

Glucose homeostasis remains altered by acute caffeine ingestion following 2 weeks of daily caffeine consumption in previously non-caffeine-consuming males

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Acute caffeine ingestion increases serum NEFA and plasma adrenaline and decreases insulin sensitivity. Although frequently suggested, it is not known if a tolerance to these alterations in glucose homeostasis is developed in habitual caffeine consumers. Our objective was to determine whether acute caffeine ingestion continued to alter insulin, glucose, NEFA and adrenaline during an oral glucose tolerance test (OGTT) following 14 d of caffeine consumption. Twelve caffeine-naïve young males underwent four OGTTs over a 4-week period. Subjects ingested a gelatin-filled placebo (PLA) capsule on the first trial day and 5 mg caffeine/kg body weight on the remaining three trial days (day 0, day 7, day 14) before a 2 h OGTT. Following day 0 and day 7, subjects were given six dosages of 5 mg caffeine/kg to consume per d between trials. Serum insulin and blood glucose area under the curve (AUC) were significantly elevated ($P < 0.05$) v. PLA on day 0 (36 and 103 %, respectively) and were not different from PLA on day 7. On day 14, insulin AUC was 29 % greater than PLA ($P < 0.05$), and glucose was greater ($P < 0.05$) during the first hour, although the 50 % elevation in glucose AUC was not different from PLA. Before the OGTT, caffeine resulted in greater ($P < 0.05$) serum NEFA and plasma adrenaline concentrations in all three caffeine trials, but both NEFA and adrenaline concentrations were decreased ($P < 0.05$) on day 14 v. day 0. Although 14 d of caffeine consumption by previously caffeine-naïve subjects reduced its impact on glucose homeostasis, carbohydrate metabolism remained disrupted.

Caffeine: Glucose homeostasis: Oral glucose tolerance test

The relationship between caffeine, insulin sensitivity and the risk of type 2 diabetes has received considerable recent attention¹. Acutely, caffeine can decrease insulin sensitivity during a hyperinsulinaemic–euglycaemic clamp^{2–6} and an oral glucose tolerance test (OGTT)^{7–10}. On the other hand, recent epidemiological studies report an inverse relationship between coffee consumption and type 2 diabetes^{11–16} and measurements of glucose tolerance^{17–20}. Several of these studies^{15–18} have speculated that a lessening or tolerance of the acute negative effects of caffeine on glucose homeostasis occurs in habitual caffeine consumers. Central and peripheral tissues demonstrate various degrees of tolerance to caffeine²¹. For example, chronic caffeine use decreased the acute effect of caffeine on mean arterial pressure and adrenaline concentrations, but not middle cerebral artery velocity²². Similarly, some individuals develop tolerance to the pressor effects of caffeine, albeit incomplete, while others do not^{23,24} and still others demonstrate no change in blood pressure with chronic caffeine consumption²⁵. To our knowledge no one has examined whether the influences of caffeine on carbohydrate homeostasis following an acute challenge is affected by daily intake of caffeine.

It is appreciated that skeletal muscle is normally the main site of glucose disposal. Thong *et al.*⁵ demonstrated that

caffeine decreased muscle glucose uptake during a hyperinsulinaemic–euglycaemic clamp by 50 %, but the mechanism(s) of this action are unknown. Caffeine is an adenosine receptor antagonist and the three most commonly proposed mechanisms for the impairment of insulin's actions on muscle are attributed to the resulting increase in NEFA^{26,27} or adrenaline¹⁰ or a direct antagonism of muscle adenosine receptors^{28,29}. Thus, with regards to possible caffeine habituation, these are three possible mechanisms that should be evaluated. Habitual caffeine intake has been associated with less mobilisation of NEFA^{30,31}. A blunting of the adrenaline response to caffeine ingestion has also been reported following several consecutive days of administration^{22,32}. An increased rate of caffeine metabolism, although unlikely to occur^{33,34}, would result in the developed tolerance. Chronic caffeine consumption can increase adenosine receptor numbers of some tissues^{35–38}. While skeletal muscle has been shown to have adenosine receptors³⁹, the influence of habitual exposure to caffeine is unknown. It is noteworthy that if habituation to caffeine does occur, it must be reversed in a brief period of time, as most studies showing a disrupted carbohydrate homeostasis have used habitual caffeine users as subjects^{2,4,10} who are required to withdraw for 48 h before a laboratory test day.

Abbreviations: AUC, area under the curve; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; PLA, placebo.

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The purpose of the present study was to determine if a tolerance to the disruption of glucose homeostasis observed following acute caffeine ingestion develops following 7 and 14 d of daily caffeine ingestion. Moreover, we looked to evaluate whether this daily exposure to caffeine was associated with changes in caffeine metabolism and/or the impact of caffeine on NEFA and/or adrenaline concentrations. We hypothesised that caffeine would still disrupt glucose homeostasis following 14 d of caffeine consumption. Furthermore, we hypothesised that NEFA and adrenaline would exhibit a developing tolerance to the effects of caffeine.

Experimental methods

Subjects

The present study received ethical approval for research involving human participants by the University of Guelph Research Ethics Board. Subject recruitment through a combined flyer and poster campaign resulted in twelve male subjects volunteering for the study, following the completion of a medical questionnaire and giving informed, written consent. All included subjects were non-smokers, healthy and recreationally active. Inclusion criteria required subjects to be non-caffeine users (consuming less than one caffeine-containing beverage or food per week). Subject characteristics are summarised in Table 1. Subjects were required to keep a 3 d food record before each testing day and to refrain from strenuous exercise and alcohol consumption 48 h before each testing day.

Experimental design

Subjects referred to the laboratory after a 10–12 h fast on four occasions each separated by 1 week. On each occasion, a catheter was inserted into an antecubital vein and kept patent with a saline drip. After an initial blood sample (–60 min), subjects were given a gelatin-filled capsule (placebo; PLA) on the first day of testing and 5 mg caffeine/kg body weight in capsule form during the remaining three experimental days (day 0, day 7, day 14). Subjects were instructed to ingest capsules with 250 ml water. At 1 h after the capsules were ingested, a blood sample was taken (0 min, 60 min after capsule was administered) and 75 g dextrose (TRUTOL 75; Custom Laboratories Inc., Baltimore, MD, USA) was ingested to initiate a 120 min OGTT. Blood was subsequently taken at 15, 30, 45, 60, 90 and 120 min post-dextrose ingestion.

Table 1. Subject characteristics.
(Mean values with their standard errors; n 12)

Characteristic	Mean	SEM
Age (years)	21.9	0.02
Height (m)	1.8	0.02
Weight (kg)	79.2	0.9
BMI (kg/m ²)	23.4	0.8
Fasting insulin (pmol/l)	52.0	9.7
Fasting glucose (mmol/l)	4.3	0.1
Fasting C-peptide (nmol/l)	0.27	0.07
Fasting NEFA (μ mol/l)	555.8	68.0
Fasting adrenaline (nmol/l)	0.39	0.04

All subjects received the initial caffeine treatment (day 0) 1 week following the PLA trial. After day 0 and day 7, subjects were given six dosages of 5 mg caffeine/kg body weight (two capsules per d) to consume at the same time each morning (before 12.00 hours) between the trial days. This resulted in 7 and 14 consecutive days of fixed caffeine consumption before the third (day 7) and fourth trial (day 14), respectively. Subjects were asked to return the empty package that previously contained the caffeine capsules to evaluate adherence. Subjects were instructed to refrain from consuming additional caffeine for the duration of the study and to maintain habitual physical activity.

Laboratory analysis

Blood samples were taken for the measurement of glucose, insulin, NEFA, C-peptide, adrenaline and methylxanthines. Whole-blood glucose was analysed immediately by a glucose oxidase method (YSI 2300 Stat Plus Glucose Analyzer; Yellow Springs International, Yellow Springs, OH, USA). Approximately 3 ml blood was collected in an untreated tube, allowed to clot, and then centrifuged for 10 min at 1200 *g* at 22°C for serum collection. Serum was stored at –20°C until analysis of insulin (RIA; Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA, USA) and NEFA (NEFA kit, Wako Chemicals, Richmond, VA, USA) was performed. An additional 2 ml blood was collected in a serum tube with added aprotinin and was centrifuged to collect serum for analysis of C-peptide (Human C-peptide RIA kit; Linco Research, St Charles, MO, USA). In addition, approximately 4 ml blood collected in a heparinised tube was centrifuged for 10 min at 1200 *g* at 22°C and a sample was stored at –80°C for the analysis of plasma methylxanthines by HPLC⁴⁰. Plasma was prepared for adrenaline analysis by adding 120 μ l 0.24 M-EGTA and reduced glutathione to the remaining blood in the heparinised tube and centrifuged at 1200 *g* for 10 min at 22°C. The supernatant fraction was stored at –80°C for later analysis (Adrenaline RIA kit; Labor Diagnostika Nord GmbH, Nordhom, Germany). All samples were analysed in duplicate.

Calculations and statistical analysis

Areas under the curve (AUC) for insulin, glucose and C-peptide were determined using the trapezoidal method executed in GraphPad Prism software (Prism v3.03; GraphPad Software Inc., San Diego, CA, USA) with $t = 0$ min as baseline. The insulin sensitivity index (ISI) was calculated as described by Matsuda & DeFronzo⁴¹. Fasting and average OGTT plasma insulin and glucose concentrations are used in the original equation⁴¹. We have used whole-blood glucose and serum insulin for the calculation and therefore acknowledge that the use of this index was not to comment on absolute values but for comparison between treatments only. Differences in fasting plasma methylxanthine concentrations were calculated by repeated-measures ANOVA on ranks. When statistical significance was indicated, a *post hoc* Dunnett's method test with PLA as a control was applied. Change in plasma methylxanthines during the first and second hours was calculated. Treatment differences in AUC, ISI and change in methylxanthines were determined using a one-way repeated-measures ANOVA. When statistical significance was indicated, a *post hoc* Bonferroni *t* test with PLA as a control was applied. Effects of treatment, time, and

treatment \times time interactions were determined using a two-way repeated-measures ANOVA. When statistical significance was indicated, a Tukey *post hoc* test was applied for multiple comparison analysis. Statistical analysis was performed using SigmaStat 2.03 (1997; Systat Software Inc., San Jose, CA, USA) with statistical significance set at $P < 0.05$ for all analyses. Results are reported as mean values with their standard errors.

Results

Plasma methylxanthines

Plasma methylxanthines were generally non-detectable (trace amounts) during PLA and at fasting during day 0, confirming compliance to the study design. Plasma methylxanthine concentrations increased significantly following caffeine ingestion on day 0, day 7 and day 14 compared with PLA ($P < 0.05$) (data not shown). Compared with PLA (0.2 (SEM 0.08) $\mu\text{mol/l}$), day 0 fasting caffeine concentration (0.1 (SEM 0.05) $\mu\text{mol/l}$) was not significantly different ($P > 0.05$), whereas day 7 (6.0 (SEM 1.9) $\mu\text{mol/l}$) and day 14 (5.9 (SEM 2.0) $\mu\text{mol/l}$) were significantly elevated ($P < 0.05$). Peak caffeine concentrations of 43.5 (SEM 2.5), 48.5 (SEM 1.8) and 48.5 (SEM 2.7) $\mu\text{mol/l}$ on day 0, day 7 and day 14, respectively, were achieved at 0 min immediately before OGTT initiation. Within caffeine treatments, fasting plasma caffeine was significantly elevated on day 7 ($P < 0.05$) and day 14 ($P < 0.05$) due to caffeine supplementation and when these values were subtracted from 0, 60 and 120 min concentrations respectively, no treatment effect was observed with the change in plasma caffeine concentration (data not shown). As expected, plasma theophylline, paraxanthine and theobromine closely paralleled the caffeine results (data not shown).

Serum insulin

Serum insulin values at -60 min and 0 min (initiation of the OGTT) did not differ between each of the four trial days (Fig. 1). As expected, caffeine ingestion alone on day 0, day 7 and day 14 did not alter fasting serum insulin concentrations (Fig. 1). Insulin AUC was significantly greater by 30% ($P < 0.05$) and 23% ($P < 0.05$) *v.* PLA on both day 0 and day 14, respectively (Table 2).

Whole-blood glucose

Blood glucose at -60 min and 0 min (initiation of the OGTT) were not different between each of the four trial days (Fig. 2). Blood glucose response on day 0 was significantly greater ($P < 0.05$) than PLA at every time point during the OGTT (Fig. 2) and day 0 glucose AUC was significantly greater ($P < 0.05$) by 100% over PLA (Fig. 2). The glucose AUC for day 7 and day 14 was not different ($P > 0.05$) from PLA (Table 2), but the day 14 glucose response was significantly higher than PLA at 30 ($P < 0.05$) and 45 min ($P < 0.05$).

Insulin sensitivity index

ISI was not significantly different between each of the four trial days despite insulin AUC being 30, 5 and 23% greater than PLA on day 0, day 7 and day 14, respectively

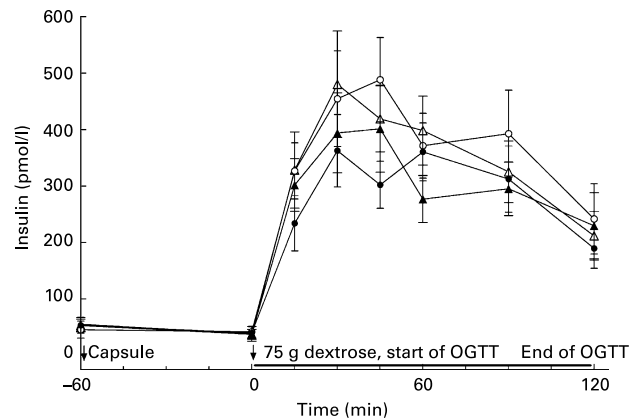


Fig. 1. Serum insulin before and during oral glucose tolerance tests (OGTT) for placebo (PLA) (●), day 0 (○), day 7 (▲) and day 14 (△) treatments. Caffeine (5 mg/kg) or placebo (gelatin) was ingested at -60 min followed by ingestion of 75 g dextrose (0 min) to initiate a 2 h OGTT. Values are means ($n = 12$), with their standard errors represented by vertical bars. A significant treatment effect exists without interaction with time (PLA *v.* day 0, PLA *v.* day 14, day 0 *v.* day 7; $P < 0.05$).

(Table 2), and the respective values for glucose AUC exceeded PLA by 100, 35 and 50% (Table 2). ISI for day 0 (10.5 (SEM 1.9), day 7 (12.7 (SEM 1.8) and day 14 (11.8 (SEM 2.2) were 17, 0.2 and 7% lower compared with PLA (12.7 (SEM 1.9), where a lower ISI indicates less insulin sensitivity.

Serum C-peptide

Serum C-peptide AUC was 44% greater ($P < 0.05$) on day 14 compared with PLA (Table 2). Although not significant, C-peptide AUC was 31% greater on day 0, and 27% greater on day 7 compared with PLA.

Serum non-esterified fatty acids

Fasting (-60 min) serum NEFA concentrations were not different between treatments (Fig. 3). Overall, day 0 elicited a significantly higher ($P < 0.05$) NEFA response compared with PLA (Fig. 3). At 0 min, NEFA was significantly higher on day 0 compared with PLA ($P < 0.05$), day 7 ($P < 0.05$) and day 14 ($P < 0.05$) were both significantly higher than PLA at time 0 min. By 60 min, there were no significant differences in serum NEFA concentrations among treatments.

Plasma adrenaline

Plasma adrenaline concentrations increased from fasting in all three caffeine treatments while remaining below fasting values during the PLA treatment (Fig. 4). Overall, plasma adrenaline concentrations during the OGTT on day 0 and day 7 were significantly greater than PLA ($P < 0.05$). At 0 min, plasma adrenaline concentrations on day 0 ($P < 0.05$), day 7 ($P < 0.05$) and day 14 ($P < 0.05$) were significantly higher than PLA, while adrenaline on day 14 was significantly lower than day 0 ($P < 0.05$). Only day 0 plasma adrenaline remained elevated at 60 ($P < 0.05$) and 120 min ($P < 0.05$) compared with PLA.

Table 2. Serum insulin, whole-blood glucose and serum C-peptide areas under the curve in men during 2 h oral glucose tolerance tests following the ingestion of placebo (PLA), caffeine (day 0) and caffeine following 7 and 14 d of caffeine ingestion(Mean values with their standard errors; *n* 12)

	PLA		Day 0		Day 7		Day 14	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Insulin (pmol/l × 2 h)	29 311	4412	38 098*	5373	30 696	3431	36 102*	4644
Glucose (mmol/l × 2 h)	126	40	256*	36	170	37	189	43
C-peptide (nmol/l × 2 h)	81	20	106	24	103	23	117*	28

* Mean value is significantly different from that for PLA ($P < 0.05$; Bonferroni *t* test following one-way repeated-measures ANOVA).

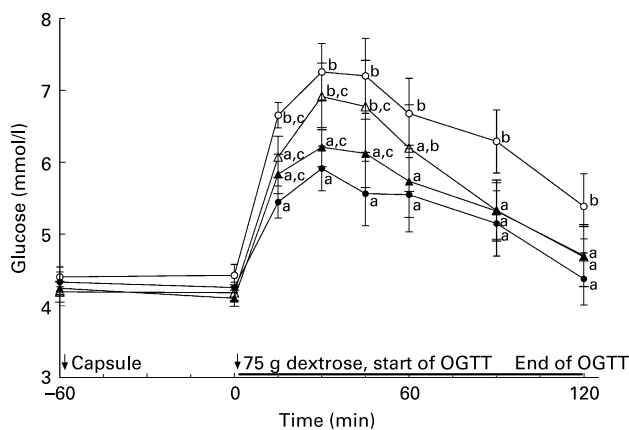
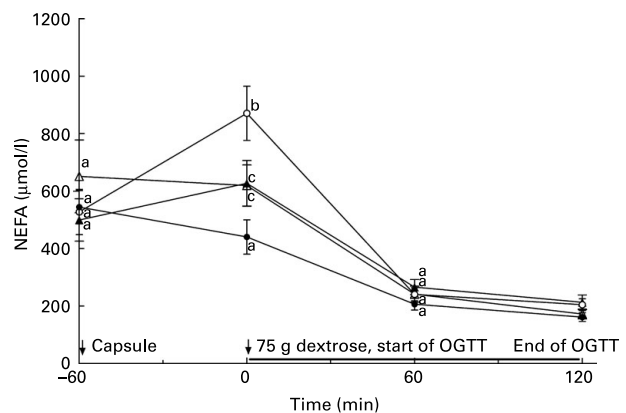
Discussion

Although caffeine has been shown to result in an acute alteration in glucose homeostasis^{7–9}, it is not known whether individuals become habituated to this effect with regular caffeine intake. The present study utilised a series of OGTTs to examine the consequences of 2 weeks of daily alkaloid caffeine ingestion on glucose tolerance in twelve healthy non-caffeine-consuming males. Even after 14 d of caffeine consumption, acute caffeine ingestion still resulted in increased NEFA and adrenaline concentrations and, when combined with an OGTT, increased serum insulin concentrations and elevated blood glucose during the first hour of the OGTT. Overall, the novel finding of the present study was that the influence of caffeine on glucose homeostasis and NEFA mobilisation was lessened, but not restored to PLA concentrations following short-term daily caffeine ingestion. In addition, the partial habituation was not associated with similar changes in NEFA, adrenaline or methylxanthine concentrations.

A comparison between PLA and day 0 blood metabolite responses demonstrated that acute caffeine ingestion resulted in significantly elevated NEFA and adrenaline before and elevated insulin and glucose response during the

administration of an OGTT. These data are very consistent with other acute caffeine OGTT^{7–10} and hyperinsulinaemic–euglycaemic clamp studies^{2–6}.

A reduction in day 7 blood insulin and glucose AUC suggests that 7 d of daily caffeine administration in previously caffeine-naïve subjects significantly reduced the acute insulin and glucose response to caffeine ingestion before an OGTT. Notably, these observed reductions in insulin and glucose concentrations occurred with plasma methylxanthine concentrations similar to day 0 and increased, albeit blunted, adrenaline and NEFA concentrations. Interestingly, caffeine did disturb the insulin and glucose responses following 14 d of caffeine administration. On day 14, a caffeine-induced elevation in blood glucose was observed during the first hour of the OGTT despite 2 weeks of daily caffeine ingestion. The 50% increase in day 14 glucose AUC was not significantly different from PLA, but was associated with a significant increase in insulin AUC. The insulin response observed on day 14 was similar to the insulin response seen following acute caffeine ingestion despite significantly less increase in NEFA and adrenaline concentrations. C-peptide AUC followed insulin AUC and was significantly higher on day 14 *v.* PLA and was increased 31% on day 0 compared with PLA, although this was not significant ($P = 0.2$). We cannot dismiss the idea that caffeine may affect hepatic extraction

**Fig. 2.** Blood glucose before and during oral glucose tolerance tests (OGTT) for placebo (PLA) (●), day 0 (○), day 7 (▲) and day 14 (△) treatments. Caffeine (5 mg/kg) or placebo (gelatin) was ingested at –60 min followed by ingestion of 75 g dextrose (0 min) to initiate a 2 h OGTT. Values are means (*n* 12), with their standard errors represented by vertical bars. Day 0 blood glucose was significantly elevated throughout the OGTT compared with PLA ($P < 0.05$), day 7 ($P < 0.05$) and day 14 ($P < 0.05$), respectively. ^{a,b,c} Mean values at the same time point with an unlike letter are significantly different ($P < 0.05$).**Fig. 3.** Serum NEFA before and during oral glucose tolerance tests (OGTT) for placebo (PLA) (●), day 0 (○), day 7 (▲) and day 14 (△) treatments. Caffeine (5 mg/kg) or placebo (gelatin) was ingested at –60 min followed by ingestion of 75 g dextrose (0 min) to initiate a 2 h OGTT. Values are means (*n* 12), with their standard errors represented by vertical bars. ^{a,b,c} Mean values at the same time point with an unlike letter are significantly different ($P < 0.05$).

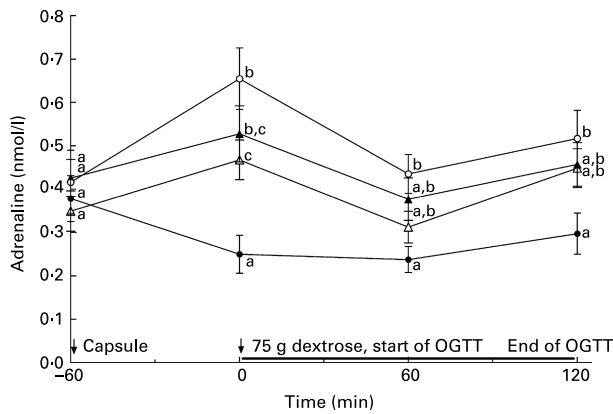


Fig. 4. Plasma adrenaline before and during oral glucose tolerance tests (OGTT) for placebo (PLA) (●), day 0 (○), day 7 (▲) and day 14 (△) treatments. Caffeine (5 mg/kg) or placebo (gelatin) was ingested at -60 min followed by ingestion of 75 g dextrose (0 min) to initiate a 2 h OGTT. Values are means (n 12), with their standard errors represented by vertical bars. ^{a,b,c} Mean values at the same time point with an unlike letter are significantly different ($P < 0.05$).

of insulin or glucose-induced insulin secretion on the basis of these results. However, these data suggest that the C-peptide response was fundamentally similar to insulin, although based on the insulin results and our previous work⁷⁻⁹ we would have expected a higher C-peptide AUC.

The data allowed us to examine several putative mechanisms for the actions of caffeine on glucose homeostasis. Elevated NEFA concentrations can decrease insulin-mediated glucose uptake by skeletal muscle^{26,27}. Prior research has shown that caffeine users exhibit an increase in NEFA concentrations following acute caffeine ingestion^{6,10,34}. On day 7 caffeine administration resulted in less of an increase in NEFA concentration than on day 0, but it was greater than PLA, implying that the lipolytic effect of caffeine^{7,8} partly persisted in previously naive subjects. Caffeine users have shown some habituation to this effect^{30,34}, which can be abolished with 4 d³⁰ and 5 d³⁴ of caffeine withdrawal. On the other hand, Denaro *et al.*⁴² have demonstrated that NEFA continue to be elevated in healthy subjects consuming a high dose of caffeine (12 mg/kg per d, greater than twice the dosage in the present study) for 5 d, suggesting that tolerance has not been established. No further reduction in serum NEFA concentration followed 14 d of caffeine administration (the concentrations at time 0 in the two trials were virtually identical) and yet in this trial, insulin and glucose were elevated compared with PLA during the OGTT. The changes at 7 and 14 d suggest that while NEFA mobilisation partly habituates, there is no direct relationship with the changes in glucose and insulin responses.

Caffeine administration for 7 d did not significantly suppress the acute rise in adrenaline following caffeine intake. Previous studies with tetraplegic individuals^{43,44} demonstrated that the caffeine-induced elevation of NEFA occurred independent of adrenaline, and this was most probably due to a direct antagonism of adenosine receptors on the adipocytes. Plasma adrenaline concentrations were elevated compared with PLA following acute caffeine ingestion in all three trials. The increase in adrenaline on day 7 was similar to that of day 0 and yet was accompanied by no significant

rise in glucose or insulin concentrations. In addition, despite very similar plasma adrenaline concentrations on day 7 and day 14, insulin and glucose concentrations were higher on day 14. The impairment of glucose tolerance by caffeine can be abolished with administration of propranolol to block β -adrenergic receptors, suggesting that the presence of adrenaline is important to the disruptions in peripheral glucose uptake observed with acute caffeine administration¹⁰. However, Battram *et al.*² observed that adrenaline infusion that achieved plasma concentrations in excess of those in the present study did not impair glucose uptake during a hyperinsulinaemic-euglycaemic clamp. The reduction in plasma adrenaline response following daily caffeine ingestion may indicate a developing, but not established, tolerance within the central nervous system to an acute dose of caffeine before an OGTT, but it is very unlikely that the disturbances in glucose homeostasis induced in the caffeine trials is a direct result of an altered adrenaline response.

Partial tolerance to the acute effects of caffeine on NEFA, adrenaline, insulin and glucose appears to have been established after 7 d of daily caffeine ingestion. However, the return of elevated insulin and glucose concentrations on day 14 accompanied by no further change in NEFA response and a decreasing adrenaline concentration suggests the influence of an additional mechanism altering glucose homeostasis following caffeine ingestion.

Daily caffeine ingestion significantly increased fasting caffeine concentrations on day 7 and day 14 due to supplementation. After adjusting for fasting concentration, we observed a comparable plasma methylxanthine concentration during each acute caffeine challenge following 0, 7 and 14 d of caffeine administration, suggesting that methylxanthine metabolism was not altered with 2 weeks of daily caffeine administration, confirming that an increased liver metabolism of caffeine via cytochrome p450 is not induced^{33,34}. The disturbance in glucose homeostasis on day 14 occurred with similar caffeine concentrations present on both day 7 and day 14, which suggests that altered caffeine metabolism is not involved in the development of this tolerance.

Caffeine is a non-specific adenosine antagonist and could be acting by affecting A1 adenosine receptors in skeletal muscle. The response of adenosine receptors to caffeine treatment varies across tissues. Prolonged caffeine treatment increases the number of adenosine receptors in the brain without leading to changes in caffeine-stimulated effects^{35,36}. Chronic caffeine administration up regulates adenosine receptors to alter aggregation in platelets^{37,38} but not lipolysis in adipose tissue³⁷. There are conflicting data discerning the role of adenosine on skeletal muscle and glucose uptake^{28,29,45,46}. In lean rodent muscle, adenosine receptor antagonist administration has been shown to increase⁴⁵, decrease^{28,29} or have no effect⁴⁶ on glucose uptake. To our knowledge, the regulation of adenosine receptors in human skeletal muscle by caffeine has not been characterised and remains a possible mechanism for the observed persistent effect of caffeine in the present study.

Caffeine is a widely consumed drug, most notably in the form of caffeinated beverages, such as coffee. Coffee has received significant recent attention because of epidemiological studies that show habitual coffee consumption can decrease the incidence of type 2 diabetes¹¹⁻¹⁶. Furthermore,

habitual caffeinated coffee consumption reduced several markers of glycaemia, including fasting C-peptide concentrations¹⁹, fasting insulin^{17,18,20} and 2 h post-OGTT plasma glucose^{17,20}. Although these studies attempt to resolve the considerable evidence that acute coffee⁷ and caffeine alone^{2–10,47} impair glucose metabolism by suggesting tolerance is developed in habitual caffeine users^{15–18}, there is a lack of supportive literature. To the contrary, van Dam *et al.*⁴⁸ showed that adverse effects of coffee on insulin–glucose homeostasis were still present following 4 weeks of daily coffee consumption. Furthermore, decaffeinated coffee may also provide type 2 diabetes risk reduction^{14,19}. Coffee contains constituents in addition to caffeine such as quinides⁴⁹, that may positively impact glucose homeostasis. The present results suggest daily caffeine ingestion for 14 d does not completely restore glucose metabolism to the PLA OGTT state. Long-term exposure to additional elements in coffee may explain the discrepancy between acute and long-term effects of coffee consumption, including the possibility of long-term beneficial metabolic effects such as slight weight reduction⁵⁰ that can not be distinguished with 14 d of exposure to caffeine alone.

The results of the present study are relevant to individuals who regularly consume caffeine, most probably in the form of caffeinated beverages. The present study design was not a randomised design, and therefore changes in dietary habits, exercise, familiarity with the testing programme or other period effects could affect the response to the OGTT. The present study did not include a control group, and while this may be considered a limitation, the purpose of the study was to evaluate the change in OGTT response following caffeine supplementation in non-consumers and not to facilitate a comparison between caffeine consumers and non-consumers. In addition, when interpreting the results, it is important to consider that measurements were made following one caffeine dosage (roughly three strong cups of coffee) and not after caffeine intake distributed throughout the day which, admittedly, may better reflect daily caffeine habits of many individuals. This makes the translation of the results to caffeinated beverages somewhat limited and future studies should employ various study designs to assess such issues.

We demonstrate a persistent effect of caffeine on glucose metabolism following regular caffeine consumption for 14 d. A possible limitation is that subjects in the present study have made the lifestyle decision to abstain from regular caffeine consumption and therefore the results may be different in habitual caffeine users. A longer period (more than 2 weeks) of caffeine administration may have to occur for complete tolerance to become established. Our data show that the relationship between caffeine and metabolic consequence is complex and requires further study. Based on the present results, it is impossible to conclude that 2 weeks is indeed long enough for a tolerance to the glucose metabolism impairment associated with acute caffeine consumption to become clearly established.

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