried out by giving short talks and placing advertisements at Complutense University campus.

The inclusion criteria was as follows: men and women with body mass index (BMI) under 25 kg/m², 18 to 45 years old, non-smokers, non-vegetarian, non-pregnant women, not-having vitamins or dietary supplements, not-having taken antibiotics six months before the start of the study and not suffering chronic disorders or pathologies, apart from hypercholesterolemia. Participants were separated in two groups attending to their total cholesterol (TC) levels: normocholesterolemic (TC< 200 mg/dL) and hypercholesterolemic (TC> 200-240 mg/dL) subjects.

Fifty-four subjects accepted to participate in the study and gave informed written consent; however, two withdrew due to personal and professional reasons. Of the remaining volunteers, twenty-five were normocholesterolemic and twenty-seven were hypercholesterolemic. Baseline characteristics of the fifty-two volunteers who finally participated in the study are presented in Table 1.

Study design

A randomized, controlled, cross-over study was carried out in free-living subjects (Figure 1). After a run-in stage (2 weeks), subjects were randomly distributed in 2 groups, so that half the participants firstly consumed the coffee product and the other half had the control drink for 8 weeks. Then, after a 3 week wash-out stage, subjects changed to drink the other beverage during the same time (8 weeks). During the coffee intervention, volunteers consumed three times a day a 2 g serving of the coffee blend dissolved in 200 mL of hot water, preferably at breakfast, midday and after lunch, without milk or sugar. At the control intervention, instead of coffee, a control drink consisting in water or an isotonic caffeine- and polyphenol-free drink was consumed 3 times a day. Blood samples, blood pressure and anthropometric measurements were taken at the end of the run-in (baseline), control and coffee stages. Blood samples were collected in BD Vacutainer® tubes (Becton, Dickinson and Company, New Jersey, USA) with or without EDTA to separate plasma and serum, respectively, and aliquots were separated and stored at -80°C until analysis.

Coffee composition

The soluble coffee product used was a commercial green/roasted mixture (35:65, w/w) that was provided and packed explicitly for the study in blind 2 g sachets by the manufacturing company. Total soluble polyphenols in the green-roasted coffee product were analysed by high performance liquid chromatography with diode array detection (HPLC-DAD) as described elsewhere [18]. Methylxanthine content in the infusions was analysed by HPLC-DAD in an Agilent 1200 series (Agilent Technologies, CA, USA) following a method previously described [19]. The coffee contained 85.1 mg/g of total hydroxycinnamic acids (57.4 mg/g corresponded to caffeoylquinic acids, mainly 5-*O*-caffeoylquinic acid), and 20.5 mg/g of total methylxanthines (20.2 mg/g were caffeine). Therefore, the daily consumption of hydroxycinnamic acids and methylxanthines was 510.6 and 123 mg, respectively.

Dietary control and compliance

Along the whole study, participants were asked to maintain their habitual lifestyle and dietary habits, except for consuming other coffee products, certain fruits and vegetables rich in polyphenols, particularly those rich in hydroxycinnamic acids (artichokes, oranges, grapes, aubergines, or soya among others) as well as products like tea, yerba mate, whole wheat products and caffeine containing drinks. The consumption of potatoes, apples, pomegranates, rice and corn was reduced to once a week.

Before starting the study, volunteers were instructed on how to fill in the dietary records. In each stage, they were asked to fill out a 72-hour food intake report. To estimate the energy intake and dietary composition, the programme DIAL (Department of Nutrition and Bromatology I, School of Pharmacy, Complutense University of Madrid, Spain) was used. Study compliance was controlled by calling the volunteers every week, and counting the number of coffee servings returned after the intervention.

Physical activity, body fat and waist circumference

Participants were asked to maintain their usual physical activity throughout the study. They filled out questionnaires before the start of the study and at each intervention to evaluate their physical activity which was estimated using the programme ADN (Department of Nutrition and Bromatology I, School of Pharmacy, Complutense University of Madrid, Spain).

At baseline and the end of each stage of the study, volunteer's percentage of total body fat and waist circumference were measured using a Tanita Segmental Body Composition Analyzer BC-418 MA (Tanita Corp. Tokyo, Japan) and a SECA 203 flexible tape (SECA Ltd, UK), respectively.

Blood pressure parameters

At the end of each stage, before blood sampling, systolic and diastolic blood pressure (BP) were measured using an automatic arm sphygmomanometer (Pic Indolor Diagnostic, BS 150, Artsana, Italy) in triplicate, waiting for 3 min between measurements. Readings were compared and accepted if in agreement within 10–15 mmHg.

Biochemical analysis and insulin resistance calculations

Triglycerides and HDL-C were determined in serum samples following reference methods or methods recommended by Sociedad Española de Bioquímica Clínica y Patología Molecular (SEQC) using a Roche Cobas Integra 400 Plus Analyser (Roche Diagnostics, Mannheim, Germany). Fasting glucose was analysed using a colorimetric kit (Sprinreact). Fasting insulin concentration was analysed using the Bio-Rad Multiplex Diabetes Kit on Bio-Plex MAGPIX system. Using fasting glucose and insulin data, Homeostasis Model Assessment index was calculated to estimate insulin resistance (HOMA-IR) according to the equation by Matthews et al [20]: $HOMA-IR = [Glucose (mg/dL) \times Insulin (mU/L)]/405$.

Adipokine biomarker analysis

Leptin, resistin, PAI-1 and visfatin were analysed in plasma samples using the Bio-Plex Pro Human Diabetes Kit. All analytes were measured in duplicate on a MAGPIX™ Multiplex reader and software Bio-Plex Manager™ MP (Luminex Corporation, Austin, USA) was used for data processing. Intra-assay %CV were 3, 3, 4 and 5 and inter-assay %CV were 4, 4, 4 and 3 for leptin, resistin, PAI-1 and visfatin respectively. Results were expressed as pg/mL plasma.

Statistical analysis

Setting the statistical power at 80%, the significance level at 0.05, and taking total cholesterol as the main variable, a sample size of 23 subjects per group was calculated based on the assumption that the within-patient standard deviation of the response variable was 10 and the difference between treatments was 6 mg/dL. Statistical analyses were carried out using the program SPSS version 20.0 (IBM Company, NY, USA). Normality of distribution and homogeneity of variance of all variables studied were evaluated using the Kolmogorov-Smirnov and Levene tests, respectively. A general linear model of variance for repeated measures was used to assess differences due to consuming coffee and the group was considered an inter-individual factor. Furthermore, differences within each group were studied using the paired Bonferroni test. Significance level was established at $p < 0.05$.

Results

In all the studied parameters, the normocholesterolemic and hypercholesterolemic exhibited the same behaviour after the consumption of coffee and thus the effect of the group was not significant except for waist circumference ($p=0.007$) and triglycerides ($p=0.027$). There was no significant coffee x group interaction in any of the biomarkers studied.

Effects on food intake

Protein intake was significantly lower at the end of the coffee intervention (Table 2), whereas none of the other dietary intake parameters showed statistical differences along the study. There was no influence due to the group.

Effects on physical activity, body fat and waist circumference

Volunteers did not show changes in their physical activity along the study (data not shown). A significant decrease ($p=0.001$) in the percentage of body fat was observed in both groups after coffee consumption (normocholesterolaemics: 22.8 ± 1.4 , 23.7 ± 1.4 and 21.5 ± 1.2 and hypercholesterolaemics: 25.4 ± 1.3 , 24.4 ± 1.2 and 23.6 ± 1.3 , at baseline, control and coffee interventions, respectively). Regarding waist circumference, there was a significant difference

between the two groups; in contrast to the normocholesterolaemic subjects, among the hypercholesterolaemic volunteers there was a tendency to reduce their waist circumference, although the reduction was not statistically significant (Table 3).

Effects on blood pressure

According to the general linear model of variance for repeated measures analysis, systolic and diastolic BP significantly decreased in both the healthy and hypercholesterolaemic group (Table 3) after coffee consumption ($p= 0.001$ and $p< 0.001$, respectively). Furthermore, when the differences within each group were studied using the paired Bonferroni test, in the latter group both parameters were statistically lower after the coffee intervention compared to baseline, although not to control values.

Effects on triglycerides and HDL-cholesterol

Regarding triglyceride levels, a significant reduction ($p= 0.017$) was observed after coffee consumption as well as a significant difference due to the group ($p= 0.027$), so that the reduction in the lipid biomarker was much greater in the hypercholesterolaemic subjects compared to the healthy. In contrast, HDL-C levels did not change.

Effects on glucose levels and insulin resistance

At the end of the coffee intervention, glucose concentration significantly decreased (Table 3, $p= 0.030$). In contrast, insulin data did not show changes (normocholesterolaemics: 8.60 ± 0.39 , 9.14 ± 0.31 and 8.01 ± 0.37 and hypercholesterolaemics: 8.95 ± 0.45 , 8.80 ± 0.36 and 8.56 ± 0.42 mU/L at baseline, control and coffee interventions, respectively). Insulin resistance, calculated according to the HOMA-IR model, decreased significantly ($p=0.011$, Figure 2).

Effects on adipokines

Leptin ($p= 0.001$), PAI-1 ($p< 0.001$) and resistin ($p= 0.034$) significantly decreased in the two study groups (Table 4). According to the Bonferroni test, after coffee consumption leptin and resistin levels were statistically lower with respect to their corresponding baseline values in both groups of volunteers. Similarly, PAI-1 levels after the coffee intervention were significantly lower in both groups of intervention with respect to the baseline, and in the healthy volunteers also with respect to the control.

Discussion

The prevalence of MetS in the Mediterranean regions is estimated to be 15%–20% in men and 19%–25% in women, with an increase as high as 50% in subjects over 70 years in certain countries [1]. These rates have further risen during the last decade due to unhealthy lifestyle and dietary habits [1] and thus it is pertinent to identify foodstuffs or beverages that can help to counteract MetS. In this sense coffee could play an important role, given its high rate of consumption and its composition, rich in polyphenols. In the present study, the intake of three cups of coffee per day was established in order to reproduce a realistic consumption rate and pattern (morning, midday and after lunch) of coffee consumers in Spain. Apart from setting the intake of coffee and restricting the consumption of certain foods rich in polyphenols and methylxanthines, the study was carried out in free-living subjects who kept their lifestyle unchanged, as far as the dietary and physical activity questionnaires filled out showed. Attending to the dietary records, their energy and macronutrient intakes were slightly below the dietary recommendations established by Moreiras et al [21] for the Spanish population, except those for proteins, lipids and saturated fatty acids that were slightly above recommended intakes. Overall, it may be considered that volunteers consumed a healthy Mediterranean diet.

The results of the present study show that the consumption of a green/roasted coffee blend is inversely related with three components of MetS: blood pressure, blood glucose and triglyceride levels, which is in agreement with the cross-sectional study by Hino et al [12]. It is noticeable that the positive effect on triglycerides was more pronounced in the hypercholesterolaemic group. On the other hand, in this study no effects were observed on central obesity or HDL-C, in contrast to the results described in another cross-sectional study when more than two cups/day of coffee were consumed [22]. Again the hypercholesterolaemic group showed a better response to coffee consumption as waist circumference tended to decrease in these subjects.

Our study supports that fasting plasma glucose and triglycerides are among the components of MetS more susceptible to the protective effect of coffee [23]. CGA is the major bioactive compound in coffee likely to be responsible for these health benefits, as the hypoglycaemic and antidiabetic effects of polyphenols are well known, stimulating glucose uptake in both insulin-sensitive and insulin-resistant adipocytes. CGA also improves glucose tolerance and insulin resistance, acting as an active principle in glucose metabolism regulation (reviewed in Meng et al [8]). In addition, CGA has shown to induce positive effects on lipid metabolism, lowering serum and hepatic cholesterol and triglyceride levels, inhibiting fat absorption, activating fat metabolism in the liver, and improving obesity related hormone levels among

other effects [8]. Relating to the bioavailability and pharmaceutical studies in humans carried out in our group using the green/roasted coffee [24, 25] the biological effects here observed should be mainly attributed to the content in phenolic compounds, without discarding the contribution of methylxanthines. Considering that in the study three doses of coffee were consumed distributed along the day (breakfast, midday and after lunch), it is likely that during the study the levels of phenolic metabolites remained in the blood stream at relatively high concentrations for a long time [24], in contrast, to caffeine and its derived methylxanthines and methyluric which circulated in plasma for a shorter period [25]. However, the transformation of the CGA during roasting and soluble coffee manufacture/brewing is complex. Drastic roasting conditions may produce losses of up to 95% of CGAs with 8–10% being lost for every 1% loss of dry matter [7]. The content of CGA in the coffee blend studied was twice as high as that reported in regular instant coffees (decaffeinated and caffeinated) which ranged between 30 – 40 mg/g [26].

This study has looked into certain adipokines as the regulators of systemic glucose and lipid homeostasis, attempting to further understand the effects on glucose and triglycerides observed. Moreover, we endeavoured to shed light in the association between coffee consumption and adipokines, with evidences scarce and inconsistent so far. Regarding the relationship between coffee consumption and circulating leptin, an inverse association [27-29] or no change in serum leptin at higher levels of coffee consumption [30] have been reported. Similarly, an inverse association between caffeine consumption and plasma leptin has been described [28, 31]. As for PAI-1, one clinical study has reported an increase in plasma PAI-1 in heavy coffee drinkers compared to light coffee drinkers [32] whereas another intervention observed a decrease in serum levels of PAI-1 with higher consumption of coffee and caffeine [28]. These authors reported no changes on resistin or visfatin levels in a cross sectional study. Our results support an inverse association between regular coffee consumption and serum concentrations of leptin, PAI-1 and resistin. The possible mechanism underlying the observed adipokine decrease may be related to the effect of phenolic compounds and/or caffeine in the coffee blend decreasing body fat mass, thereby decreasing the number of adipocytes and the subsequent secretion of the studied adipokines. This would be in agreement with the results of an experiment on the efficacy of CGA altering body fat in high-fat diet induced obese mice in which CGA significantly lowered body weight, visceral fat mass and plasma leptin compared to the high-fat control group that consumed caffeic acid instead of CGA [33]. Leptin is a hormone exclusively excreted by adipocytes. Although the pathophysiological significance of blood leptin levels is not fully understood, it is positively correlated with obesity and inflammatory

markers [29]. In contrast, resistin is produced in adipocytes and macrophages, and elevated circulating resistin has been associated with higher risk of type 2 diabetes, inflammation and atherosclerosis [34]. It cannot be ruled out that the observed beneficial effect of coffee consumption on glucose levels has operated through an improved adipokine profile. Visfatin concentrations did not show changes along the study, in agreement with the lack of changes in the visceral fat (waist circumference) where this adipokine is synthesized.

It is noteworthy that according to the results of the present study, the component of MetS most susceptible to the protective effect of coffee was blood pressure. The reduction of systolic and diastolic BP was -5.2 and -5.6 mmHg in the hypercholesterolaemic group, and -3.4 and -2.3 mmHg in the normocholesterolaemic subjects, respectively, which is highly significant considering that volunteers had normal BP values at baseline (Table 1). The magnitude of the reduction in BP is comparable to that in systolic BP (9.2 mmHg) in mild-hypertensive subjects after the intake of 300 mg/d of hydroxycinnamic acids during 8 weeks reported by Ochiai et al [35]. Considering this, the intake of the green/roasted coffee might be a promising strategy to reduce both systolic and diastolic BP.

Caffeine has an acute pressor effect, particularly in hypertensive subjects, which may be regulated through many biological mechanisms, including binding to the adenosine receptor, activating the sympathetic nervous system via elevated plasma concentration of catecholamines and stimulating the pituitary-adrenocortical response to increase cortisol production. However, changes in the caffeine-induced pressor effect develop in habitual coffee drinkers, as there are a complex set of counterregulatory hormones that maintain blood pressure and may cause tolerance to the humoral and hemodynamic effects of caffeine [36]. Nevertheless, other ingredients in coffee may also have blood control effects, mainly CGA. A group of studies support that CGA in coffee counteracts the effect of caffeine. Among these, a placebo-controlled, randomized clinical trial carried out in patients with mild hypertension showed that CGA (140 mg/day) reduced blood pressure (systolic and diastolic) after 12 week consumption [37]. The same research group previously demonstrated that the short term ingestion of GCE had hypotensive effects and reported a dose-response relationship from 70 mg to 280 mg CGA/day [38]. Similarly, in spontaneous hypertensive rats, the ingestion of GCE reduced blood pressure in a dose-dependent manner [39]. Contrarily, in normotensive subjects the consumption of a drink containing green coffee bean extract (140 CGA mg/day) for 4 months did not induce hypotensive effects, although improved vasoreactivity was observed through an increase in the vasodilative response in ischemic reactive hyperemia [40]. Therefore, the hypotensive effect of CGA in normotensive subjects is not clear, although the

present study supports that 345mg/d of CGA does induce such positive effect in healthy subjects as well as a pilot crossover study carried out in healthy subjects who consumed a green coffee rich in CGA compared to black coffee [41]. Other components in coffee formed during roasting, may play a role in coffee's effect on blood pressure, such as hydroxyhydroquinone (HHQ). Yamaguchi et al [42] compared the effects of ordinary coffee to HHQ-free coffee, the former had no effect on blood pressure after 4 weeks, in contrast the HHQ-free coffee reduced blood pressure in subjects with mild hypertension which points to HHQ inhibiting the effect of CGA. Once again, in hypertensive rats, HHQ inhibited the hypotensive effects of CGA [43]. In addition, quinides in coffee may counteract caffeine-induced adenosine receptor antagonism by inhibiting the adenosine receptor transporter [45].

Limitations of this study: the study population was healthy or relatively healthy, being the prevalence of metabolic syndrome null. Strengths of the study: Lifestyle characteristics and social status of the volunteers were homogenous. Other background features were controlled such as volunteers' physical activity and dietary habits. Intake of the coffee was well controlled.

Conclusion

Regular consumption of a green/roasted coffee blend may be recommended to healthy and hypercholesterolaemic subjects to prevent MetS, as it produces positive effects on blood pressure, blood glucose and triglycerides levels, being particularly interesting for hypercholesterolaemic subjects as coffee improved the lipid profile.

The authors declare that there is no conflict of interest.

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497 **Table 1.** Subject characteristics

	Normocholesterolaemic (n=25)		Hypercholesterolaemic (n=27)	
	Men (n=10)	Women (n=15)	Men (n=10)	Women (n=17)
Age (years)	25 ± 6	27 ± 8	35 ± 9	33 ± 6
Weight (kg)	76.8 ± 13.4	57.6 ± 7.0	78.2 ± 7.7	56.7 ± 5.1
BMI (kg/m ²)	24.2 ± 2.8	21.9 ± 2.5	24.9 ± 2.3	21.4 ± 2.2
Systolic BP (mmHg)	123.1 ± 8.3	107.8 ± 8.3	128.8 ± 9.4	111.5 ± 5.5
Diastolic BP (mmHg)	74.2 ± 6.2	67.6 ± 5.5	83.1 ± 8.8	71.3 ± 5.4

505 Values represent mean ± standard deviation. BMI: body mass index; BP: blood pressure

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Table 2. Intake of energy and macronutrients during the study

	Normocholesterolaemic (n=25)			Hypercholesterolaemic (n=27)			
g/day	Baseline	Control	Coffee	Baseline	Control	Coffee	P*
Energy#	1878±110	1854±79	1817±79	1860±83	1857±107	1779±95	N.S.
Dietary fibre	13.6±1.0	14.7±0.9	14.2±0.8	17.3±1.2	17.6±1.4	17.1±1.3	N.S.
Carbohydrates	180.0±12.3	180.6±7.9	177.0±10.2	184.2±8.1	167.8±11.8	169.9±11.1	N.S.
Lipids	77.9±5.1	76.0±3.4	74.7±4.5	78.0±3.2	71.9±3.5	69.0±5.4	N.S.
Proteins	87.2±4.8	79.3±4.3	78.9±4.2	79.7±4.4	75.9±3.9	73.5±3.6	0.025
Cholesterol#	295.1±15.9	286.8±19.5	286.9±22.3	282.9±20.9	246.6±25.5	246.1±31.4	N.S.
Saturated FA	26.9±1.7	26.8±1.6	25.5±1.9	25.4±1.4	26.7±1.5	23.0±2.3	N.S.
Monounsaturated FA	33.9±2.1	36.9±1.8	33.3±2.1	32.1±1.8	28.4±2.4	28.9±2.2	N.S.
Polyunsaturated FA	9.6±0.7	9.9±0.6	10.2±0.6	10.8±0.9	10.0±0.8	10.3±0.9	N.S.

#Energy values are expressed in Kcal/day and cholesterol in mg/day. Volunteers completed a 72-h food intake report and energy and dietary composition was calculated using the programme DIAL (Department of Nutrition and Bromatology I. School of Pharmacy, Complutense University of Madrid, Spain). Values represent mean ± standard error of mean. *P values correspond to the effect of consuming coffee which was studied using a general linear model of the variance for repeated measures analysis. The effects of the group and coffee x group interaction were non-significant in all parameters. Mean values within a row with unlike superscripts correspond to significant differences within either the normocholesterolaemic or hypercholesterolaemic group according to Bonferroni test. FA: fatty acids. N.S.: non-significant.

522 **Table 3.** Effect of the consumption of the roasted/green coffee on components of the metabolic syndrome

	Normocholesterolaemic (n=25)			Hypercholesterolaemic (n=27)			
cm	Baseline	Control	Coffee	Baseline	Control	Coffee	p*
Waist circumference	70.4±1.3	70.6±1.4	70.9±1.4	76.8±2.5	76.6±2.5	75.6 ±2.5	N.S.
mmHg							
Systolic BP	113.3±2.1	112.6±2.0	109.9±2.1	119.4±2.9 ^a	115.8±2.5 ^{ab}	114.2±3.1 ^b	0.001
Diastolic BP	69.5±1.2	69.2±1.0	67.2±1.2	76.8±2.2 ^a	73.3±1.9 ^b	71.2±2.2 ^b	<0.001
mg/dL							
Triglycerides	71.3±7.1	70.0±5.2	71.0±5.7	103.3±7.5 ^a	86.6±5.5 ^{ab}	82.9±6.1 ^b	0.017
HDL-C	55.9±2.9	56.7±2.4	58.2±2.7	63.1±3.1	59.4±2.5	62.7±2.9	N.S.
Glucose	74.0±1.5	74.9±1.4	71.0±1.6	76.95±1.6	74.57±1.4	73.20±1.7	0.030

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524 *P values correspond to the effect of consuming coffee which was studied using the general linear model of the
525 variance for repeated measures analysis. The effects of the group [except for the waist circumference
526 (p=0.007) and triglycerides (p=0.027)] and coffee x group interaction were non-significant in all parameters
527 Mean values within a row with unlike superscripts correspond to significant differences within either the
528 normocholesterolaemic or hypercholesterolaemic group according to Bonferroni test. N.S.: non significant.
529

530 **Table 4.** Effects of regularly consuming the green/roasted coffee on adipokines

	Normocholesterolaemic (n=25)			Hypercholesterolaemic (n=27)			
pg/mL	Baseline	Control	Coffee	Baseline	Control	Coffee	p*
Leptin	3341±379 ^a	2854±367 ^b	2685±349 ^b	3393±381 ^a	2581±310 ^{ab}	2566±306 ^b	0.001
PAI-1	3579±175 ^a	3397±207 ^a	2757±158 ^b	3980±260 ^a	3652±205 ^{ab}	3165±233 ^b	<0.001
Resistin	36660±1520 ^a	34553±2006 ^{ab}	31587±1631 ^b	37033±1904 ^a	34968±1299 ^{ab}	34951±2607 ^b	0.029
Visfatin	3302±322 ^a	2721±318 ^{ab}	2417±397 ^b	3480±240	3263±342	3185±395	N.S.

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532 *P values correspond to the effect of consuming coffee which was studied using the general linear model of the

533 variance for repeated measures analysis. The effects of the group and coffee x group interaction were non-

534 significant in all parameters. Mean values within a row with unlike superscripts correspond to significant

535 differences within either the normocholesterolaemic or hypercholesterolaemic group according to Bonferroni test.

536 N.S.: non-significant.

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