Consumption of caffeinated coffee and a high carbohydrate meal affects postprandial metabolism of a subsequent oral glucose tolerance test in young, healthy males

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Caffeine and caffeinated coffee (CC) elicit acute insulin insensitivity when ingested before a carbohydrate load. The effects of CC on glucose tolerance and insulin sensitivity when co-ingested with a high carbohydrate meal and on postprandial metabolism of a subsequent (second) carbohydrate load have not been studied. In a randomised, crossover design, ten healthy males ingested either CC (5 mg caffeine/kg body weight), decaffeinated coffee (DC) or water (W; equal volume) co-ingested with a high glycaemic index cereal followed 3 h later by a 75 g oral glucose tolerance test. After the initial meal, insulin area under the curve (AUC) and insulin sensitivity index did not differ between treatments, although glucose AUC for CC (107 (SEM 18) mmol/l × 3 h) and DC (74 (SEM 15) mmol/l × 3 h) was greater than W (-0.2 (SEM 29) mmol/l × 3 h, $P < 0.05$). After the second carbohydrate load, insulin AUC for CC was 49 % and 57 % greater ($P < 0.01$) than for DC and W, respectively. Despite the greater insulin response, glucose AUC for CC (217 (SEM 24) mmol/l × 2 h) was greater than both DC (126 (SEM 11) mmol/l × 2 h, $P = 0.01$) and W (55 (SEM 34) mmol/l × 2 h, $P < 0.001$). Insulin sensitivity index after the second meal was lower after CC (8.2 (SEM 0.9)) compared with both DC (12.4 (SEM 1.2), $P < 0.01$) and W (13.4 (SEM 1.4), $P < 0.001$). Co-ingestion of CC with one meal resulted in insulin insensitivity during the postprandial phase of a second meal in the absence of further CC ingestion. Thus, CC may play a role in daily glycaemic management.

Coffee: Caffeine: Insulin sensitivity: Oral glucose tolerance test

Caffeine (1,3,7-trimethylxanthine) is the world's most widely consumed psychoactive substance and it is estimated that 60–75 % of total caffeine intake comes from coffee in North American adults[1]. Epidemiological evidence consistently suggests that heavy, habitual coffee consumption (at least 3–4 cups of coffee per day) may be protective against the development of type 2 diabetes (T2DM)[2–7]. While such findings could support the inclusion of coffee in dietary guidelines for individuals with or at risk of developing T2DM, a number of investigations have found either caffeine[8–12] or caffeinated coffee (CC)[12,13] to produce an acute insulin insensitive environment when consumed before ingesting carbohydrate. We have shown that CC, when ingested 1 h before a carbohydrate meal, elicits 147 and 29 % greater area under the curves (AUC) for glucose and insulin, respectively, in comparison with decaffeinated coffee (DC) in healthy males[13]. Lane *et al.*[14] have recently reported that consuming CC together with a liquid meal resulted in greater blood insulin and glucose concentrations than when DC was consumed. Given that the half life of caffeine is 4–6 h[15], it is conceivable that it could have metabolic actions well beyond those of a first meal. While previous studies have investigated the acute effects of caffeine and/or coffee consumed 1 h before carbohydrate ingestion, to our knowledge, there are no studies that have investigated the effects of CC on glycaemic control when co-ingested with a meal. Furthermore, the prolonged metabolic effects of coffee consumption beyond one meal have not been investigated.

The objectives of the present study were: (1) to determine the effect of caffeinated and DC on glucose tolerance and insulin sensitivity when co-ingested with a carbohydrate meal, and (2) to determine whether prolonged effects of CC on glucose management exist after ingestion of a second carbohydrate load in the form of an oral glucose tolerance test (OGTT). We hypothesised that ingestion of CC with a carbohydrate meal would negatively affect blood glucose management after both the initial and subsequent carbohydrate loads.

Experimental methods

Subjects

Subjects were recruited through the use of poster advertisements on the University of Guelph campus. Males between 18 and 50 years of age were candidates for the study and participants were excluded if any of the following criteria were present: (1) known diagnosis of diabetes or impaired glucose tolerance, (2) fasting blood glucose > 6.0 mmol/l,

(3) use of any medications known to alter glucose metabolism, or (4) BMI ≤ 18.5 or $> 30 \, \text{kg/m}^2$. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Guelph Human Research Ethics Board. Written informed consent was obtained from all subjects. In a preliminary session, all participants were given instructions for completing dietary records and were provided with a list of substances they could not consume 48 h before each trial. In addition, participants wore light clothing and removed their shoes to allow weight (in kg) and height (in cm) to be measured. Upon completion of the study, subjects received a financial honorarium.

Experimental design

The study followed a randomised, crossover design with each subject receiving three different study treatments on separate days separated by 1 week. Subjects (n 10) were required to ingest a meal (79·1 g Kellogg's Crispix® (Kellogg Canada Inc., London, ON, Canada) mixed with 150 ml skim milk (William Neilson Ltd, Halton Hills, ON, Canada) calculated to have a mixed meal glycaemic index of 81 using a methodology described elsewhere[16]) providing 75 g available carbohydrate along with either CC, DC or water (W). Available carbohydrate was calculated by difference, thus the amount of dietary fibre present in the mixed meal was subtracted from the total carbohydrate content; the proportions of cereal and milk provided ensured our mixed meal provided 75 g available carbohydrate. At 3 h postmeal ingestion, subjects underwent a 2 h OGTT designed to simulate consumption of a second, high carbohydrate meal. For the OGTT, subjects were required to consume an oral glucose drink (TRUTOL®, Custom Laboratories Inc., Baltimore, MD, USA) providing 75 g carbohydrate in the form of dextrose. In association with the first meal, subjects received either a volume of CC (Maxwell House Original Roast®, Kraft Canada, Don Mills, ON, Canada) that was calculated to provide 5 mg caffeine/kg body weight, the equivalent volume of DC of the same brand or W. Previously, our laboratory has established a method of preparing drip-filtered coffee with a known caffeine concentration[17]. Preparation of coffee utilising this method yields 62·1 mg caffeine per 100 ml of brewed coffee. Before the present experimental trials, we determined the exact volume of CC (and subsequent volume of DC and W) for each subject. The volume of coffee ingested by each subject during the trials ranged from 535 to 812 ml.

The principal researchers were blinded to all three experimental treatments, but the subjects were not blinded to the W treatment. Each trial was separated by 1–2 weeks and for a given subject, each trial was conducted at the same time of the day. For 48 h before each trial, subjects were required to abstain from exercise and consumption of any alcohol or caffeine-containing products, and to maintain thorough dietary records. After the first trial, subjects were asked to ingest the same diet (based on their initial 2-d dietary records) during the 2 d before all subsequent trials. To help facilitate compliance, subjects were provided with the same dinner (sandwich) and snack (granola bar) the day and evening before each of the trials. Subjects reported to the laboratory the following morning after a 10–12 h overnight fast.

Dietary records were later analysed for macronutrient composition consumed before each experiment.

Experimental protocol

A medically trained technician inserted a Teflon catheter into an antecubital vein and kept it patent with a normal saline infusion. A fasting venous blood sample was taken (time = -13 min), immediately after which subjects were required to ingest the first meal together with the test beverage. The beverage was ingested in two equal amounts: subjects drank the first half of the beverage, ingested the meal and then consumed the rest of the beverage. During the first trial, the feeding time was recorded and subjects were required to consume the first meal in the same amount of time in all subsequent trials. It took subjects an average of 13 min to ingest the first meal, and thus the initial baseline time point was denoted by -13 min. Subsequently, a second blood sample ($t = 0$) was taken and further samples were obtained at 15, 30, 45, 60, 90, 120, 150 and 180 min. In 3 h postmeal ingestion ($t = 180$ min), subjects ingested the second meal within a 5 min period. Blood samples were then taken for a further 2 h (185, 200, 215, 230, 245, 275 and 305 min).

Laboratory analysis

At each time point, approximately 8 ml blood was withdrawn and partitioned for analysis of whole-blood glucose, serum insulin, serum C-peptide, serum NEFA and plasma methylxanthines. At each blood collection time point, approximately 2 ml was drawn into a sodium heparinised tube and immediately analysed for blood glucose using a glucose oxidase method (YSI 2300 Stat Plus Glucose Analyzer, Yellow Springs, OH, USA). A 200 μl aliquot of heparinised blood was centrifuged at room temperature for 10 min at 1200 g, and plasma was stored at $-80°C$ for later analysis of methylxanthines by HPLC[18]. Another 3 ml blood was drawn into a separate tube with no additive. This blood was allowed to clot at room temperature and then centrifuged at 1200 g for 10 min at room temperature. Serum supernatant was aliquoted and frozen at $-20°C$ for subsequent insulin (Intermedico 'Coat-a-count' RIA Kit, Diagnostic Products Corporation, Los Angeles, CA, USA) and NEFA analysis (NEFA kit, Wako Bioproducts, Richmond, VA, USA). An additional 3 ml blood was partitioned into a non-treated tube containing 80 μl aprotinin (a trypsinogen inhibitor). This tube was kept at room temperature for 30 min to allow the blood to clot after which it was centrifuged (1200 g) at room temperature for 10 min. The serum obtained from this sample was stored at $-20°C$ for subsequent C-peptide analysis (Human C-peptide RIA Kit, Linco Research Inc., St Charles, MO, USA). The minimal detectable limits for insulin and C-peptide are 1·2 μIU/ml and 0·1 ng/ml, respectively.

Statistical analysis

For all analyses, ingestion of the initial cereal meal and treatment beverages will be referred to as the 'initial meal', and ingestion of the Trutol® at 180 min will be referred to as the OGTT. AUC was calculated using the trapezoidal method[19]

and was determined separately for glucose, insulin and C-peptide after both the initial meal (time $= -13$ to 180 min) and OGTT (time $= 180$ to 305 min). AUC after the OGTT was determined in two ways: (1) using the parameter concentration at 180 min as the baseline value and (2) using the fasting ($t = -13$ min) concentration as baseline. Whole-body insulin sensitivity was estimated separately for the first and second meals using the equation described by Matsuda & DeFronzo[20]. This equation calculates an insulin sensitivity index that is significantly correlated (r 0.73, $P < 0.0001$) with the rate of whole-body glucose disposal during a hyperinsulinaemic–euglycaemic clamp[20].

Whole-blood glucose, serum insulin and serum C-peptide AUC data were analysed for treatment effects using a one-way ANOVA for repeated measures with differences identified using the Tukey–Kramer *post hoc* analysis. Differences between treatments at each time point for NEFA were analysed using a one-way ANOVA for repeated measures with differences identified using Tukey's *post hoc* analysis. Due to the nature of the treatments, methylxanthine data were not analysed statistically, but was used to confirm subject compliance and to document the plasma caffeine concentration achieved in the CC trial and its temporal pattern. Statistical analysis was performed using all ten subjects; however, due to technical reasons, two subjects were unable to complete the CC trial; therefore, statistical analysis in the CC group was performed with eight subjects. All statistical analyses were carried out using the Statistical Analysis System, version 8.2 (SAS Institute, Inc., Cary, NC, USA), and differences were accepted as significant if $P \leq 0.05$. All results are presented as means with their standard errors.

Results

Subject characteristics

The men were 20–27 years of age (23 (SEM 1) years) and had an average weight, height and BMI of 79.5 (SEM 3.6) kg, 182 (SEM 2) cm and 23.9 (SEM 0.8) kg/m², respectively. All subjects met the fasting blood glucose requirement of < 6.0 mmol/l. Three out of the ten subjects did not normally consume coffee, and the remaining subjects were light to moderate consumers of coffee (1–3 cups of coffee per day) and caffeine (0–2 caffeine-containing beverages other than coffee per day). Out of the three non-coffee drinkers, two reported consuming 0–1 caffeine-containing beverages per day, whereas one subject did not regularly consume products containing caffeine.

Dietary analysis

Dietary analysis of self-reported food records for 2 d before each trial showed that total average daily energy and carbohydrate intake did not differ among subjects between the randomised trials (11 360 (SEM 728), 10 167 (SEM 410) and 10 598 (SEM 715) kJ for trials 1, 2 and 3, respectively, $P = 0.35$; 355 (SEM 26), 343 (SEM 13) and 347 (SEM 24) g carbohydrate for trials 1, 2 and 3, respectively, $P = 0.87$) and treatments (11 125 (SEM 757), 10 489 (SEM 728) and 10 171 (SEM 657) kJ for treatments CC, DC and W, respectively, $P = 0.39$; 363 (SEM 24), 346 (SEM 17) and 338 (SEM 27) g

carbohydrate for treatments CC, DC and W respectively, $P = 0.39$). None of the subjects reported intake of caffeine-containing foods on their diet records and subject compliance was confirmed by baseline plasma methylxanthine analysis (Fig. 1).

Response to the initial meal (−13 to 180 min)

Insulin. Mean fasting insulin concentrations were 45.2, 42.0 and 43.0 pmol/l for CC, DC and W, respectively ($P = 0.99$). Serum insulin concentrations rose after ingestion of the first meal (Fig. 2). Overall, there was no significant difference in insulin AUC between CC, DC and W (Table 1). Immediately after ingestion of the first meal ($t = 0$), insulin concentrations for CC and DC were 125 and 134 % greater than W, respectively (Fig. 2).

C-peptide. Mean fasting C-peptide concentrations were 0.36, 0.31 and 0.30 nmol/l for CC, DC and W, respectively ($P = 0.63$). Similarly, serum C-peptide concentrations rose (Fig. 3), but there was no significant difference in AUC between CC, DC and W (Table 1). In association with the noted changes in insulin, at $t = 0$ min, average C-peptide response was 64 and 59 % greater than W for CC and DC, respectively (Fig. 3).

Glucose. Mean fasting glucose concentrations were 4.2, 4.1 and 4.4 mmol/l for CC, DC and W, respectively ($P = 0.46$) and after consumption of the initial meal, blood glucose concentrations rose similarly in all three treatments (Fig. 4). It is of interest that in the first hour after the meal, glucose concentrations were very similar between treatments, but between 90 and 180 min, glucose concentrations appeared to stabilise in the CC treatment, while they continued to decline in the DC and W treatments. AUC for both CC and DC was significantly greater than W (Table 1). The AUC for CC was 45 % greater than DC, but this difference was not significant ($P = 0.72$).
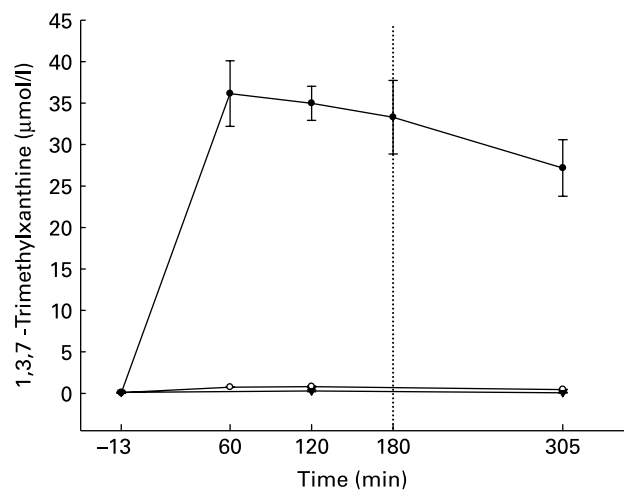

Fig. 1. 1,3,7-Trimethylxanthine after consumption of a 75 g carbohydrate meal with either caffeinated coffee (CC, ●), decaffeinated coffee (DC, ○) or water (W, ▼), followed by an oral glucose tolerance test. CC (5 mg caffeine/kg body weight) or the equivalent volume of DC or W was ingested with a cereal meal at $t = -13$ min. At 3 h later ($t = 180$ min), participants ingested 75 g dextrose to complete an oral glucose tolerance test (denoted by the ⋮). 1,3,7-Trimethylxanthine data were not analysed statistically.
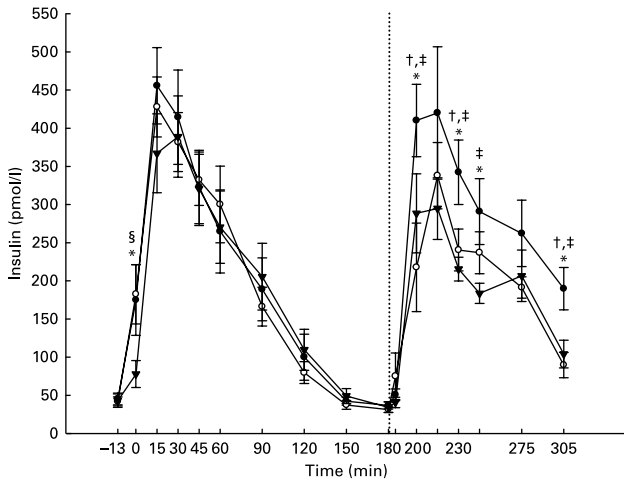
Fig. 2. Serum insulin after consumption of a 75 g carbohydrate meal with either caffeinated coffee (CC, ●), decaffeinated coffee (DC, ○) or water (W, ▼), followed by an oral glucose tolerance test. CC (5 mg caffeine/kg body weight) or the equivalent volume of DC or W was ingested with a cereal meal at $t = -13$ min. At 3 h later ($t = 180$ min), participants ingested 75 g dextrose to complete an oral glucose tolerance test (denoted by the ⋮). Data are presented as means (n 10 for all groups except the CC treatment where n 8) with their standard errors represented by the vertical bars. Data were analysed using a one-way ANOVA for repeated measures with differences identified using the Tukey–Kramer *post hoc* analysis. * Overall significance for the time point ($P < 0.05$). † Significant difference between CC and DC. ‡ Significant difference between CC and W. § Denotes a significant difference between DC and W.
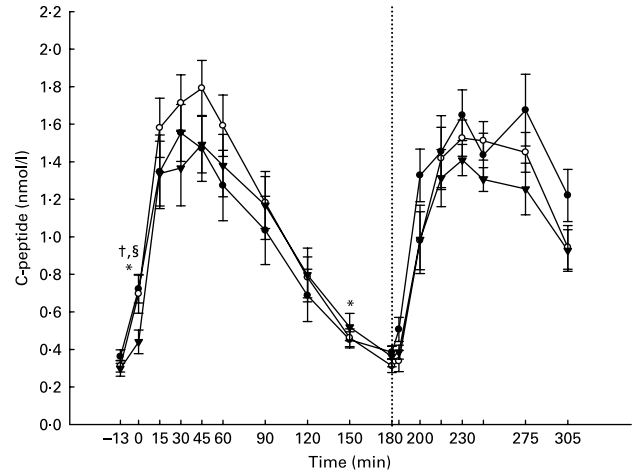
NEFA. Mean fasting NEFA concentrations were not significantly different between the treatment groups (426, 578 and 498 μmol/l for CC, DC and W, respectively, $P = 0.36$). Serum NEFA decreased for 2 h after meal ingestion, with no significant treatment differences. After $t = 120$ min, NEFA concentrations rose such that at $t = 180$ min, NEFA in the CC treatment were significantly greater than both the DC and W treatments ($P < 0.05$; Fig. 5).

Response to the oral glucose tolerance test (180–305 min)

Insulin. Immediately before the ingestion of TRUTOL® ($t = 180$ min), mean insulin concentrations were not found

Table 1. Calculated area under the curve (AUC) for glucose, insulin and C-peptide concentrations during the initial meal ($t = -13$ to 180 min) in healthy males

(Mean values with their standard errors)

	Insulin (pmol/l × 3 h)		C-peptide (nmol/l × 3 h)		Glucose (mmol/l × 3 h)	
	Mean	SEM	Mean	SEM	Mean	SEM
CC	28 369	4603	126	15	107[a]	18
DC	27 577	3425	144	15	74[a]	15
W	26 811	4069	136	17	−0.2[b]	29
P value	0.84		0.14		0.01	

CC, caffeinated coffee; DC, decaffeinated coffee; W, water.
[a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$; one-way ANOVA for repeated measures with differences identified using the Tukey–Kramer *post hoc* analysis).

Fig. 3. Serum C-peptide after consumption of a 75 g carbohydrate meal with either caffeinated coffee (CC, ●), decaffeinated coffee (DC, ○) or water (W, ▼), followed by an oral glucose tolerance test. CC (5 mg caffeine/kg body weight) or the equivalent volume of DC or W was ingested with a cereal meal at $t = -13$ min. At 3 h later ($t = 180$ min), participants ingested 75 g dextrose to complete an oral glucose tolerance test (denoted by the ⋮). Data are presented as means (n 10 for all groups except the CC treatment where n 8) with their standard errors represented by the vertical bars. Data were analysed using a one-way ANOVA for repeated measures with differences identified using the Tukey–Kramer *post hoc* analysis. * Overall significance for the time point ($P < 0.05$). † Significant difference between CC and DC. ‡ Significant difference between CC and W. § Significant difference between DC and W.

to be significantly different across the treatment groups (36.8, 31.4 and 34.3 pmol/l for CC, DC and W, respectively; $P = 0.75$; Fig. 2). After ingestion of the TRUTOL®, insulin concentrations increased with peak concentrations reached at
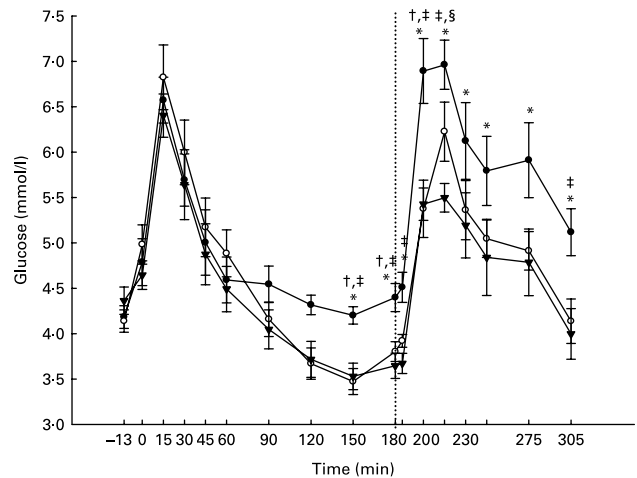

Fig. 4. Blood glucose after consumption of a 75 g carbohydrate meal with either caffeinated coffee (CC, ●), decaffeinated coffee (DC, ○) or water (W, ▼), followed by an oral glucose tolerance test. CC (5 mg caffeine/kg body weight) or the equivalent volume of DC or W was ingested with a cereal meal at $t = -13$ min. At 3 h later ($t = 180$ min), participants ingested 75 g dextrose to complete an oral glucose tolerance test (denoted by the ⋮). Data are presented as means (n 10 for all groups except the CC treatment where n 8) with their standard errors represented by the vertical bars. Data were analysed using a one-way ANOVA for repeated measures with differences identified using the Tukey–Kramer *post hoc* analysis. * Overall significance for the time point ($P < 0.05$). † Significant difference between CC and DC. ‡ Significant difference between CC and W. § Significant difference between DC and W.
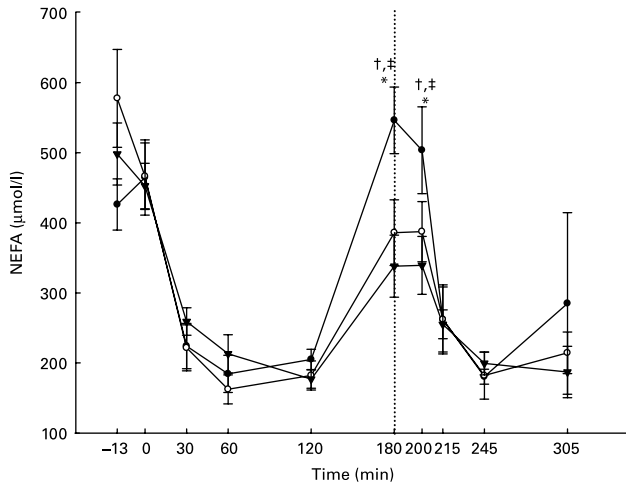
Fig. 5. NEFA after consumption of a 75 g carbohydrate meal with either caffeinated coffee (CC, ●), decaffeinated coffee (DC, ○) or water (W, ▼), followed by an oral glucose tolerance test. CC (5 mg caffeine/kg body weight) or the equivalent volume of DC or W was ingested with a cereal meal at $t = -13$ min. At 3 h later ($t = 180$ min), participants ingested 75 g dextrose to complete an oral glucose tolerance test (denoted by the ⋮). Data are presented as means (n 10 for all groups except the CC treatment where n 8) with their standard errors represented by the vertical bars. Data were analysed using a one-way ANOVA for repeated measures with differences identified using Tukey's *post hoc* analysis. * Overall significance for the time point ($P<0.05$). † Significant difference between CC and DC. ‡ Significant difference between CC and W.

$t = 215$ min in all three treatments (Fig. 2). At this time, the mean insulin concentration in the CC treatment was 24 % greater than DC (420 (SEM 87) *v.* 338 (SEM 44) pmol/l, respectively) and 42 % greater than W (295 (SEM 40) pmol/l). Using mean insulin concentration at either −13 or 180 min as baseline, the AUC for CC was greater than both DC ($P<0.01$) and W ($P<0.001$), while there was no difference between DC and W (Table 2). At $t = 305$ min, the insulin concentration for the CC treatment remained 111 % greater than for DC (190 (SEM 28) *v.* 90 (SEM 17) pmol/l, respectively) and 83 % greater than for W (104 (SEM 18) pmol/l; Fig. 2).

C-peptide. The pattern of the C-peptide response during the OGTT was similar to that of insulin and at $t = 180$ min, mean C-peptide concentrations for CC, DC and W were not

significantly different (0.39, 0.31 and 0.36 nmol/l, respectively, $P = 0.12$). Using the C-peptide concentration at time 180 min as baseline (Table 2), AUC for both CC and DC was significantly greater than W ($P<0.01$ and $P=0.05$, respectively). Although AUC for CC was 12 % greater than that of DC, this difference was not significant ($P=0.30$). When AUC was analysed using the fasting ($t = -13$) C-peptide concentration as baseline DC was not different from either of the other treatments; however, the CC treatment remained greater than the W treatment ($P<0.05$). At the end of the experiment ($t = 305$), C-peptide concentration in the CC treatment was 30 and 31 % higher than both the DC and W treatments (Fig. 3).

Glucose. In contrast to both insulin and C-peptide, mean blood glucose concentration at $t = 180$ min was significantly higher in the CC treatment compared with both DC and W (4.4, 3.8 and 3.6 mmol/l for CC, DC and W, respectively; $P<0.05$). While the OGTT was characterised by elevated insulin concentrations in the CC trial, this did not result in a reduced blood glucose response. Glucose concentrations rose after meal ingestion, and peak concentrations were reached at $t = 215$ min in all three treatments (Fig. 4) with the concentration for the CC treatment being 16 % greater than DC and 22 % greater than W. When AUC was calculated using the fasting ($t = -13$ min) baseline (Table 2), glucose AUC for CC was greater than that for either DC ($P=0.01$) or W ($P<0.001$). In addition, there was a strong trend towards increased glucose AUC in the DC *v.* W treatment ($P=0.07$). When the concentration at $t = 180$ min was used as baseline, the differences were reduced to that of a strong trend ($P=0.07$; Table 2). At the conclusion of the present experiment, mean glucose concentration in the CC condition was 19 % greater than the DC treatment and 28 % greater than the W condition (Fig. 4).

NEFA. At $t = 180$ min, mean serum NEFA concentrations in the CC treatment (546 μmol/l) were significantly greater than both the DC (386 μmol/l) and W (338 μmol/l) treatments ($P<0.001$) and remained significantly elevated compared with the DC and W treatments ($P<0.05$) up to $t = 200$ min (Fig. 5). Thereafter, there were no significant differences between any of the treatments.

Insulin sensitivity. Insulin sensitivity index was calculated separately for the initial meal ($t = -13$ to 180 min) and the OGTT ($t = 180$ to 305 min). After ingestion of the initial

Table 2. Calculated area under the curve (AUC) for glucose, insulin and C-peptide concentrations during the oral glucose tolerance test (OGTT; $t = 180$ to 305 min) in healthy males

(Mean values with their standard errors)

Baseline*	Insulin (pmol/l × 2 h)				C-peptide (nmol/l × 2 h)				Glucose (mmol/l × 2 h)			
	180 min		Fasting (−13 min)		180 min		Fasting (−13 min)		180 min		Fasting (−13 min)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CC	30 837[a]	2879	29 816[a]	2774	131[a]	9	134[a]	8	184	29	217[a]	24
DC	21 259[b]	2172	19 960[b]	2265	117[a]	8	117[a,b]	8	168	19	126[b]	11
W	20 075[b]	1976	19 009[b]	1738	97[b]	7	106[b]	9	141	27	55[b]	34
P value	<0.001		<0.001		<0.005		<0.05		0.07		<0.001	

CC, caffeinated coffee; DC, decaffeinated coffee; W, water.
[a,b] Mean values within a column with unlike superscript letters were significantly different ($P<0.05$, one-way ANOVA for repeated measures with differences identified using the Tukey–Kramer *post hoc* analysis).
* AUC during the OGTT was determined in two ways: (1) using the parameter concentration at 180 min as the baseline value and (2) using the fasting ($t = -13$ min) concentration as baseline.

meal, there was no difference in insulin sensitivity index between CC, DC and W (13·7 (SEM 4·0), 11·9 (SEM 1·1) and 13·4 (SEM 2·2), respectively, $P = 0.45$). In contrast, whole-body insulin sensitivity during the OGTT decreased significantly in the CC treatment (8·2 (SEM 0·9)) when compared with the DC (12·4 (SEM 1·2)) and W (13·4 (SEM 1·4)) treatments ($P < 0.01$ and < 0.001, respectively). The DC and W treatments were not significantly different from each other during this period ($P = 0.40$).

1,3,7-Trimethylxanthine (caffeine). As previously noted, methylxanthine analysis confirmed compliance to pretest diet instructions and also confirmed the treatments. Specifically, plasma 1,3,7-trimethylxanthine concentration rose quickly after CC ingestion peaking at approximately 36 μmol/l at $t = 60$ min (Fig. 1). Immediately before the OGTT ($t = 180$ min), mean 1,3,7-trimethylxanthine concentration was 33·3 (SEM 4·4) μmol/l and at the end of the treatment, it was still at 27·1 (SEM 3·4) μmol/l.

Discussion

Contrary to our first hypothesis, co-ingestion of CC with a high glycaemic index meal did not result in either exaggerated insulin or C-peptide responses or decreased insulin sensitivity, although the plasma caffeine concentration was elevated early in the treatment and was at a concentration similar to that of previous studies[17,21]. Despite no difference in insulin between the treatments, blood glucose in the CC treatment was higher than both the DC and W treatments, although this difference only became apparent during the last 90 min of the first meal treatment. In contrast, the present findings supported our second hypothesis in that individuals who consumed CC with breakfast experienced a significant impairment in blood glucose management and a 30–40 % decrease in insulin sensitivity after ingestion of a second carbohydrate load 3 h later. At the end of the trial, which was 5 h after CC ingestion, serum insulin remained 112 and 82 % greater in the CC treatment compared with the DC and W treatments, respectively.

There are several possible explanations for the lack of a caffeine effect on glucose homeostasis after the initial meal. Caffeine, when taken in the form of CC, may elicit insulin insensitivity to a lesser extent than pure caffeine as has been previously reported[12]. Secondly, most of the studies investigating the effect of caffeine or CC on blood glucose management have used liquid dextrose (i.e. an OGTT) as the carbohydrate load[8–10,12,22], whereas we chose to use a high glycaemic index cereal that produces an overall lower glycaemic response in comparison with dextrose. Finally, caffeine or CC is traditionally ingested 30–60 min before the carbohydrate challenge, yet we sought to investigate the co-ingestion of CC with a high carbohydrate load. It does not appear that tissue exposure to caffeine was impaired by the nature of our nutritional challenge as plasma caffeine concentrations were substantial within 60 min of CC ingestion. However, by this time, insulin was approximately 200 % above fasting concentrations. As discussed below, it is possible that other biologically active compounds in coffee interfered with the initial effects of caffeine. In contrast, Lane et al.[14] recently reported that the simultaneous consumption of CC and a liquid meal resulted in greater blood insulin and glucose

responses than when DC was consumed. Unfortunately, they did not determine plasma caffeine concentration. In addition, their subjects consumed a liquid rather than a solid meal, and were middle-aged individuals with T2DM as compared with the young, healthy adults in the present investigation. Lane et al.[14] noted that the magnitude of the caffeine effect was directly related to the duration of T2DM, and thus it may well be that one would see less of an immediate effect with young, healthy subjects in the present study.

We did observe a significant reduction in insulin sensitivity in the CC treatment over the second postprandial period, whereas there were no differences between the DC and W treatments. These findings corroborate previous work suggesting that caffeine, ingested in the form of coffee, impairs acute blood glucose management[12,13]. Despite 45–57 % greater insulin in the CC treatment throughout the OGTT, this was not associated with a reduction in blood glucose in the CC treatment, but rather it was elevated. This reduced insulin sensitivity is consistent with previous work in which caffeine[8–12,22] or CC[12,13] has been ingested before consuming carbohydrate. This acute reduction in insulin sensitivity is also consistent with studies examining the effects of caffeine on insulin sensitivity utilising the euglycaemic insulin clamp technique[23–27]. In contrast, the acute effects of caffeine and/or CC have never been examined beyond one meal period. To our knowledge, the present study is the first to report reduced insulin sensitivity to coffee after ingestion of a second carbohydrate challenge (in this case, an OGTT). While the 'second meal' was in the form of liquid dextrose, this nevertheless provided a carbohydrate challenge for the purpose of assessing the postprandial response. Since our goal was to assess whether CC elicited prolonged metabolic effects to carbohydrate intake, the use of a standard OGTT provided the simplest means as a mixed meal with various macronutrients would have been a more complex situation, thereby inhibiting our ability to interpret the present results.

In the present study, we chose to calculate AUC for the second meal in two separate ways using different baseline values. Baseline is usually regarded as the last time point before carbohydrate administration and typically does not differ among treatments. We were presented with a unique methodological challenge in determining how AUC could be quantified for the OGTT and were not aware of previous studies that have measured second meal effects along a continuous timeline. Previous work examining a second meal effect is limited and usually examined either the effects of an evening meal on breakfast glycaemic response[28,29] or glucose tolerance at lunch following a test breakfast[30–33]. The proper selection of baseline was important with respect to the experimental question we posed. We felt it necessary to calculate AUC using the initial fasting baseline as this reflects the absolute physiological rise in glucose, insulin and C-peptide responses throughout the entire experiment. Regardless of which baseline was used for insulin and C-peptide, the CC treatment resulted in significantly elevated AUC values. However, for glucose, a significantly greater AUC for the CC condition was only observed when the fasting (as opposed to $t = 180$ min) baseline was used. Regardless of the baseline employed, there is no evidence of the high insulin response in the CC trial resulting in lower glucose concentrations.

It may be argued that the volume of coffee and/or quantity of caffeine administered in the present study is not representative of typical amounts ingested by the average Western consumer. The volume of coffee administered to our subjects was measured to provide 5 mg caffeine/kg body weight and in the present study ranged between 535 and 812 ml, providing between 332 and 503 mg caffeine. While the volume of coffee ingested is likely higher than the typical consumer would consume in one sitting, the amount of caffeine consumed is not necessarily atypical. The amount of caffeine in coffee varies significantly and is affected by a number of determinants, for example, type of bean and brewing method. A cup (237 ml) of coffee yields between 74 and 250 mg caffeine; however, the consumption of a 'medium'-sized coffee (for example, 474 ml) from a specialty coffee shop can yield up to 550 mg caffeine[34]. Additionally, consumer caffeine consumption is likely to increase secondary to the increased production of caffeine-containing novelty foods and beverages (for example, W, chewing gum, energy drinks and alcoholic beverages). In the present study, no subjects were heavy coffee/caffeine consumers and would generally be classified as ranging from non-users (not consuming caffeine regularly) to light–moderate consumers. In our protocol, subjects abstained from caffeine consumption for 48 h before testing. It is not known whether these factors influenced the data, but there were no reports of side effects and the data were not highly variable.

While the purpose of the present study was not to investigate the mechanisms by which caffeine or CC precipitates insulin insensitivity, there are several factors that may be involved. The primary action of caffeine in the range of 25–40 μmol/l is adenosine receptor antagonism[35]. Thong et al.[23] demonstrated that skeletal muscle is the major tissue where caffeine impedes glucose uptake, and it has been suggested that caffeine mediates its inhibitory effects on glucose transport via A_1 adenosine receptor antagonism[36]. Thong et al.[37] have verified that rodent skeletal muscle does have A_1 adenosine receptors in the plasmalemma and that the removal of adenosine or antagonising of the A1 receptors suppressed insulin-mediated glucose uptake by 30–50 %. In addition, the ingestion of caffeine is known to stimulate the release of epinephrine and at the whole-body level, there is evidence to suggest that epinephrine exerts the opposite effects of insulin through β-adrenergic receptor activation[38–40]. When caffeine is ingested in the presence of propranolol, a β-adrenergic receptor antagonist, the insulin antagonistic effects are abolished suggesting that caffeine may act indirectly via the elevation of epinephrine[24]. Recently, Battram et al.[21] have shown that during a hyperinsulinaemic–euglycaemic clamp, the infusion of epinephrine to achieve the same plasma concentration of that reached by the ingestion of caffeine resulted in a significant decrease in insulin sensitivity. However, they also demonstrated that the ingestion of caffeine and the infusion of epinephrine resulted in a more pronounced decrease in whole-body glucose disposal than did epinephrine alone[41]. These findings illustrate that epinephrine is not solely responsible for the caffeine-induced insulin insensitivity. While it is speculative, in the present study, insulin concentrations were considerably elevated within 15 min after ingestion of the first meal. This may have allowed insulin to initiate GLUT 4 translocation and promotion of glucose uptake before caffeine blocking the A1 receptors. Consistent with this, there was a modest effect on blood glucose late in the initial postprandial period. Subsequently, there was considerable caffeine present in the circulation before initiation of the OGTT.

Although we report a prolonged, negative effect on glucose homeostasis after the ingestion of CC with a cereal meal, this could very well be compatible with the findings in the epidemiological investigations that heavy, habitual coffee consumption reduces the risk of T2DM[2–7]. Over 600 volatile components have been identified in coffee (caffeinated or decaffeinated)[42]. While caffeine, which makes up only 1·1–2·2 % (by weight) of roasted coffee[43], elicits negative effects on glucose management, it is possible that separate compounds also found in coffee may elicit opposite or positive effects on glucose management. An example of such compounds is chlorogenic acids or chlorogenic acid-derived quinones. Gastric infusion of 3,4-diferuloyl-1,5-quinide (a synthetic quinide) to conscious Sprague–Dawley rats during a hyperinsulinaemic, euglycaemic clamp has been shown to increase whole-body glucose disposal in comparison with normal saline and DC infusions[44]. In addition, Shearer et al.[45] performed hyperinsulinaemic, euglycaemic clamps on Sprague–Dawley rats that were fed a 4-week high-fat diet in combination with either placebo (W), DC or DC with caffeine. They reported greater glucose infusion rates and increased whole-body insulin sensitivity in rats fed DC in comparison with the other treatments. These findings suggest that DC contains compounds that negate the negative effects of caffeine on insulin sensitivity.

We acknowledge that the present study is limited by a small sample size restricted to young, healthy males; however, the present findings are consistent with previous similar investigations[12,13]. Future investigations are warranted to examine the prolonged effects of caffeine or coffee throughout the day when additional mixed meals would be consumed. The clinical relevance of the present findings has yet to be determined, and future, larger-scale randomised control trials including both males and females are warranted to investigate the acute and long-term effects of CC consumption on glycaemic management, particularly in higher risk populations. These results are of particular significance to those conducting research in the area of human metabolism and more specifically, glycaemic response and glycaemic index research. Individuals completing research in such areas should be aware of the metabolic effects of caffeine and coffee and must control for these substances during testing.

In conclusion, our data suggest that individuals may experience prolonged negative caffeine-induced effects with respect to glucose and insulin responses and highlight the importance of recognising metabolic interactions that occur with foods commonly ingested in the typical Western diet. The present findings further suggest that the simple substitution of CC with DC has the potential to improve acute glycaemic control in young, healthy males, and we speculate that this effect may be of additional benefit to other populations, such as those who are obese, insulin resistant or have T2DM.

Acknowledgements

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