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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1694 – 1698
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Metabolic and hormonal effects of caffeine: randomized, double-blind, placebo-controlled crossover trial

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Received 13 March 2007; accepted 16 July 2007

Abstract

In short-term studies, caffeine has been shown to increase insulin levels, reduce insulin sensitivity, and increase cortisol levels. However, epidemiological studies have indicated that long-term consumption of beverages containing caffeine such as coffee and green tea is associated with a reduced risk of type 2 diabetes mellitus. There is a paucity of randomized studies addressing the metabolic and hormonal effects of consuming caffeine over periods of more than 1 day. We evaluated the effect of oral intake of 200 mg of caffeine taken twice a day for 7 days on glucose metabolism, as well as on serum cortisol, dehydroepiandrosterone (DHEA), and androstenedione, and on nighttime salivary melatonin. A double-blind, randomized, placebo-controlled crossover study with periods of 7 days and washouts of 5 days comparing caffeine with placebo capsules was conducted. Participants were 16 healthy adults aged 18 to 22 years with a history of caffeine consumption. Blood samples from each subject were assayed for glucose, insulin, serum cortisol, DHEA, and androstenedione on the eighth day of each period after an overnight fast. Nighttime salivary melatonin was also measured. Insulin levels were significantly higher (by 1.80 μU/mL; 95% confidence interval, 0.33-3.28) after caffeine intake than after placebo. The homeostasis model assessment index of insulin sensitivity was reduced by 35% (95% confidence interval, 7%-62%) by caffeine. There were no differences in glucose, DHEA, androstenedione, and melatonin between treatment periods. This study provides evidence that daily caffeine intake reduces insulin sensitivity; the effect persists for at least a week and is evident up to 12 hours after administration.
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1. Introduction

Caffeine is an ingredient of many widely used beverages and foods, including coffee, tea, many soft drinks, and chocolate. The average consumption of caffeine in the United States is 170 mg/d [1], the equivalent of about 350 mL of brewed coffee. One reason for the popularity of caffeine-containing beverages is the stimulation of the central nervous system that they provide [1].

However, caffeine may have other effects, including metabolic and hormonal ones. With short-term dosing, caffeine has been shown to impair glucose metabolism in nondiabetic persons [2-5] and in persons with type 2 diabetes mellitus [3,6,7]. The effects of dosing over more than 1 day have been less well studied, although at least one investigation of decaffeinated vs caffeinated coffee suggested that caffeine consumed over more than 1 day also deteriorates glucose metabolism [8].

Effects on other hormonal systems have not been as well investigated. However, cortisol levels may increase after short-term administration of caffeine in healthy subjects [9-12] or in those with elevated blood pressure [13,14], although this effect was not seen in habitual coffee drinkers [15,16] or in habitual smokers [17]. Although it is known that caffeine induces wakefulness by blocking adenosine receptors, the effect of caffeine on melatonin levels is unclear [18-22].

The purpose of our study was to examine the effect of 1 week of moderate caffeine intake on fasting glucose

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.07.013

metabolism in healthy young adults. In addition, we evaluated effects on salivary melatonin and serum levels of adrenocortical hormones.

2. Material and methods

2.1. Study design

We conducted a double-blind, placebo-controlled, randomized crossover trial among healthy, nonsmoking, nonpregnant students 18 years or older. To ensure that subjects could comfortably complete the study, we required each participant to have a history of tolerance to caffeinated beverages or antifatigue caffeine tablets and no history of cardiac arrhythmia or seizures. Subjects were recruited from the student body at Dartmouth College through e-mail announcements and posters. Each subject signed an informed consent document, which was approved together with the protocol by the Institutional Review Board at Dartmouth-Hitchcock Medical Center.

Consenting participants were randomized to one of two sequences: placebo followed by caffeine or caffeine followed by placebo. The randomization list was completed using a random number generator. The active agent phase of each sequence consisted of taking a 200-mg tablet of caffeine twice (total of 400 mg) daily for 7 days. The placebo phase consisted of taking an identical placebo on the same schedule.

All participants made 5 visits. At the first visit (day −5), eligibility was confirmed, participants gave informed consent, and baseline caffeine intake was assessed using a questionnaire. Subjects were asked to abstain from all products containing caffeine for the subsequent 5 days. A list of caffeine-containing products was provided to aid in this effort. At visit 2 (day 0), after the 5-day washout, patients were randomized to one of the two sequences. They were given a 7-day supply of study capsules and instructed to take one capsule twice daily—one between 8:00 and 10:00 AM and one between 4:00 and 6:00 PM. They were again instructed to refrain from caffeine-containing products and were asked to return 7 days later in the morning after an overnight fast. At visit 3 (between 8:00 and 9:00 AM of day 7), participants had blood drawn for measurement of study end points and were instructed to return 5 days later while abstaining from all caffeine-containing products. At visit 4 (day 11), after the second washout period, patients were entered on another 7-day study period, conducted as in the first. This was completed at visit 5, when patients returned between 8:00 and 9:00 AM on day 18 for phlebotomy. Subjects and study personnel were blind to study agent assignments.

Between midnight and 2:00 AM of day 7 of each crossover cycle, study staff obtained saliva samples for melatonin measurement from each subject in his/her regular bedroom. Subjects were instructed to remain in their bedrooms between midnight and 1:00 AM and to keep the room dark. Study staff obtained the specimens in the darkened room, using a flashlight for minimal illumination, to avoid short-term decreases in melatonin [23].

2.2. Study end points

Nighttime saliva samples were assayed for melatonin. All other end points were measured on serum drawn on the morning of the eighth day of each of the 2 periods, after an overnight fast. The study end points related to glucose metabolism were fasting serum insulin and blood glucose, the ratio of insulin to glucose, and the homeostasis model assessment (HOMA) indices HOMA%S and HOMA%B [24-26]. Glucose and insulin were measured twice, 10 minutes apart, at each blood draw session; and the means of these 2 measurements were used in all analyses involving those analytes. Calculations for the 2 HOMA indices were performed using software downloaded from the Web site http://www.dtu.ox.ac.uk. The other end points were serum cortisol, dehydroepiandrosterone (DHEA), and androstenedione.

2.3. Laboratory methods

Fasting blood glucose was measured by an enzymatic hexokinase method using a Hitachi 917 chemistry analyzer (Roche Diagnostics, Indianapolis, IN). The dynamic range of measurements is 2 to 750 mg/dL (0.11-41.6 mmol/L), with a reference range for adults of 74 to 106 mg/dL (4.11-5.89 mmol/L). The interassay coefficient of variation (CV) is <2% for control sera containing from 118 to 253 mg/dL.

Androstenedione was assayed by solid phase radioimmunoassay (Diagnostic Products, Los Angeles, CA) with an analytical detection limit of 0.04 ng/mL and a dynamic range of 0.04 to 10 ng/mL. Manufacturer-supplied quality controls (2 levels) were tested in each assay. Specimens and quality control sera were tested in duplicate. The intraassay CV for all specimens was <5%. Based upon the quality control sera, the interassay CV was 8.9% and 5.7% for androstenedione levels of 1.05 and 4.93 ng/dL, respectively. The reference range is 0.8 to 3.0 ng/mL for reproductively healthy adult men and 0.5 to 3.7 ng/mL for adult women.

Dehydroepiandrosterone was also assayed by double antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX) with an analytic detection limit of 0.009 ng/mL and a dynamic range from 0.009 to 30 ng/mL. Manufacturer-supplied quality controls (3 levels) were tested in each assay. Specimens and quality control sera were tested in duplicate. The intraassay CV for all specimens was <5% (response). Based upon the quality control sera, the interassay CV is 6.9%, 8.6%, and 3.8% for DHEA levels of 1.02, 2.56, and 9.68 ng/mL, respectively. The reference range for adult men is 1.4 to 12.5 ng/mL.

Serum insulin and cortisol levels were measured using a fully automated chemiluminescent immunoassay system (Immulite, Diagnostic Products). The assay had a detection

Table 1
Baseline characteristics of subjects

	Mean (SD) or percentage (n)
Female	50% (8)
Age (y)	20.5 (1.2)
Height (m)	1.70 (0.10)
Weight (kg)	76.2 (12.8)
BMI (kg/m^2)	25.6 (3.6)
Self-reported intake of	
Caffeine (mg/wk)	358 (434)
Alcohol (drinks/wk)	13.3 (14.6)

BMI indicates body mass index.

limit of 0.2 μg/dL and a dynamic range of 0.2 to 50 μg/dL. The interassay CVs were 11.8%, 7.3%, and 5.8% for control sera containing from 4.5, 12.2, and 35.0 μg/dL, respectively. Daytime levels of cortisol in adults are 5 to 25 μg/dL by this method. The limit of detection of the insulin assay is 2 μIU/mL, and the dynamic range of the assay is 2 to 300 μIU/mL. The interassay CV was 5.5% and 4.7% for control sera containing 10.8 and 45.5 μIU/mL, respectively. The upper 95% confidence limit for apparently healthy, fasting volunteers was 28.4 μIU/mL.

Melatonin was assayed by a direct saliva enzyme-linked immunosorbent assay (Salimetrics, State College, PA). The assay had a range of sensitivity of 1 to 81 pg/mL, with a CV of 5.6%.

2.4. Statistical methods

The method of generalized estimating equations was used to model the effect of the study agent, the effects of period, and the interaction of study agent and period, with subjects specified as the cluster. There were no significant ($P > .05$) interactions (indicating no evidence of carryover effects [27,28]) or significant period effects for any of the end points. The point and interval estimates and P values estimated from models with period effects were very similar to those obtained from the raw means and the results of the paired t tests, and we report the results of the latter. Analyses were performed using the statistical software R.1.1.1 [29].

3. Results

Sixteen participants completed the study out of 20 randomized. Three subjects dropped out during the first treatment phase because they could not attend the scheduled blood draws, whereas one did not complete the second treatment phase because of an acute illness unrelated to the study protocol. Table 1 presents a description of the 16 subjects who completed the study. Half of the participants were female, and the mean age was 20.5 years (range, 18-22 years). All reported regular use of more than 200 mg/d of caffeine at baseline, with a mean of 358 mg daily. There were no significant carryover effects for any of the end points.

Mean fasting insulin was significantly higher after caffeine intake than after placebo (8.5 vs 6.7 μU/mL, P for difference = .02) (Table 2). Mean fasting glucose was also higher after caffeine, but the difference was not statistically significant. The mean HOMA index of insulin sensitivity was significantly lower during the caffeine phase.

Cortisol levels were about 12% higher after caffeine than after placebo, a nonsignificant difference. Treatment differences for androstenedione and DHEA were even smaller. Nighttime salivary melatonin was also similar in the 2 phases of the study (Table 2).

4. Discussion

In this randomized crossover trial, we found that 1 week of 200 mg of caffeine consumed twice a day (approximately equivalent to drinking 2 mugs of coffee per day) resulted in decreased insulin sensitivity in young adults. We found no effects of caffeine on serum cortisol, androstenedione, or DHEA, or on salivary melatonin.

Previous randomized studies have documented that a single dose of caffeine (3-5 mg/kg) causes some degree

Table 2
Mean (SD) of the metabolic and hormonal end points after 7 days of caffeine or placebo and an overnight fast[a]

	Placebo	Caffeine	Difference	95% CI	P
Glucose metabolism					
Serum insulin (μU/mL)	6.72 (2.64)	8.53 (2.34)	1.80	0.33 to 3.28	.020
Serum glucose (mg/dL)	92.9 (5.43)	94.4 (5.08)	1.4	−1.6 to 4.5	.33
HOMA%S	130.8 (51.2)	96.0 (26.8)	−34.8	−62.2 to −7.2	.017
HOMA%B	80.1 (20.2)	92.6 (18.6)	12.5	+0.3 to 24.6	.045
Steroid hormones					
Serum cortisol (μIU/mL)	11.8 (5.3)	13.5 (5.3)	1.8	−1.9 to 5.4	.32
Serum androstenedione (ng/mL)	3.02 (0.72)	2.77 (0.71)	−0.24	−0.59 to 0.10	.15
Serum DHEA (ng/mL)	265 (127)	275 (123)	10	−17 to 38	.43
Pineal hormone					
Nighttime salivary melatonin (log pg/mL)	1.57 (1.10)	1.46 (1.09)	−0.11	−0.83 to 0.61	.75

The means are based on the 16 observations from the placebo phase and 16 observations from the caffeine phase. The P values are from paired t tests. There were no significant period or carryover effects. CI indicates confidence interval.

[a] Melatonin measured at night.

of insulin resistance [2,4-8,30-32] and glucose intolerance [3,5-7,33,34] over the subsequent 2 to 3 hours, although one investigation reported a tendency to decreased insulin levels [35]. Our findings document that this effect remains after 7 days of caffeine and persists even after an overnight fast, when subjects could conceivably be experiencing some caffeine withdrawal. Previous investigations that evaluated the effects of caffeine or coffee consumed over multiple days [36-38] did not find an increase in glucose levels, although one of these studies [37] reported higher fasting insulin levels after 2 weeks of caffeine consumption, indicating decreased insulin sensitivity.

It has been hypothesized that the effect of caffeine on glucose metabolism is mediated by an increase in epinephrine release [4]; but a recent study concluded that there are other, unidentified mechanisms [39]. One such mechanism might involve increased levels of free fatty acids associated with caffeine intake, which can lead to insulin resistance [40].

The interplay between caffeine and beverages containing caffeine, such as coffee and green tea, and their effects on glucose metabolism is unclear. Consumption of green tea has been associated with reduced risk of diabetes [41]. There is also consistent evidence from observational studies that coffee intake is associated with a reduced risk of diabetes mellitus and improved glucose metabolism [42,43]. One possible explanation for the discrepancy between the epidemiological findings and the experimental studies could be an antidiabetogenic effect of the chlorogenic acid in coffee, a polyphenol that is degraded into quinides, which have been reported to increase insulin sensitivity in rats [42]. There is some evidence that the beneficial association of habitual coffee consumption with glucose metabolism is unrelated to caffeine [44]. Decaffeinated coffee may have a short-term hypoglycemic effect [5], and habitual consumption of decaffeinated coffee may lower the risk of diabetes more than the caffeinated coffee consumption [45]. Of course, it is also possible that some other characteristic of coffee drinkers might explain the decreased risk of diabetes or that the effects seen in the randomized studies do not persist over periods of years.

Previous studies have reported that caffeine causes short-term increases of cortisol levels [9-11,46] in healthy subjects. We observed a modest, nonsignificant increase after 7 days of caffeine. The lack of substantial difference between the treatments could be due to the development of tolerance. However, the confidence limits around our estimate of caffeine's effect on cortisol are compatible with the reported short-term effects; so our negative results could simply be the result of lack of statistical power. We found no effect of caffeine on DHEA or androstenedione.

We found a small nonsignificant decrease in nighttime melatonin levels. Significant decreases in nighttime melatonin were observed after short-term caffeine intake in 3 randomized studies [18,20,22], whereas a significant increase was observed in another [21] and a pharmacokinetic study found that caffeine increased the bioavailability of oral melatonin [47]. Caffeine is known to cause wakefulness by blocking adenosine receptors, but it remains unclear if a second reason for the wakefulness is a decrease in melatonin levels [19].

Our trial has the advantage of a randomized, crossover design, which permitted an efficient assessment of the effects of caffeine. However, the study was limited to healthy young adults and examined end points after fasting (or at night for melatonin), not throughout the day. It does not address the effects of habitual caffeine intake over more than 1 week. It is conceivable that the differences in glucose metabolism that we observed were due to the effect of caffeine withdrawal in the placebo period: caffeine withdrawal has been estimated to peak at 20 to 51 hours after cessation of intake and have an entire duration of 2 to 9 days [48]. Subjects in the placebo periods of our study had had no caffeine for 10 days at the time of insulin measurement and so were unlikely to be affected by withdrawal. Another limitation of our study was the lack of plasma caffeine measurements to document compliance of subjects with the protocol. Furthermore, there was no control for exercise in our protocol, although there is no reason to believe there was an imbalance across treatment arms.

In summary, caffeine has adverse effects on insulin resistance that are maintained for at least a week. Further study is needed to study longer-term effects and to clarify the differences between findings from intervention studies such as ours and those from epidemiological studies suggesting that chronic caffeine consumption may have a protective effect on the risk of diabetes mellitus.

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