Nutrition 32 (2016) 928–936

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

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

Filippo Giorgio Di Girolamo Ph.D., Pharma.D. [a], Sara Mazzucco Ph.D., Pharma.D. [a], Roberta Situlin M.D. [a], Nina Mohorko Ph.D. [b], Zala Jenko-Pražnikar Ph.D. [c], Ana Petelin Ph.D. [c], Marcello Tence M.D. [a], Rado Pišot Ph.D. [b], Luciano Navarini Chem.D. [d], Gianni Biolo Ph.D., M.D. [a,*]

[a] Department of Medical, Surgical and Health Sciences, Clinica Medica AOUTS, University of Trieste, Trieste, Italy
[b] Institute for Kinesiology Research, Science and Research Center of Koper, University of Primorska, Koper, Slovenia
[c] Faculty of Health Sciences, University of Primorska, Izola, Slovenia
[d] illycaffe S.p.A., Trieste, Italy

ARTICLE INFO

Article history:
Received 23 November 2015
Accepted 1 February 2016

Keywords:
Oxidative stress
Roasting intensity
Type-2 diabetes mellitus
Insulin resistance
Coffee
Omega-3 fatty acids

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.

Methods: We performed a double-blind, crossover intervention study. Fourteen healthy male volunteers consumed four cups daily of light roasted coffee (LRC) and dark roasted coffee (DRC), each for 1 wk (intervention period 1 and 2 respectively). One wk washout, with total abstinence from coffee and other possible caffeine sources, preceded each intervention. 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–oral glucose tolerance test, were significantly lower after 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, were significantly greater after DRC than LRC (34.7 ± 25.0 and -18.8 ± 21.0, $P = 0.03$). The initial (30 min) post–oral glucose tolerance test area under the curve 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 ($+1437 \pm 371$ 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 postload 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.

This work is part of a joint project with illycaffe s.p.a. within the framework of the "Nutrigenomica e consumo di caffe: Effetti fisiologici, genetica del gusto e genetica della pianta", partially supported by the POR-FESR 2007-2013 Regione Autonoma Friuli Venezia Giulia, Italy. F.G.D.G. conducted research, analyzed data, performed statistical analysis, and wrote the paper; S.M. conducted research, analyzed data, and performed statistical analysis; R.S. conducted research wrote the paper; N.M. conducted research; Z.J.P. conducted research; A.P. conducted research; M.T. conducted research; R.P. designed research; L.N. designed research and analyzed data; G.B. designed research, analyzed data, performed statistical analysis, wrote the paper, and had primary responsibility for final content. All authors have read and approved the final manuscript. We acknowledge all the subjects for volunteering for these studies; Mariella Sturma, University of Trieste Research Laboratory, for her excellent technical support; and Stefano Mammi and Elisabetta Schievano, University of Padua, for measurement of NMP and niacin content by NMR.

* Corresponding author. Tel.: +39 040 399 4532; fax: +39 040 399 4593.
E-mail address: biolo@units.it (G. Biolo).

http://dx.doi.org/10.1016/j.nut.2016.02.001
0899-9007/

Introduction

Coffee is one of the most widely consumed beverages in the world. Several epidemiologic 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 (T2-DM), 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 T2-DM 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 [14]. Coffee, both with or without caffeine, also contributes to the postprandial insulin secretion by acutely stimulating the release of glucagon-like peptide-1 (GLP-1), an incretin secreted by the small intestine [15,16]; furthermore, coffee contains over a thousand bioactive antioxidant substances, making this beverage a major dietary antioxidant supplier in western countries [17]. Among coffee components, chlorogenic acid, melanoidins, quinides, and N-methylpyridinium (NMP) have clearly shown the potential to affect glucose and insulin metabolism [18–24].

The roasting process has relevant effects on the relative content of coffee's biochemical makeup. 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, whereas that of quinides, NMP, and melanoidins, produced by Maillard reaction, increases [25]. 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 [26]. 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 [19]. NMP has been shown to control oxidative stress through induction of the nuclear factor E2-related factor 2 and the antioxidant response element pathway [23]. The effects of quinides and NMP on glucose metabolism have been poorly investigated in humans. NMP promoted glucose uptake in vitro, and quinides improved insulin action in rats [21,24].

The 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 lasting 2 wk and each inclusive of 1 wk washout followed by a 1 wk intervention period, with the intake of LRC (intervention 1) and DRC (intervention 2) products as four cups daily of espresso coffee.

Materials and methods

Study participants and design

Fourteen healthy male volunteers were recruited (age 39 ± 2 y; body mass index 25.0 ± 0.4 kg/m^2) according to the following inclusion criteria: habitual coffee drinker, absence of chronic and acute illnesses, no pharmacologic 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 [13,27].

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 follow these rules: exclusion of caffeine-containing foods and drinks other than the test coffees, and decaffeinated coffee; maintenance of habitual exercise levels and eating patterns; avoidance of nutrient supplements, herbal products, and medications; and daily compilation of food and exercise logs, checked weekly by the dietitian.

The study lasted 4 wk and was organized as a double-blind, crossover intervention, inclusive of two subsequent phases. Each phase involved a washout period of 7 d, during which the participants abstained from drinking any coffee or caffeinated products, followed by an intervention period (7 d), during which subjects drank four espresso coffees daily (at breakfast, midmorning, after lunch, and in the afternoon), first as LRC and then as DRC (Fig. 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 *C. arabica* varieties. Green coffee beans were roasted at two different intensities, as determined by color measurement (Colorette 3 B, 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 [28,29].

N-methylpyridinium and niacin levels were determined by 1 H-nuclear magnetic resonance. Coffee samples were prepared by adding 50 μL of D_2O, containing 3-trimethylsilyl-[2,2,3,3-2 H_4] propionate as chemical shift reference, to 500 μL of beverage in a 5-mm precision glass NMR tubes (535-pp,Wilmad-LabGlass, Vineland, NJ, USA). A Bruker Avance DMX600 spectrometer (Rheinstetten, Germany), operating at 599.90 MHz for 1 h 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 and using a consensus vocabulary, by a panel of eight experts. Scores (0–7) from testing evaluation were statistically processed (Fizz Network 2.31 G Biosystem, Couternon, France). The two products were described as being significantly (by analysis of variance) different for 8 out of 11 taste and flavor attributes.

Metabolic assessment

An oral glucose tolerance test (OGTT) was performed at the end of each washout and intervention period 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 postabsorptive state −30 min, −15 min, and immediately before the glucose load. Then, each subject received 75 g of glucose

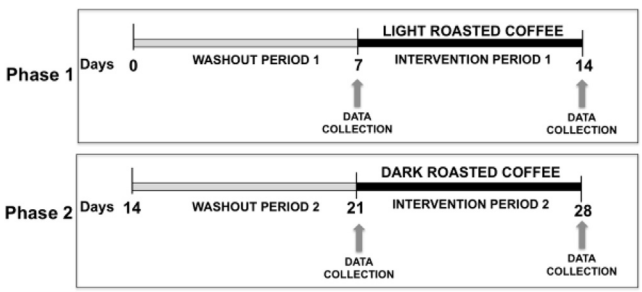

Fig. 1. Study protocol.