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Roasting intensity of naturally low-caffeine *Laurina* coffee modulates glucose metabolism and redox balance in humans

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ABSTRACT

Objective. Coffee consumption is negatively associated with risk of type 2 diabetes and cardiovascular mortality. Coffee roasting can greatly modify the quality-quantitative characteristics of bioactive compounds. We compared the effects of two different roasting intensities of the same naturally low-caffeine Arabica coffee variety (*Laurina*), on glucose and lipid metabolism as well as oxidative stress.

Research Methods & Procedures. We performed a double-blind, crossover intervention study. 14 healthy male volunteers consumed 4 cups/day of Light Roasted Coffee (LRC) and Dark Roasted Coffee (DRC) for one-week (intervention period 1 and 2 respectively). One-week washout, with total abstinence from coffee and other possible caffeine sources, preceded each interventions. Data were collected at the end of washout and intervention periods.

Results. Changes between washout and intervention periods in glucose concentrations at 2-h post-OGTT, were significantly lower following DRC than LRC intake (-0.6±0.3 and 0.4±0.3 mmol/l, $p<0.03$). Changes in β-cell function, assessed as insulin secretion-sensitivity index-2 (ISSI2), were significantly greater following DRC than LRC (34.7±25.0 and -18.8±21.0, $p=0.03$). The initial (30 minutes) post-OGTT AUC of glucagon-like peptide-1 was 24±9% greater ($p=0.03$) after DRC than LRC. LRC or DRC did not affect insulin sensitivity. Changes from basal of reduced-to-oxidized glutathione ratio (GSH/GSSG) in erythrocytes were significantly greater after DRC than LRC (+1437±371 and -152±30, $p<0.05$). The omega-3 index in erythrocyte membranes was 16±4% greater ($p<0.001$) after DRC than LRC.

Conclusions. DRC consumption improved post-load glucose metabolism by increasing incretin and insulin secretions. DRC compared to LRC improved redox balance and increased omega-3 fatty acids. Thus, we suggest greater metabolic benefits related to DRC.

INTRODUCTION

Coffee is one of the most widely consumed beverages in the world. Several epidemiological studies have shown clear associations between coffee intake and reduced risk for cardiovascular and all-cause mortality [1]. Other studies have underlined the potential role of coffee consumption in reducing the risk of type-2 diabetes mellitus (T2DM), characterized by an ever-increasing prevalence. Prospective studies in different countries and meta-analyses have shown an inverse dose-dependent correlation between long-term consumption of coffee, both regular and decaffeinated, and T2DM risk [2-9]. Even though some studies have shown that caffeine acutely increases blood glucose and decreases insulin sensitivity [10-13], long-term caffeine intake has positive metabolic effects, such as increased secretion from adipocyte of adiponectin, a hormone with insulin sensitizing properties. [15]. Coffee, both with or without caffeine, contributes also to the postprandial insulin secretion by acutely stimulating the release of glucagon-like peptide-1 (GLP-1), an incretin secreted by the small intestine, [24, 25], furthermore, coffee contains over a thousand bioactive antioxidant substances, making this beverage a major dietary antioxidant supplier in western countries [16]. Among coffee components, chlorogenic acid (GCA), melanoidins, quinides and N-methylpyridinium (NMP) have clearly shown the potential to affect glucose and insulin metabolism [17-23].

The roasting process has relevant effects on the relative content of coffee biochemicals. Green beans and light roasted coffee (LRC) have a high content of CGA and trigonelline. These compounds directly improved insulin sensitivity and secretion, as well as glucose uptake, in experimental conditions. With roasting, the concentration of CGA and trigonelline decreases, while that of quinides, NMP and melanoidins, produced by Maillard reaction, increases [26]. These changes have been shown to influence the antioxidant capacity of coffee and its impact on physiological systems. The high antioxidant capacity of dark roasted coffee (DRC) has been especially associated with its melanoidin and NMP content [27]. The gastrointestinal

tract is the major site of melanoidin antioxidant action. Dietary melanoidins have been demonstrated to reduce the formation of lipid hydroperoxides and advanced lipid oxidation end products during meal digestion [18]. NMP has been shown to control oxidative stress through induction of the nuclear factor E2-related factor 2 (Nrf2) and the antioxidant response element (ARE) pathway [22]. The effects of quinides and NMP on glucose metabolism have been poorly investigated in humans. NMP promoted glucose uptake in vitro, while quinides improved insulin action in rats [20, 23].

Principal aim of the present study was to compare the effects of two different roasting intensities of the same naturally low-caffeine Arabica coffee variety (*Laurina*), on glucose and lipid metabolism and oxidative stress in healthy volunteers. We used a crossover, double-blind experimental design. Subjects underwent two consecutive study phases, each one lasting two weeks and each inclusive of one-week washout followed by one-week intervention period, with the intake of LRC (intervention 1) and DRC (intervention 2) products, as 4 cups/day of espresso coffee per day.

METHODS

Study participants and design

Fourteen healthy male volunteers were recruited (age 39±2 y; BMI 25.0±0.4 kg/m^2) according to the following inclusion criteria: habitual coffee drinker, absence of chronic and acute illnesses, no pharmacological treatment and no smoking habits. A physician confirmed the health conditions of the participants through a complete medical history and physical examination. The study was approved by the National Ethics Committee of Slovenia. All the participants signed an informed consent. Measurements were performed at the Institute for Kinesiology of the Primorska University (Koper, Slovenia). As reported elsewhere, during long-term consumption of different coffee products, a sample size lower than that used in the present study provided 90% power in detecting differences in glucose and lipid metabolism, with a probability of 0.05 [13, 14].

Before the study, an expert dietitian assessed eating and physical activity habits of each participant. To minimize potential individual lifestyle confounding variables, subjects were asked to followed some simple rules including: exclusion of caffeine containing foods and drinks, besides the test-coffees, and decaffeinated coffee; maintenance of habitual exercise levels and eating pattern; avoidance of nutrient supplements, herbal products, and medications; and daily compilation of food and exercise logs, checked weekly by the dietitian.

The study lasted four weeks and was organized as a double-blind, crossover intervention, inclusive of two subsequent phases. Each phase involved a washout period of 7-days, during which the participants abstained from drinking any coffee or caffeinated products, followed by an intervention period (7-days), during which subjects drank 4 espresso coffees/day (at breakfast, midmorning, after lunch and in the afternoon), first as LRC and then as DRC (Figure 1).

All LRCs and DRCs used in the study came from the same green coffee batch. Before each intervention period, subjects were provided with specific coffee capsules, filled with the coffee type matched to the study phase. To standardize the brewing process, all participants received the same *espresso* coffee machine (X7.1 IPERESPRESSO, illycaffè spa, Trieste, Italia) and were instructed on its proper use.

Coffee characteristics

The coffee products were obtained from a *Coffea Arabica* variety known as *Laurina* (or *Bourbon Pointu*), characterized by a lower content of caffeine than other *Coffea Arabica* varieties. Green coffee beans were roasted at two different intensities, as determined by color measurement (Colorette 3B, Probat): LRC (color 108±3 A.U.) and DRC (color 82±3 A.U.). Bioactive compound content in LRCs and DRCs (Table 1), including quinolactones (measured as 5-O-caffeoyl-epi-δ-quinide) were measured as previously described [27, 28].

NMP and niacin levels were determined by 1H-Nuclear Magnetic Resonance. Coffee samples were prepared by adding 50µL of D_2O, containing 3-trimethylsilyl-[2,2,3,3-$2H_4$] propionate as chemical shift reference, to 500µL of beverage in a 5mm precision glass NMR tubes (535-pp,Wilmad). A Bruker Avance DMX600 spectrometer (Rheinstetten, Germany), operating at 599.90 MHz for 1H and equipped with a 5 mm TXI xyz-triple gradient probe, was used for quantification (expressed as molar ratio with caffeine).

The coffee component descriptive profiles (Table 2) of LRCs and DRCs were carried out in duplicate, in a sensory laboratory, designed in accordance with ISO8589, using a consensus vocabulary, by a panel of eight experts. Scores (0-7) from testing evaluation were statistically processed (Fizz Network 2.31G Biosystem, Couternon, France). The two products were described as being significantly (ANOVA) different for 8, out of 11, taste and flavor attributes.

Metabolic assessment

An OGTT was performed at the end of each washout and intervention periods in study phases 1 and 2. In the morning of the test day, a catheter was positioned in a forearm vein for blood drawing. Blood samples were collected in the post-absorptive state -30min, -15min and immediately before the glucose load. Then, each subject received 75g of glucose dissolved in 300 ml of water. Post-load blood samples were collected at 30, 60, 90 and 120min in EDTA

tubes and immediately centrifuged (3000g, 4°C) for 10 minutes. Plasma, red blood cells and serum were collected, treated in accordance to the different analytical protocols and stored at -80°C, until measurements.

Analyses and calculations

Glucose, insulin and GLP-1 plasma concentrations were determined in the post-absorptive state and during OGTT. Glucose and insulin were analyzed by standard procedures in a certified external laboratory (Synlab Italia Srl, Italy). GLP-1 levels were determined by a commercially available ELISA kit (TemaRicerca, Bologna, Italia). Insulin resistance in the post-absorptive state was measured by the HOMA-IR, i.e., HOMA-IR=(fasting-glucose × fasting-insulin/22.5) [30]. The trapezoid method was used to calculate AUC for glucose, insulin and GLP-1 during 120min post-OGTT [30]. Early post-OGTT levels of glucose, insulin and GLP-1 were assessed through calculation of 30min post-OGTT AUC [31]. Insulin sensitivity was calculated with the Matsuda index (IS_{OGTT}) [32], a measure of whole-body insulin sensitivity, validated against the euglycaemic-hyperinsulinaemic clamp, as follows: IS_{OGTT}=[10000/√(fasting-glucose × fasting-insulin × $average_{OGTT}$-insulin × $average_{OGTT}$-glucose)]; Insulin sensitivity was also determined as ratio between 120min post-OGTT $AUC_{insulin}$ and $AUC_{glucose}$ [30]. β-cell function during OGTT was assessed with the insulin secretion-sensitivity index-2 as follows: ISSI-2=(IS_{OGTT} ×$AUC_{insulin}$/$AUC_{glucose}$) [30, 33]. The other biochemical indices were measured in the post-absorptive state. Adiponectin levels were determined using a commercial ELISA kit (BioVendor, Lab. Med. Inc., Brno, Czech Republic). Total and HDL cholesterol and triglyceride plasma levels were assessed with standard methods by a certified external laboratory (Synlab Italia Srl, Italy). Homocysteine, 5-oxoproline and amino acid concentrations were assessed by gas-chromatography mass-spectrometry (GC-MS, HP5890, Agilent Technologies, Santa Clara, CA), using the internal standard technique, as previously described [34, 35]. For each compound, a known amount of

stable isotope (Cambridge Isotope Laboratories) was added as internal standard to a known volume of plasma. Silylated derivatives were measured under electron-impact ionization by selective ion monitoring. Total glutathione concentrations in erythrocytes were evaluated by GC-MS using the internal standard technique as previously described [36]. The GSH/GSSG ratio in erythrocytes was determined by a commercially available kit (Prodotti Gianni, Milano, Italia) [36]. The relative fatty acid (FA) contents in erythrocyte membranes were determined by gas-chromatography flame-ionization-detection (GC-FID; GC6850 Agilent Technologies, Santa Clara, CA, USA), as previously reported [37]. Red blood cell membrane levels of FAs were expressed as percent ratio between AUC of each FA peak and the sum of all FA peaks. Δ-5 and Δ-9 desaturase indices (arachidonic/dihomo-γ-linolenic acid ratio and oleic/stearic acid ratio respectively, in erythrocyte membranes) are markers of insulin sensitivity. The omega-3 index is a marker of cardiovascular risk, defined as sum of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in erythrocyte membranes.

Body weight and composition. At the end of each washout and intervention period, body weight was recorded and body composition was measured by a mono-frequency bioelectrical impedance apparatus and its software (BIA101 and Software Bodygram®, Akernsrl, Firenze, Italy).

Statistics

Data are reported as mean±SEM. In order to evaluate coffee effects and coffee×roasting interactions, we have used repeated measures ANOVA, or ANCOVA, where appropriate (washout values were used as covariates). When the results of coffee×roasting interactions were significant ($p \leq 0.05$), the changes induced by the coffee products, evaluated as the difference between the values obtained at the end of the intervention and the washout periods (delta values), were assessed through paired *T*-test. Values were logarithmically transformed

169 when appropriate; $p<0.05$ was considered statistically significant. Statistical analysis was
170 performed using SPSS software (v12; SPSS, Inc., Chicago, IL).
171

RESULTS

All participants were compliant to the study. Body weight and composition, as expected, did not change significantly throughout the protocol (Table 3).

As shown in Table 4, fasting glucose, insulin and GLP-1 concentrations and HOMA-IR did not change significantly after the coffee intake phases.

Following OGTT, we found significant coffee×roasting interaction on glucose concentrations at 120min post OGTT. Changes in the 2h post OGTT glucose values (Figure 2A), comparing basal with the intervention periods, were significantly lower after DRC intake than after LRC (-0.6±0.3 and 0.4±0.3 mmol/l, $p<0.03$, Student paired t-test). Furthermore there was significant coffee×roasting interaction on delta changes from fasting values of glucose concentrations. The early (30min) and the total (120min) post-OGTT AUC of plasma glucose and insulin (Figure 2A and B) and the post-OGTT marker of insulin sensitivity (IS_{OGTT}) were not significantly modified by the coffee different roasting intensities.

There was a significant coffee×roasting interaction on ISSI2, an index of β-cell insulin secretion-sensitivity. Absolute delta values of this index, calculated after DRC administration, were significantly greater than those observed after LRC intake (34.7±25.0 and -18.8±21.0, $p=0.03$, Student paired t-test).

During OGTT, in all conditions (washout and intervention periods), GLP-1 plasma concentrations, at 30min increased by 5-6 times the fasting values and after 60min, decreased toward basal values. GLP-1 values at 30min were influenced by both coffee intake and roasting intensity (Figure 2C). One week of DRC significantly increased (5±2%) GLP-1 concentrations ($p<0.02$, paired Student t-test with Bonferroni correction), similarly a week of LRC increased this incretin concentration but not significantly. At 30min post-OGTT, there was also significant coffee×roasting interaction on delta changes from the fasting GLP-1 values. Furthermore there were significant coffee effects and coffee×roasting interaction on

early (30min) post-OGTT AUC of GLP-1. Coffee administration at different roasting degree did not significantly changed total (120min) post-OGTT AUC of GLP-1.

The effects of LRC and DRC consumption, on glutathione availability and redox status in erythrocytes are shown in Table 5. Coffee consumption significantly increased total glutathione concentrations in erythrocytes, without a significant coffee×roasting interaction, which resulted significant for the GSH/GSSG ratio. Absolute delta values of the GSH/GSSG ratio after DRC administration were significantly greater than those observed after the intake of LRC (1437±371 and -152±30, $p<0.05$, Student paired t-test). There were neither coffee nor coffee×roasting interaction effects on 5-oxoproline plasma concentrations, a precursor of glutamic acid in the γ-glutamyl cycle. However, we found a significant coffee×roasting interaction on the 5-oxoproline-to-glutamate ratio (Table 5). Delta changes between washout and intervention periods of this ratio were significantly greater following DRC than LRC. No changes were observed in the plasma lipid profile (Table 5).

The effects of LRC and DRC on adiponectin plasma levels are shown in Table 5. Coffee consumption significantly increased adiponectin concentrations, with no coffee×roasting interaction. Adiponectin concentrations increased by ~11±2% after both LRC and DRC intake. C-reactive protein (CRP) concentrations did not change significantly throughout the study. Table 6 shows the effects of LRC and DRC on plasma amino acids. There was a significant coffee×roasting interaction on both cysteine and homocysteine plasma concentrations, which were significantly greater after DRC than LRC consumption. The other amino acid plasma levels were not influenced by either interventions.

The effects of LRC and DRC on fatty acid relative composition in red blood cell membranes are shown in Table 7. There was a significant coffee×roasting interaction effect on erythrocyte membrane content for: DHA, the sum of the measured n-3 polyunsaturated fatty acids (PUFA) and the omega-3 index. The sum of n-3 PUFAs, DHA concentration and the

222 omega-3 index in erythrocytes were significantly higher after DRC than LRC intake, while no
223 significant changes were observed for other membrane FAs.
224

DISCUSSION

Epidemiological studies have shown that long-term, habitual coffee consumption reduces the risk of T2DM [2-9]. Nonetheless, the mechanisms of such association are not well defined [17]. Caffeine acutely increases glucose levels [10-13], while it may improve insulin sensitivity in long-term studies [38]. Besides caffeine, coffee contains a mixture of bioactive compounds, with antioxidant and/or glucose metabolism regulating properties, whose levels are influenced by the roasting process [16]. Changes in roasting intensity may modify the glucoregulatory and antioxidant coffee effects. To perform the present study, we selected a naturally low-caffeine *Laurina* coffee variety, to preserve taste and functional effects of caffeine while, at the same time, minimizing undesirable properties. In our study, caffeine amount was equivalent in both products (Table 1), the observed different effects between the two coffees products can be therefore attributed to roasting related changes in bioactive compounds other than caffeine.

Our results indicate a strong association between DRC intake and improved post-load glucose metabolism, as shown by lower glucose concentrations, increased insulinogenic index and enhanced early secretion of GLP-1. In agreement with previous observations [8], coffee had no effects on fasting glucose metabolism.

Early GLP-1 response during the first 30 minutes after oral glucose intake may be selectively altered in non-obese subjects with impaired glucose tolerance [31]. This suggests that the rapidity of GLP-1 response to oral glucose may be delayed in pre-diabetic conditions, that may lead to a defective insulin secretion. In our study, one-week of DRC intake enhanced initial post-OGTT GLP-1 secretion, possibly causing the observed increased insulin secretion and decreased glycaemia. The acute effects of decaffeinated coffee on glucose mediated GLP-1 secretion have been previously described [24, 25]. We underline that in our study coffee-induced changes in OGTT response were observed at least 12 hours after the intake of the last

coffee. This suggests an adaptive metabolic mechanism to a prolonged coffee consumption, rather than an acute response and may explain the favorable association between long-term coffee intake and T2DM prevention.

Thus, the improved postprandial glucose metabolism, associated with DRC consumption, was accounted by an enhanced insulin secretion rather than an improved insulin sensitivity. This is confirmed by the observation that fasting HOMA-IR and post-load indices of insulin sensitivity (IS_{OGTT} and $AUC_{insulin}$-to-$AUC_{glucose}$ ratio) were not significantly affected by coffee ingestion.

Several cross-sectional studies have tested the associations between a long-term intake of different types of caffeinated or decaffeinated coffees and indices of insulin secretion and sensitivity [40, 41] in subjects both healthy or with impaired glucose tolerance. While nearly all studies observed beneficial effects on post-load glycaemia, results point to mechanisms other than insulin secretion or sensitivity [17, 20, 23-25, 40, 41], related to differences in coffee composition and roasting degree. In our study while caffeine content was equivalent both in DRC and LRC, the proportions of quinides, derived from CGA, and NMP, derived from trigonelline, were, as expected, much higher in DRC. Evidences in vitro and in animal models clearly showed that quinides and NMP directly influence glucose metabolism [20, 23]. Quinides seem to inhibit the adenosine membrane transport systems, thus decreasing adenosine uptake and increasing its extracellular levels [42]. Therefore quinides could contribute to counteract the caffeine pleiotropic effect mediated by adenosine activity competition [42]. In the islets of Langerhans, increased extra-cellular adenosine levels may stimulate insulin secretion as well as β-cell proliferation and survival, through activation of the A1 adenosine receptors [43, 44]. We hypothesize that, in our study, DRC intake may have, at least in part, enhanced glucose-mediated insulin secretion through this mechanism.

Beneficial coffee effects have been also related to the well-known antioxidant activity of its bioactive compounds. Several factors [45-47] however can influence this effect. With roasting, the concentrations of different phenolic compounds, including CGA, present in green coffee, are reduced to various extent [26], while antioxidant properties are maintained from the higher levels of NMP and melanoidins [48]. In our study, *Laurina* coffees showed the expected roasting-induced changes in the quantities of the bioactive compound (Table 1). Both LRC and DRC intake, in agreement with other studies [49], increased the red blood cell (RBC) total glutathione concentration, the most relevant antioxidant in the body. It was suggested that some coffee phenolic compounds might increase GSH concentration through the γ-glutamyl-cysteine synthetase activation, the rate-limiting enzyme in GSH synthesis [50]. Other studies have shown a pronounced antioxidant effect in vivo, with dark roasted, NMP rich, coffee consumption [27]. Moreover several of the polyphenols may become part of the melanoidins, contributing to the preservation of antioxidant content and capacity of DRC, even after a decrease in the phenolic compound levels [26]. In our coffee products, the increased concentration of melanoidins with roasting is evident by the reduction in colorimetric values [51], as reported in the section on “coffee characteristics”. Our data show, as in other studies [49, 52], that DRC specifically increased the availability of the reduced form of glutathione, as indicated by the higher GSH/GSSG ratio (Figure 3). In addition, we have observed changes in plasma concentration of precursor amino acids involved in the GSH synthesis. In particular, plasma levels of cysteine, the glutathione key precursor, were significantly enhanced after a week of DRC intake, while glycine and glutamic acid, the other two glutathione precursors, showed an increase without achieving statistical significance. Furthermore, plasma concentration of 5-oxoproline, an intermediate in the γ-glutamyl cycle, considered a marker of glutathione catabolism in vivo [35, 53], was significantly reduced in relation to its product, glutamic acid (Figure 3).

Homocysteine is synthetized from methionine through a transmethylation reaction. Its metabolic fate includes recycling to methionine, though remethylation, or transulphuration to cystathionine and ultimately to cysteine (Figure 3). In parallel to cysteine concentration changes induced by DRC, we have observed also, in agreement with other authors, significant increases in homocysteine plasma levels [52, 53]. The mechanism of such association is unknown. However, given the beneficial effects of coffee intake on cardiovascular mortality [1] coffee-related increases in homocysteine levels do not seem to represent a risk factor.

In western countries, coffee-drinking accounts for a significant proportion of daily antioxidant intake [16], however other factors such as physical exercise, nutrition and energy balance can affect glucose metabolism and oxidative stress. In our study these variables were standardized and monitored throughout the washout and intervention periods by an expert dietitian, using food and activity logs, filled daily by the volunteers.

The Omega-3 index, a recently introduced marker, inversely related with the cardiovascular risk [55], was significantly affected by the roasting intensity. A higher omega-3 index was significantly associated with DRC consumption in comparison with LRC intake. This is in accordance with previous evidence showing that a higher plasma antioxidant capacity is positively associated with PUFA n-3 concentration in RBC membranes [56].

In agreement with previous studies, we found that one week of coffee consumption increased adiponectin plasma concentration [57]. Caffeine, CGA, NMP and other coffee compounds have the potential to stimulate adiponectin secretion from adipose tissue. It has been suggested that this hormone, through modulation of inflammation and insulin resistance, may mediate some of the beneficial coffee effects [15]. We found that roasting intensity did not significantly affected adiponectin concentrations.

This was a crossover, double-blind intervention study. Because of technical reasons, related to the manufacturer, we could not follow a randomization protocol, however we found no

significant differences between baseline values obtained at the end of each washout period, of the same length, which preceded both intervention periods.

In summary DRC, as compared to LRC, both derived from naturally-low caffeine *Laurina* coffee, showed positive effects on glucose metabolism, oxidative stress and cardiovascular risk markers. In addition, DRC presented more desirable organoleptic characteristics (Table 2).

Abbreviations:

CGA, chlorogenic acid

DRC, dark roasted coffee

FA, fatty acid

GLP-1, glucagon-like peptide-1

GSH, reduced glutathione

GSSG, oxidized glutathione

IS_{OGTT}, insulin sensitivity index

ISSI2, insulin secretion-sensitivity index-2

LRC, light roasted coffee

NMP, N-methylpyridinium

PUFA, polyunsaturated fatty acids

T2DM, type 2 diabetes mellitus

Conflict of interest.

The authors declare no competing financial interest.

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Authors' contributions to manuscript: FGDG, conducted research, analyzed data and performed statistical analysis, wrote paper; SM, conducted research, analyzed data and performed statistical analysis; RS, conducted research, wrote paper; NM, conducted research;

ZJP, conducted research; AP, conducted research; MT, conducted research; RP, designed research; LN, designed research, analyzed data; GB, designed research, analyzed data, performed statistical analysis, wrote paper and had primary responsibility for final content. All authors have read and approved the final manuscript.

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Table 1. Daily intake of coffee bioactive compounds.

Bioactive compounds [a]	Coffee roasting intensity	
	LRC	DRC
Caffeine	197	197
Caffeic acid	1.6	1.2
Ferulic acid	0.4	0.4
Mono-caffeoylquinic acid isomers	430	226
Mono-feruloylquinic acid isomers	42.4	30.0
Di-caffeoylquinic acid isomers	11.2	2.8
Trigonelline	334	191
5-O-caffeoyl-epi-δ-quinide	1.59	2.00
N-methylpyridinium	17.2	35.2
Niacin	10.0	10.9

[a] mg of bioactive compounds in the total volume of coffee drank daily during the intervention periods; error between 5 - 10%. LRC, light roasted coffee; DRC, dark roasted coffee

Table 2. **Components of the descriptive profile of coffee.**

Descriptive profile	Coffee roasting intensity		p-value[a]
	LRC	DRC	
Bitter	4.44	5.37	<0.001
Sour	5.71	4.64	<0.001
Sweet	4.69	4.39	0.06
Texture	4.73	5.52	<0.01
Global aroma	5.56	6.12	0.07
Chocolate	2.8	4.04	<0.001
Caramel	2.35	3.08	<0.01
Toasted bread	1.78	2.94	<0.001
Flower/fruit	2.27	1.71	0.06
Burnt	1.31	2.25	<0.05
Global quality	4.61	5.95	<0.05

[a] p-values (ANOVA). Results are expressed as mean of scores, from 0 to 7, given by a panel of eight experts. LRC, light roasted coffee; DRC, dark roasted coffee

Table 3. Effect of *Coffea Arabica variety Laurina* coffee at different roasting intensity on body composition and anthropometric data.

	Study phase 1		Study phase 2		p-value[a]	
	Washout	**LRC**	**Washout**	**DRC**	**Coffee effect**	**Coffee × roasting intensity**
Weight (kg)	79.8±2.3	80.1±2.2	80.1±2.2	79.8±2.2	0.92	0.39
FFM (kg)	61.6±1.7	60.9±1.9	62.2±1.5	61.5±1.5	0.14	0.52
FM (kg)	18.2±1.2	17.9±1.4	17.9±1.3	18.3±1.3	0.24	0.50

N=14. Data are expressed as mean ± SEM. [a]ANOVA or ANCOVA (washout values as covariates) were used where appropriate. Values were log transformed when appropriate. LRC, light roasted coffee; DRC, dark roasted coffee; FFM, Fat-Free Mass; FM, Fat Mass.

Table 4. Effect of *Coffea Arabica variety Laurina* coffee at different roasting intensity on glucose metabolism.

	Study phase 1		Study phase 2		p-value[a]	
	Washout	**LRC**	**Washout**	**DRC**	**Coffee effect**	**Coffee × Roasting intensity**
FASTING STATE						
Glucose (mmol/L)	5.3±0.1	5.4±0.1	5.4±0.1	5.5±0.1	0.06	0.67
Insulin (pmol/L)	43.2±6.6	41.8±3.5	46.3±6.1	43.3±5.2	0.80	0.95
GLP-1 (mg/dL)	2.0±0.5	2.3±0.3	2.1±0.4	2.8±0.5	0.18	0.70
Insulin resistance						
HOMA-IR	0.6±0.1	0.6±0.01	0.6±0.1	0.6±0.1	0.77	0.88
ORAL GLUCOSE TOLERANCE TEST						
Glucose						
120-min concentration (mmol/L)	5.1±0.2	5.6±0.3	5.7±0.4	5.0±0.2	0.57	<0.02
Δ 120min concentration (mmol/L)	-0.2±0.2	0.1±0.2	0.3±0.4	-0.4±0.2	0.32	0.03
30min AUC	3.2±0.1	3.3±0.1	3.3±0.1	3.4±0.1	0.20	0.95
120min AUC	13.2±0.5	12.9±0.6	13.5±0.6	13.1±0.3	0.21	0.67
Insulin						
30min AUC	75.9±12.0	78.7±6.5	82.3±8.2	94.2±10.9	0.12	0.52
120min AUC	502.6±57.3	459.2±38.6	478.4±44.9	508.6±39.6	0.80	0.21
Insulin sensitivity						
IS_{OGTT}	7.74±1.04	7.02±0.63	6.78±0.67	6.80±0.57	0.54	0.25
Insulin resistance						
$AUC_{insulin}/AUC_{glucose}$ ratio	37.6±3.6	36.3±3.3	35.5±3.0	39.0±2.9	0.43	0.26
β-cell function						
ISSI2	261.0±25.9	242.2±21.2	220.4±13.7	255.1±21.5	0.70	0.03
GLP-1 response						
30min concentration	20.1±3.5	21.9±2.7	21.8±2.8	26.8±3.3	0.08	<0.01
Δ 30min concentration (mg/dL)	18.1±3.2	19.6±2.6	19.7±2.6	24.0±3.0	0.16	0.03
30min AUC	5.5±0.9	6.1±0.7	6.0±0.8	7.4±0.9	0.05	<0.01
120min AUC	38.7±4.3	38.2±3.5	37.4±3.6	39.3±3.6	0.07	0.29

N=14. Data are expressed as mean ± SEM. [a]ANOVA or ANCOVA (washout values as covariates) were used where appropriate. Values were log transformed when appropriate. LRC, light roasted coffee; DRC, dark roasted coffee; GLP-1, glucagon-like peptide-1; IS_{OGTT}, insulin sensitivity or Matsuda index; ISSI2, insulin secretion-sensitivity index-2

Table 5. Effect of *Coffea Arabica variety Laurina* coffee at different roasting intensity on indices of oxidative stress, inflammation and lipids metabolism.

	Study phase 1		Study phase 2		p-value[a]	
	Washout	LRC	Washout	DRC	Coffee effect	Coffee × roasting intensity
Oxidative stress						
Total glutathione (µmol/L RBC)	2190±55	2257±57	2287±63	2326±61	0.03	0.47
GSH/GSSG (ratio)	614±202	463±172	942±343	2380±714	0.14	<0.05
Inflammation						
CRP (mg/L)	0.10±0.03	0.07±0.02	0.27±0.14	0.07±0.02	0.16	0.23
Adiponectin (µg/mL)	6.39±0.69	7.01±0.73	6.79±0.67	7.40±0.70	0.002	0.98
Lipid metabolism						
HDL cholesterol (mg/dL)	58.0±3.9	56.2±3.7	56.9±4.3	56.6±4.0	0.30	0.45
LDL cholesterol (mg/dL)	109±9	104±7	108±9	103±6	0.12	0.90
Triglycerides (mg/dL)	63±7	73±7	62±6	67±6	0.15	0.45

N=14. Data are expressed as mean ± SEM. [a]ANOVA or ANCOVA (washout values as covariates) were used where appropriate. Values were log transformed when appropriate. LRC, light roasted coffee; DRC, dark roasted coffee; GSH/GSSG, ratio between reduced and oxidized glutathione; RBC, red blood cell; CRP, C reactive protein.

Table 6. Effect of *Coffea Arabica variety Laurina* coffee at different roasting intensity on major fatty acids in erythrocyte membranes (%).

	Study phase 1		Study phase 2		p-value[a]	
	Washout	**LRC**	**Washout**	**DRC**	**Coffee effect**	**Coffee × roasting intensity**
Saturated fatty acids						
Myristic 14:00	0.25±0.03	0.27±0.02	0.25±0.02	0.25±0.03	0.70	0.53
Palmitic 16:00	23.0±0.5	23.9±0.5	22.6±0.4	22.5±0.5	0.17	0.12
Stearic 18:00	19.5±0.3	19.9±0.3	19.1±0.3	19.1±0.3	0.19	0.23
Sum	42.8±0.7	44.1±0.8	42.0±0.6	41.8±0.7	0.15	0.14
Monounsaturated fatty acids						
Palmitoleic 16:1 n-7	0.23±0.02	0.26±0.01	0.23±0.01	0.23±0.01	0.40	0.27
Oleic 18:1 n-9	15.6±0.3	16.1±0.3	15.3±0.3	15.3±0.3	0.11	0.15
Elaidic trans 18:1n-9	1.1±0.03	1.2±0.04	1.1±0.02	1.1±0.02	0.10	0.84
Eicosenoic 20:1n-9	0.32±0.04	0.30±0.02	0.28±0.01	0.27±0.01	0.59	0.70
Sum	17.3±0.3	17.8±0.4	16.9±0.3	16.9±0.3	0.11	0.18
n-3 Polyunsaturated fatty acids						
Eicosapentaenoic 20:5n-3	0.46±0.04	0.43±0.04	0.51±0.05	0.52±0.06	0.79	0.26
Docosapentaenoic 22:5n-3	2.11±0.10	1.94±0.10	2.20±0.08	2.23±0.10	0.14	0.09
Docosahexaenoic 22:6n-3	4.62±0.25	4.23±0.29	4.78±0.27	4.88±0.31	0.15	0.04
Sum	7.19±0.33	6.61±0.39	7.49±0.35	7.63±0.42	0.18	0.05
n-6 Polyunsaturated fatty acids						
Linoleic 18:2 n6	11.0±0.3	11.1±0.2	11.0±0.2	10.9±0.3	0.64	0.34
Eicosadienoic 20:2n-6	0.28±0.01	0.29±0.01	0.29±0.01	0.30±0.01	0.10	0.76
Dihomo-γ-linolenic 20:3n-6	1.74±0.06	1.65±0.06	1.83±0.05	1.81±0.05	0.03	0.29
Arachidonic 20:4n-6	15.3±0.5	14.4±0.6	15.8±0.4	16.0±0.5	0.22	0.14
Adrenic 22:4n-6	3.6±0.3	3.3±0.2	3.8±0.3	3.8±0.2	0.09	0.03
Docosapentaenoic 22:5n-6	0.8±0.04	0.9±0.1	0.9±0.04	0.9±0.04	0.33	0.53
Sum	32.7±0.7	31.5±0.8	33.6±0.7	33.6±0.7	0.23	0.34
Omega-3 index (20:5 n-3 + 22:6 n-3)	5.08±0.26	4.66±0.33	5.29±0.32	5.40±0.37	0.19	0.04
Δ9-Desaturase index (18:1 n-9 / 18:00)	0.80±0.02	0.81±0.02	0.80±0.02	0.80±0.02	0.68	0.57

Δ5-Desaturase index (20:4 n-6 / 20:3 n-6)	8.86±0.39	8.73±0.40	8.74±0.36	8.97±0.41	0.54	0.13

N=14. Data are expressed as mean ± SEM. [a]ANOVA or ANCOVA (washout values as covariates) were used where appropriate. Values were log transformed when appropriate. LRC, light roasted coffee; DRC, dark roasted coffee;

Table 7. Effect of *Coffea Arabica variety Laurina* coffee at different roasting intensity on plasma amino acids concentrations.

	Study phase 1		Study phase 2		p-value[a]	
Plasma amino acid (μmol×L^{-1})	**Washout**	**LRC**	**Washout**	**DRC**	**Coffee effect**	**Coffee × roasting intensity**
Alanine	379±20	382±14	400±22	395±16	0.93	0.69
Cysteine	554±43	520±42	538±38	590±44	0.63	0.04
Glutamic acid	142±12	125±8	136±6	139±7	0.25	0.12
Glutamine	548±34	518±26	555±32	575±36	0.76	0.10
Glycine	660±41	605±36	672±48	678±24	0.47	0.12
Leucine	153±8	143±6	161±10	167±10	0.77	0.18
Methionine	30.3±1.8	28.4±1.2	32.4±1.8	34.1±2.7	0.92	0.12
Phenylalanine	73.6±4.9	71.4±4.7	77.5±5.3	77.9±4.7	0.74	0.59
Proline	229±14	245±18	249±14	274±21	0.15	0.55
Serine	208±11	194±12	211±12	221±10	0.84	0.39
Threonine	172±8	166±7	182±8	192±8	0.78	0.14
Tyrosine	106±8	100±4	108±6	115±6	0.95	0.09
5-oxoproline	149±14	186±20	174±18	168±14	0.21	0.14
Homocysteine	20.8±5.7	20.2±5.8	21.4±6.1	23.1±6.3	0.14	0.001
5-oxoproline/glutamate	1.06±0.03	1.54±0.18	1.25±0.09	1.20±0.07	0.08	<0.02

N=14. Data are expressed as mean ± SEM. [a]ANOVA or ANCOVA (washout values as covariates) were used where appropriate. Values were log transformed when appropriate. LRC, light roasted coffee; DRC, dark roasted coffee;

FIGURES AND FIGURE LEGENDS

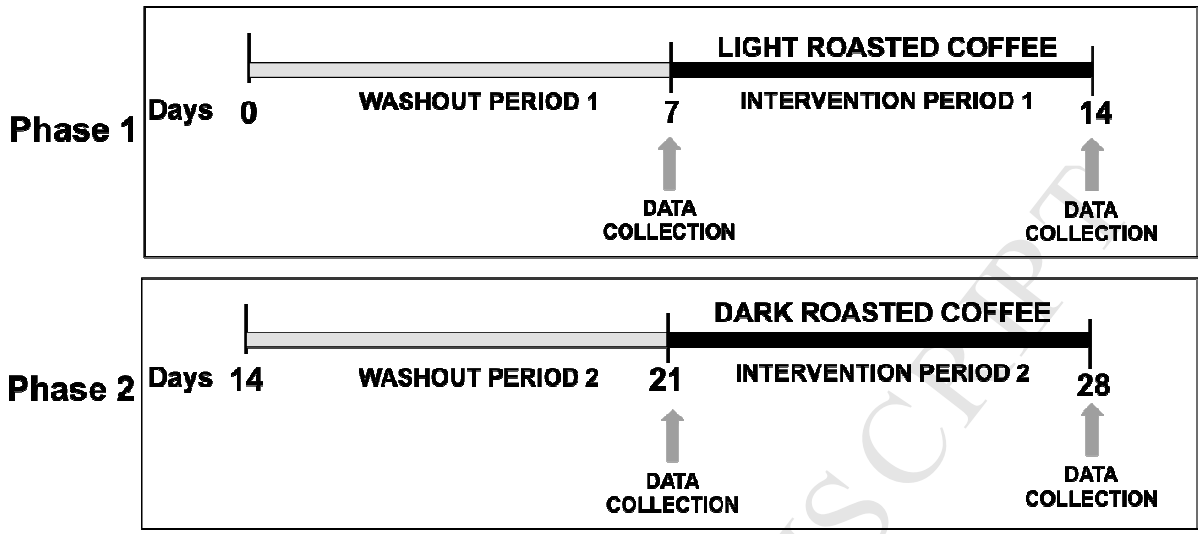

Figure 1. Study protocol.

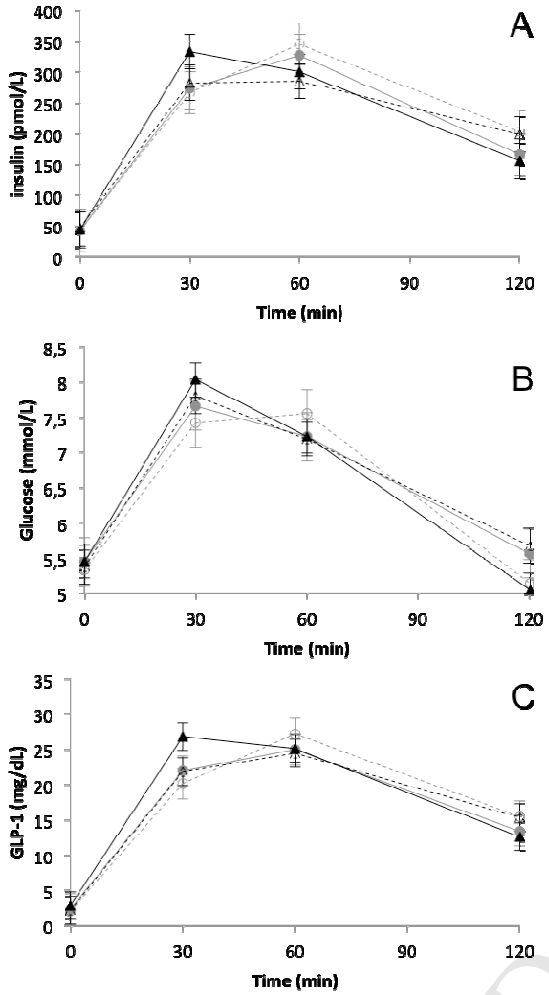

Figure 2. Effect of *Coffea Arabica variety Laurina* coffee at different roasting intensity on glucose metabolism during OGTT.

Plasma insulin (A), glucose (B) and glucagon-like peptide-1 (GLP-1) (C) concentrations.

--△--: washout period before dark roasted coffee (DRC); —▲—: intervention period with DRC;

--○--: washout period before light roasted coffee (LRC); —●—: intervention period with LRC.

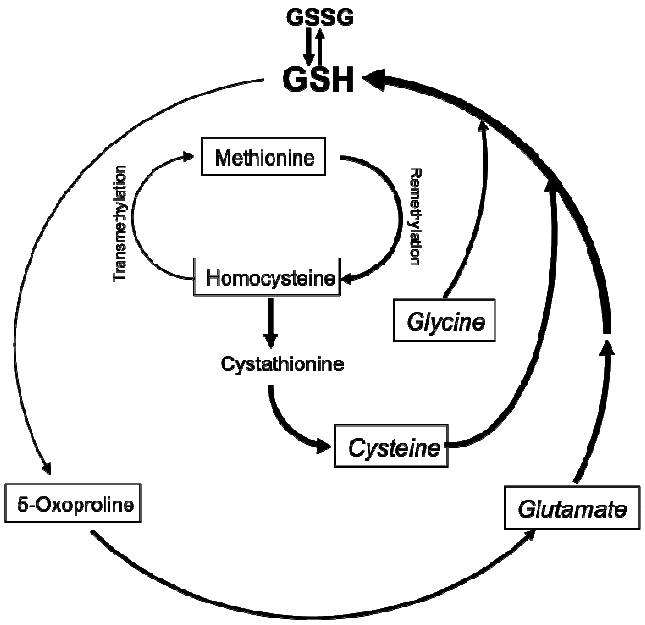

Figure 3. Dark roasted coffee (DRC) effects on the γ-glutamyl cycle.
DRC enhanced the availability of reduced glutathione (GSH). Glycine, cysteine and glutamate are direct GSH precursors. Thick-lines indicate potentially up-regulated metabolic pathways; thin-lines indicate potentially down-regulated precursors.

HIGHLIGHTS

- Long-term coffee intake modifies glucose metabolism and redox balance in humans
- Coffee benefits are most likely mediated by compounds other than caffeine
- Roasting intensity modulates the content of coffee bioactive compounds
- Dark roasted coffee showed greatest metabolic benefits and taste characteristics