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Basic nutritional investigation

Timing of caffeine ingestion alters postprandial metabolism in rats

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ABSTRACT

Objective: The association between caffeine intake and the risk for chronic diseases, namely type 2 diabetes, has not been consistent, and may be influenced by the timing of caffeine ingestion. The aim of this study was to investigate the acute effect of caffeine administered in different scenarios of meal ingestion on postprandial glycemic and lipidemic status, concomitant with changes in body glycogen stores.

Methods: Forty overnight-fasted rats were randomly divided into five groups (meal-ingested, caffeine-administered, post-caffeine meal-ingested, co-caffeine meal-ingested, post-meal caffeine-administered), and tube-fed the appropriate intervention, then sacrificed 2 h later. Livers and gastrocnemius muscles were analyzed for glycogen content; blood samples were analyzed for glucose, insulin, triglycerides, and non-esterified fatty acid concentrations.

Results: Postprandial plasma glucose concentrations were similar between groups, while significantly higher levels of insulin were witnessed following caffeine administration, irrespective of the timing of meal ingestion. Triglyceride concentrations were significantly lower in the caffeine-administered groups. Regarding glycogen status, although caffeine administration before meal ingestion reduced hepatic glycogen content, co- and post-meal caffeine administration failed to produce such an effect. Muscle glycogen content was not significantly affected by caffeine administration.

Conclusions: Caffeine administration seems to decrease insulin sensitivity as indicated by the sustenance of glucose status despite the presence of high insulin levels. The lower triglyceride levels in the presence of caffeine support the theory of retarded postprandial triglyceride absorption. Caffeine seems to play a biphasic role in glucose metabolism, as indicated by its ability to variably influence hepatic glycogen status.

Introduction

Coffee has attracted considerable attention in the field of nutritional sciences, where an increasing body of evidence supports a protective role of habitual consumption on risk factors of non-communicable diseases, namely type 2 diabetes [1,2]. There has been much debate, however, over the effect of coffee on glucose and insulin responses, with some studies suggesting no postprandial changes [3–6], whereas others report significant increases in postprandial glycemic and insulinemic responses [7,8]. Coffee contains numerous bioactive compounds that may influence glucose and insulin homeostasis, including chlorogenic acid [9] and quinides [10], as well as the mineral magnesium [11]. Coffee, however, also contains caffeine, a methylxanthine that has been found to impair glucose tolerance and insulin sensitivity among healthy individuals and those with type 2 diabetes in short-term metabolic studies [12–14], as well as longer-term studies [15,16].

Liver and muscle glycogen are the main reservoirs of stored glucose in the body, acting as the key suppliers of glucose to maintain homeostasis in the body [17]. Caffeine has the ability to influence body stores of glycogen, although results have been conflicting given caffeine's simultaneous ability to prevent and induce glycogen breakdown through its varying role on different enzymatic systems in the liver and muscle. On the one hand, caffeine has been found to be a strong inhibitor of hepatic and skeletal muscle glycogen phosphorylase, the enzyme responsible for glycogenolysis [18]; competing for the binding of glycogen phosphorylse a in a synergistic manner [19,20]. On the other hand, caffeine has been documented to stimulate the

OAO designed the study and performed the statistical analysis. SFJ performed the biochemical analytical methods. Both authors conducted the research experiment and wrote the paper.

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sympathetic nervous system [21], leading to increased release of catecholamines (epinephrine and norepinephrine), which are known to stimulate glycogenolysis [22].

There is controversy surrounding the effects of caffeine on glycemic control and glycogen sparing, and there is limited evidence of the effect of caffeine on glycemic changes concomitant with changes in body glycogen stores. The aim of this study was to examine the effect of a moderate dose of caffeine on blood glucose and insulin levels, concomitant with changes in liver and skeletal muscle glycogen content, as well as blood triglyceride (TG) and non-esterified fatty acid (NEFA) levels in different scenarios of the ingestion of a control meal. Because most dietary sources of caffeine are habitually consumed in circumstances where food may be present, investigating the effect of caffeine with, before, and after a meal may, therefore, shed some light on whether caffeine leads to different physiological effects on glycemic control, while explaining the results in the context of changes in hepatic and muscle glycogen stores and other biochemical markers.

Materials and methods

Animal housing

Forty 6-wk-old male Sprague-Dawley rats (Animal House, American University of Beirut, Lebanon) were individually housed in separate wire-bottomed cages in a temperature-controlled (22°C ± 1°C) and light-controlled room (12:12 dark/light cycle; light on at 0700 h) for 1 wk. During this period, rats were allowed free access to tap water and ad libitum intake of a semi-synthetic powder control meal adapted from an earlier study [23] (Table 1), and both body weight and food intake was monitored.

Experimental protocol

The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of the medical school at the American University of Beirut. On the experimental day, overnight-fasted rats (to eliminate the effect of residual food in the stomach on postprandial metabolism), with a mean body weight of 222 ± 3.09 g, were randomly divided into five groups (n = 8) and tube-fed the appropriate intervention as follows: the meal-ingested group (ML) received 1 g of the control meal; the caffeine-administered group (CAF) received 10 mg caffeine; the post-caffeine meal ingested group (CAF-ML^{+1}) received 10 mg caffeine followed 1 h later by 1 g of the control meal; the co-caffeine meal-ingested group (ML+CAF) simultaneously received 1 g of the control meal and 10 mg of caffeine; and the post-meal caffeine-administered group (ML-CAF^{+1}) received 1 g of the control meal followed 1 h later by 10 mg of caffeine. The 1 g of the control meal and 10 mg of caffeine were individually mixed in 3 mL of water before each administration to each of the rats for better dissolution, providing equal volumes of feedings. The 10 mg of caffeine supplemented is equivalent to around 45 mg/kg body weight in rats.

Two hours after the experimental intervention, rats were sacrificed and blood was drained from the neck vessels into tubes containing ethylenediaminetetraacetic acid (EDTA) and regular plasma tubes with no EDTA. Blood samples were kept on ice until they were centrifuged for plasma collection within 2 h then were subsequently frozen at −80°C until time of analysis. Livers and gastrocnemius muscles were removed and frozen in liquid nitrogen then stored at −70°C until analysis [23].

Table 1
Composition of the semisynthetic control meal

Ingredients	Quantity (g/kg)
Sucrose	300
Cornstarch	300
Casein	198
Corn oil	100
Alphacel (cellulose)	55
Mineral mix (AIN-93G)*	35
Vitamin mix (AIN-76A)*	10
DL-methionine	2

* Purchased from Dyets Inc. experimental diets & ingredients for laboratory animals.

Analytical methods

Blood was collected at the time of sacrifice and kept on ice, then centrifuged at 3000*g* for 15 min at 4°C. Plasma was collected and frozen at −80°C until analysis. Glucose and TG concentrations were determined using an enzymatic colorimetric method on the Vitros DT 60 II Chemistry System (Ortho-Clinical Diagnostics, Johnson and Johnson, New York, NY, USA). Insulin concentration was determined using the Rat/Mouse Insulin Elisa Kit 96-well plate for the quantification of non-radioactive insulin (Millipore, Billerica, MA, USA), whereas NEFA concentration was determined using an in vitro enzymatic colorimetric method assay via the NEFA-HR(2) diagnostic kit (Wako diagnostics, Neuss, Germany). Hepatic glycogen extraction took place following standard methods described in detail elsewhere [24,25]. Gastrocnemius muscle glycogen extraction followed the process whereby whole muscles were weighed and mechanically homogenized in a Bench-Top PRO Scientific 300 homogenizer (Oxford, CT, USA) with 5 mL 0.5 M perchloric acid at high speed for a few minutes. Extracts (5 mL) were precipitated with 0.8 mL 0.2M potassium hydroxide. Samples were then centrifuged at 3000*g* for 10 min. Muscle extracts of 5 mL were then transferred to individual conical tubes where 10 mL ethanol (96%) was added on ice. The obtained solutions were vortexed and then placed overnight in the refrigerator to allow for precipitation. The steps that followed were similar to those for hepatic glycogen extraction.

Statistical analysis

Data were analyzed using the statistical program Mini-Tab 16.1 for Windows. Statistical treatment of the data included unpaired *t* test for comparing results of group ML with the CAF group and the CAF-ML^{+1} group, while one-way analysis of variance (ANOVA) was used to compare results of the ML group with the ML+CAF and ML-CAF^{+1} groups because all retained the meal for 2 h. A probability of $P < 0.05$ was considered statistically significant. Results are displayed as mean ± SEM.

Results

Body weights and food intake of rats were similar among the different groups, indicating consistent growth and weight gain before the experimental day.

Effect of post-caffeine meal ingestion

The effect of caffeine administration alone on postprandial metabolism was assessed by comparing the ML and CAF groups (Table 2). Plasma glucose concentration was similar between the two groups, whereas plasma insulin concentration of the CAF group was significantly higher than that of the ML group ($P = 0.005$). Plasma NEFA levels were similar between groups, although the CAF group showed slightly, but not significantly, higher levels than the ML group. Moreover, the CAF group showed significantly lower plasma TG levels than the ML group ($P = 0.005$). Mean liver weights of the ML group were higher than that of the CAF group ($P = 0.03$). Likewise, hepatic glycogen contents per gram in the ML group were significantly higher than the CAF group ($P = 0.002$). No significant differences were observed in terms of muscle weight and glycogen contents between the two groups.

The effect of meal ingestion 1 h after caffeine administration (post-caffeine meal ingestion) on postprandial metabolism was assessed by comparing groups CAF and CAF-ML^{+1}. Measured plasma metabolites, organ weights, and glycogen contents were similar between groups. Thus, meal ingestion after caffeine administration failed to alter postprandial metabolism.

Effect of co- and post-meal caffeine administration

The effect of caffeine administration co- and post-meal ingestion on post-prandial metabolism was assessed by comparing groups ML, ML+CAF, and ML-CAF^{+1} (Table 3). Plasma glucose concentration was similar between groups, indicating that caffeine administration co- and post-meal failed to alter