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Caffeinated and decaffeinated instant coffee consumption partially reverses high-fat diet-induced metabolic alterations in mice

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ABSTRACT

Epidemiological data has associated coffee consumption with a lower prevalence of type 2 diabetes, metabolic syndrome and chronic liver disease. However, the mechanisms and coffee substances responsible for these effects remain unclear. In the present study, mice received caffeinated or decaffeinated instant coffee *ad libitum* during a two week period after inducing obesity by introducing a high-fat diet over a 10 week period. Body weight, glucose homeostasis, and liver and visceral adipose tissue inflammation were assessed. In addition, AKT signaling, the fatty acid profile and liver histopathological analyses were performed. Ingestion of caffeinated or decaffeinated coffee for 2 weeks resulted in reductions in glucose and insulin blood levels, and insulin tolerance was improved without reductions in final body weight or adiposity. Only caffeinated coffee modified the adipokine profile in visceral adipose tissue, resulting in a restoration of adiponectin levels. However, deleterious liver alterations, which manifested as reductions in steatosis, inhibition of iNOS expression and restoration of insulin inducing-AKT phosphorylation, were reversed by the ingestion of both caffeinated and decaffeinated coffee. Serum AST and ALT levels were also improved in mice after coffee ingestion, while the fatty acid profile in the liver and the cytokine profiles in adipose and liver tissues were not altered by coffee ingestion. Thus, instant coffee consumption reverses obesity-induced alterations in mice, and our results corroborate those of epidemiological studies that associated coffee consumption with a lower prevalence of obesity-related pathologies.

Introduction

Epidemiological studies have demonstrated that coffee consumption decreases the risk of type 2 diabetes (Bhupathiraju et al., 2013; Huxley et al., 2009; van Dam & Hu, 2005). In addition to its effects on type 2 diabetes, coffee consumption may delay the establishment of metabolic syndrome, which is characterized by visceral obesity, decreased plasma HDL cholesterol, hypertriacylglycerolemia, hypertension and hyperglycemia (Hino et al., 2007; Mure et al., 2013). Experimental data from obese mice or rat models fed a high-fat diet also revealed that coffee consumption has a beneficial effect. The ingestion of coffee or a caffeine solution by mice fed a high-fat diet for 5 or 17 weeks improved insulin resistance and lowered inflammatory adipokine production (Matsuda et al., 2011; Yamauchi et al., 2010). The administration of soluble or decaffeinated coffee to mice fed a high-fat diet also reduced body weight, adiposity, hepatic and adipose tissue inflammation (Fukushima, Kasuga, Nakao, Shimomura, & Matsuzawa, 2009). Rats fed a high-fat diet for 4 weeks in combination with decaffeinated coffee or decaffeinated coffee/caffeine in the drinking water displayed increases in insulin sensitivity (Shearer, Sellars, Farah, Graham, & Wasserman, 2007). In summary, previously published experimental studies demonstrated that both caffeinated and decaffeinated coffee ingestion concomitant with obesity induction protected animals from establishing the deleterious effects of a high-fat diet.

Coffee beans contain many substances, including lipids, proteins carbohydrates, vitamins and minerals (Nunes & Coimbra, 2001). In addition, coffee beans are not only abundant in chlorogenic acids but also contain lignans, melanoidins and other Maillard reaction products that have different biological activities (Nunes & Coimbra, 2007; Ramirez-Coronel et al., 2004). However, of all of the constituents of

Abbreviations: AKT, protein kinase B; ALT, alanine aminotransferase; AST, Aspartate aminotransferase; HDL, high-density lipoprotein; HFD, high-fat diet; iNOS, inducible nitric oxide synthase; IL, interleukin; ipGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; LCPUFA, long-chain polyunsaturated fatty acids; MUFA, monounsaturated fatty acid; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PPAR-α, peroxisome proliferator-activated receptor-α; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SREBP-1, sterol regulatory element binding protein-1; TNF-α, tumor necrosis factor-α.

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coffee beans, the biological effects of caffeine have been the most extensively studied. Caffeine acts as an adenosine A_{2A} receptor antagonist, promoting wakefulness (Huang et al., 2005). Caffeine also increases energy expenditure by promoting thermogenesis (Kogure et al., 2002). The biological responses to caffeine alone or the caffeine found in sources other than coffee appear to differ from those that occur when caffeine is consumed as a component of coffee (Tunnicliffe & Shearer, 2008), indicating the importance of studies that employ coffee because it is commonly consumed worldwide.

The probable mechanisms by which coffee consumption exerts its protective effects on the development of metabolic syndrome and type 2 diabetes are diverse. Weight management and alterations of body composition constitute one hypothesis, but little evidence exists to support it (Fukushima et al., 2009; Matsuda et al., 2011; Yamauchi et al., 2010). Coffee consumption-mediated anti-inflammatory responses may contribute to reducing the establishment of metabolic syndrome and have also been associated with decaffeinated coffee (Fukushima et al., 2009). Therefore, we decided to evaluate whether caffeinated and decaffeinated instant coffee consumption over a short-time period of time is able to reverse previously established alterations in obesity.

Materials and methods

Caffeine analysis by HPLC (CLAE-DAD)

The presence of caffeine on the decaffeinated and caffeinated samples was tested by HPLC (CLAE-DAD) analyses. The coffee solution samples (50 μL) were dissolved in 600 μL of methanol and 350 μL of Milli-Q water, and then the samples were filtrated on 0.45 μm membranes.

A Dionex UltiMate 3000 (Thermo Scientific, Germany) liquid chromatography, equipped with a C-18 Atlantis® (Waters, 5 μm, 4.6 × 150 mm) column maintained at 30 °C by a thermostat, was used. The detection was carried out using a UV/VIS (DAD-3000) (Caridi et al., 2007). The solvents were: A (water, 60% v/v) and B (methanol, 40% v/v), with a flow of 1.0 mL/min, in isocratic conditions. The spectra were obtained at 190 and 480 nm and the chromatograms processed at 260 nm.

Animals and experimental design

Male, 6-week-old Swiss mice, free of specific pathogens, were obtained from Centro Multidisciplinar de Investigação Biológica (CEMIB, State University of Campinas, Campinas, São Paulo, Brazil). The experiments were performed in accordance with the principles outlined by the Brazilian College for Animal Experimentation and received approval from the Ethics Committee of São Francisco University, Bragança Paulista, São Paulo, Brazil (001.01.11). The animals were maintained on a 12 h:12 h artificial light–dark cycle and housed individually.

After random selection, the mice were initially divided into two groups, with one group receiving standard rodent diet (control, 15% energy from fat) and the other receiving a high-fat diet (HFD), which was prepared as previously described (DeOliveira et al., 2012). The high-fat diet was composed of: 60% of the energy derived from fat (lard was the primary source of fat), 15% of the energy derived from protein and 25% of the energy derived from carbohydrates. After 10 weeks, a control group (n = 5) and HFD mice (n = 5) were sacrificed to characterize obesity-related alterations. The HFD group was divided in three: one group received caffeinated coffee (HFD + CC, n = 5), one group received decaffeinated coffee (HFD + DC, m = 5) and the other group received water (HFD, n = 5) for the final 2 weeks. Caffeinated and decaffeinated coffee (Nescafe®, Nestle, Brazil) was prepared by dissolving 0.5 g of coffee/100 ml of hot water and was made fresh every day. The rats had *ad libitum* access to caffeinated and decaffeinated coffee in the drinking water. During the intervention period, food and beverage intake was recorded.

Blood glucose level measurements, and insulin- and glucose-tolerance tests

The mice were anesthetized after 6 h of fasting, and blood samples were collected from the tails. Insulin (1.5 U/kg) was administered *via* i.p. injection, and blood samples were collected for serum glucose determinations at 0, 10, 15, 20 and 30 min. The rate constant for glucose disappearance during an insulin tolerance test (K_{ITT}) was calculated using the formula $0.693/t_{1/2}$. The glucose $t_{1/2}$ was calculated from the slope of the least squares analysis of the plasma glucose concentrations during the linear decay phase (Bonora, Manicardi, Zavaroni, Coscelli, & Butturini, 1987). The glucose tolerance test (ipGTT) was performed after 6 h of fasting and after the anesthesia procedure described above had been applied. After collection of a blood sample (time 0), a solution of 20% glucose (2.0 g/kg body weight) was administered into the peritoneal cavity. To determine the serum glucose concentrations, blood samples were collected from the tails at 30, 60, 90 and 120 min. A glucose curve was established, and the area under curve was calculated (DeOliveira et al., 2012).

Necropsy

After 6 h of fasting, mice were anesthetized; small blood samples were collected by cardiac puncture in tubes without anticoagulant and centrifuged to isolate the serum for analyses. Liver, epididymal and perirenal adipose tissues were carefully dissected, weighed and stored for further analyses.

Determination of hepatic enzyme levels in the serum

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the serum were determined using the Cobas-Mira System (Roche Diagnostics, Basel, Switzerland).

Measurements of TNF-α, IL-10, leptin, adiponectin and iNOS

Biopsies of adipose tissue and liver were homogenized in solubilizing buffer at 4 °C [1% Triton X-100, 100 mM Tris–HCl (pH 7.4), 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium orthovanadate, 2.0 mM PMSF and 0.1 mg aprotinin/ml]. The extracts were centrifuged at 15,000 rpm at 4 °C for 45 min. The supernatants were collected and used directly in a commercial ELISA kit to quantify TNF-α, leptin, adiponectin, and IL-10. The supernatant that was collected from liver biopsies was also employed for Western blot experiments. Laemmli buffer (0.1% bromophenol blue, 1 M sodium phosphate, pH 7.0, 50% glycerol, and 10% SDS — sodium dodecyl sodium sulfate) containing 100 mM DTT was added to the supernatants, and the samples were heated in boiling water for 5 min, applied to polyacrylamide gels (SDS-PAGE) and separated using electrophoresis. After electrophoresis, the proteins were transferred to nitrocellulose membranes. The nitrocellulose membranes were incubated with anti-iNOS and anti-β-actin primary antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA) overnight at 4 °C before being developed using commercial chemiluminescence kits (GE Healthcare Bio-Sciences AB, UK). Band intensities were quantitated using optical densitometry (Scion Image software, ScionCorp, Frederick, MD, USA) of the developed autoradiographs (DeOliveira et al., 2012).

Insulin signaling analyses

To evaluate insulin signal transduction in the liver after 6 h of fasting, the mice were anesthetized, and their abdominal cavities were opened. The animals then received a bulk injection of insulin (100 μL, 10^{-6} mol/L) in the portal vein, and after 30 s, fragments of the liver were excised. The fragments were processed as described and Western blot analyses were performed using anti-phospho-Akt

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