The effect of caffeine on glucose kinetics in humans – influence of adrenaline

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While caffeine impedes insulin-mediated glucose disposal in humans, its effect on endogenous glucose production (EGP) remains unknown. In addition, the mechanism involved in these effects is unclear, but may be due to the accompanying increase in adrenaline concentration. We studied the effect of caffeine on EGP and glucose infusion rates (GIR), and whether or not adrenaline can account for all of caffeine's effects. Subjects completed three isoglycaemic–hyperinsulinaemic clamps (with 3-[^{3}H]glucose infusion) 30 min after ingesting: (1) placebo capsules ($n = 12$); (2) caffeine capsules (5 mg kg^{-1}) ($n = 12$); and either (3) placebo plus a high-dose adrenaline infusion (HAdr; adrenaline concentration, 1.2 nM; $n = 8$) or (4) placebo plus a low-dose adrenaline infusion (LAdr; adrenaline concentration, 0.75 nM; $n = 6$). With caffeine, adrenaline increased to 0.6 nM but no effect on EGP was observed. While caffeine and HAdr decreased GIR by 13 ($P < 0.05$) and 34% ($P < 0.05$) *versus* the placebo, respectively, LAdr did not result in a significant reduction (5%) in GIR *versus* the placebo. Due to the fact that both caffeine and LAdr resulted in similar adrenaline concentrations, but resulted in different decreases in GIR, it is concluded that adrenaline alone does not account for the effects of caffeine and additional mechanisms must be involved.

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Acute caffeine ingestion induces a decrease in insulin-mediated glucose uptake (Graham *et al.* 2001; Greer *et al.* 2001; Keijzers *et al.* 2002; Thong *et al.* 2002; Lee *et al.* 2005). It remains unknown whether or not this effect is mediated either entirely or in part by the small, albeit significant, increase in adrenaline concentration (0.6 nM) following caffeine ingestion (Graham *et al.* 2001; Thong & Graham, 2002). Thong and Graham (2002) demonstrated previously that the inhibitory effect of caffeine on insulin-mediated glucose uptake was abolished when propranolol (a non-selective beta-adrenergic antagonist) was administered simultaneously. These data suggest that caffeine's inhibitory effect on insulin-mediated glucose uptake can be solely attributed to the accompanying increase in adrenaline concentrations. This conclusion (Thong & Graham, 2002), however, was based upon oral glucose tolerance test experiments, which are non-steady-state tests and thus a true measure of insulin-mediated glucose uptake is difficult to obtain.

Apart from the attenuating effects of caffeine on insulin-mediated glucose uptake rates, the possibility also exists that caffeine may influence another prominent effect of insulin, the inhibition of endogenous glucose production (EGP). If caffeine exerts a general suppressive effect on insulin action, one would intuitively expect that EGP would be less inhibited by insulin, i.e. an increase in EGP would be observed. In addition, a caffeine-induced increase in sympathetic activity may stimulate EGP (Clutter *et al.* 1980; Galster *et al.* 1981; Sacca *et al.* 1983). The potential effect of caffeine on EGP has never been examined in humans; however, in dogs, intraportal caffeine infusion enhanced net hepatic glucose output (Pencek *et al.* 2004). Net hepatic glucose uptake during insulin stimulation was also further increased when caffeine was added (Pencek *et al.* 2004), but the combined effect of caffeine and insulin on hepatic glucose production is unknown. In addition, the arterial caffeine concentration achieved in this study was double that used in previous studies examining the effects of caffeine on glucose metabolism in humans. Thus, extrapolating these findings from the dog to humans should be done with caution.

The purpose of the present study, therefore, was: (1) to determine the effects of acute caffeine ingestion on

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Table 1. Subject characteristics

Parameter	Mean (S.E.M.)
Age (years)	23 ± 1
Weight (kg)	78.8 ± 2.8
BMI (kg m^{-2})	23.8 ± 0.8
Body fat (%)	16.7 ± 2.4
Glucose (mM)	93 ± 2
Lactate (mM)	0.7 ± 0.1
Insulin (pM)	35.3 ± 4.5
Adrenaline (nM)	0.28 ± 0.04
Noradrenaline (nM)	0.36 ± 0.04
Caffeine (μM)	0.02 ± 0.01
Haematocrit (%)	45.0 ± 0.8
Haemoglobin (mM)	9.1 ± 0.2

Data are means ± S.E.M. Blood chemistry values are in the fasting state measured in arterialized blood. BMI, body mass index.

glucose kinetics under conditions where plasma insulin and glucose concentrations are kept constant, i.e. by use of the isoglycaemic–hyperinsulinaemic clamp technique; and (2) to ascertain whether or not these effects of caffeine on glucose kinetics are secondary to the accompanying increase in adrenaline concentration. To investigate the latter, we infused adrenaline at a rate predetermined to achieve a level of adrenaline (0.6 nM) previously reported to accompany caffeine ingestion.

Methods

Subjects

Twelve healthy, recreationally active male subjects from the University of Copenhagen community volunteered to participate in this study. Subject characteristics are given in Table 1. All subjects, except three, were non-smokers. Ten out of the twelve subjects were considered caffeine users, defined as the consumption of more than two caffeinated coffee or tea beverages and/or five caffeine-containing soft drinks per week. The study was approved by the local Danish ethical committee.

Pre-experimental procedures

Prior to experimental testing, subjects underwent a dual-energy X-ray absorptiometry (DEXA; Lunar DPX-IQ, Lunar Corp., Madison, WI, USA) scan for the determination of percentage body fat and lean tissue mass. Three days prior to each trial subjects were required to consume their regular diet (with an emphasis on carbohydrate intake), to maintain regular activity patterns and to follow this diet and activity schedule prior to each trial. In addition, no caffeine- containing products and/or alcohol were to be consumed 2 days before entering the laboratory. On the day directly prior to the experiments, subjects were required to refrain from performing any strenuous activity to avoid the depletion of muscle glycogen stores.

Experimental protocol

This study involved 3 days of investigation. Subjects arrived at the laboratory after a 10–12 h fast. Upon arrival, the subject's body weight and height was measured. For infusions of 3-[^{3}H]glucose (caffeine, high-dose adrenaline (HAdr) and placebo trials), insulin, 20% glucose and adrenaline, two catheters were placed in the antecubital veins. In addition a catheter was inserted in the retrograde direction in a dorsal hand vein, which was placed in a heating pad for sampling of arterialized blood and a baseline blood sample was taken. Following this sample a primed dose of 3-[^{3}H]glucose, adjusted to the subject's baseline glucose concentration, was administered and immediately followed by a constant infusion at a rate of 10 kBq min^{-1} ($t = -120$ min). Following a 2 h equilibration period, subjects consumed either placebo (gelatin) or caffeine capsules providing a dose of 5 mg kg^{-1} body weight. For the adrenaline trials, in addition to consuming placebo capsules, adrenaline was infused at a constant rate adjusted to each subject's body weight. Immediately following capsule ingestion, adrenaline was infused at two rates, 0.006 and 0.003 μg min^{-1} kg^{-1}, in order to achieve the desired plasma adrenaline concentrations of 1.2 and 0.6 nM for the HAdr and low-dose adrenaline (LAdr) trials, respectively. All adrenaline infusates were prepared from a stock solution of 1 mg ml^{-1} diluted in isotonic saline and the infusion lines were covered with aluminium foil to prevent oxidation of adrenaline by daylight.

All caffeine and placebo capsules were consumed with 250 ml of water. All placebo and caffeine trials ($n = 12$) were double-blinded and given in a randomized order; however, due to the fact that adrenaline was administered as an infusion, all adrenaline trials were given in an unblinded manner. In terms of randomization, the first few subjects ($n = 3$) completed the adrenaline trial as their first trial, in order to analyse their plasma samples for adrenaline concentration to ensure that the infusion rate was achieving the desired concentration. After this, all other subjects received the adrenaline trial in a randomized order ($n = 8$ for HAdr, $n = 6$ for LAdr). Also, it should be noted that two subjects completed four trials; i.e. they completed the placebo and caffeine trials, and both adrenaline (LAdr and HAdr) trials.

At 30 min after capsule ingestion ($t = 30$ min) a one-step isoglycaemic–hyperinsulinaemic clamp was initiated. For each trial the insulin infusate was prepared using 2.5 ml of the subject's plasma collected at baseline and isotonic saline. A 2 ml priming dose of insulin was given, which was then immediately followed by a constant infusion at a rate of 40 mU m^{-2} min^{-1} for

120 min. Arterialized blood glucose was measured using an automated glucose analyser (ABL 700, Radiometer, Copenhagen, Denmark) at 5 min intervals throughout the clamp procedure in order to maintain isoglycaemia, and the glucose infusion rate was adjusted as required. Arterialized blood samples were collected every 10 min from 1 h prior to capsule ingestion ($t = -60$ min) throughout the remainder of the experiment for determination of glucose specific activity (Bq mg^{-1}). In addition, samples were also collected for the measurement of various hormones and metabolites at the following times: immediately prior to capsule ingestion ($t = 0$ min), at 10, 20 and 30 min post-ingestion, and every 30 min throughout the clamp procedure. A small muscle biopsy (Tru-Core Biopsy needle, Medical Device Technologies, Inc., Gainesville, FL, USA) was taken from a subset of subjects ($n = 8$) at the beginning of the experiment for the determination of resting muscle glycogen concentrations. The sample was immediately frozen in liquid nitrogen.

Blood sampling

Arterialized blood for measurement of metabolites and hormones was sampled from the catheter in the hand vein. Blood for determination of glucose was sampled in pre-heparinized syringes (PICO 50, Radiometer) and analysed on an automatic analyser (ABL 700, Radiometer). Blood for determination of caffeine, metabolites and hormones was collected in iced tubes and immediately centrifuged at 4°C. Blood for determination of FFAs and caffeine was stabilized with 10 IU heparin (ml blood)$^{-1}$. Blood for determination of insulin was stabilized with 500 Kalikrenin inhibitory units aprotinin (Trasylol) and 1.5 mg EDTA (ml blood)$^{-1}$. Blood for determination of catecholamines was stabilized with 5 μM EGTA and 4 μM reduced glutathione in 20 μl of 0.6 N sodium hydroxide (ml blood)$^{-1}$. Plasma samples for insulin were stored at −20°C and those for free fatty acids (FFAs), catecholamines and caffeine were stored at −80°C.

Analytical procedures and calculations

Plasma adrenaline and noradrenaline concentrations were determined by a radioimmunoassay kit (KatCombi Radioimmunoassay, Immunobiological Laboratories, Hamburg, Germany). Plasma concentrations of insulin were determined with sandwich ELISA performed according to the manufacturer's instructions (DakoCytomatics, Glostrup, Denmark). Plasma FFA analysis was carried out by an enzyme colour assay (ACS-ACOD, WAKO, Denmark) according to manufacturer's instructions. Plasma caffeine concentrations were determined by high-performance liquid chromatography as previously described (Aldridge *et al.* 1979). Glycogen was determined by the hexokinase method (Karlsson *et al.* 1971). The average glucose infusion rate (GIR) was calculated every 5 min during the 120 min isoglycaemic–hyperinsulinaemic clamp and also during the final 30 min of the clamp ($t = 120$–150 min). Plasma 3-[^{3}H]glucose activity and calculation of the rate of glucose appearance (R_a) and disappearance (R_d) were determined according to previous descriptions (Altszuler *et al.* 1956; Cherrington & Vranic, 1973). Endogenous glucose production (EGP) was then calculated as EGP $= R_a -$ GIR. For technical reasons caffeine was not measured in the LAdr trial.

Statistics

The data are presented as means ± S.E.M. A two-way ANOVA was used to determine both time and treatment effects for insulin, FFA, lactate, adrenaline, noradrenaline, GIR (every 5 min during the clamp), EGP, heart rate and blood pressure. Caffeine concentrations were examined for time effects during the caffeine trial only by use of a one-way ANOVA; to compare caffeine concentrations between treatments (placebo, caffeine and HAdr only), a two-way repeated measures ANOVA was used. Treatment effects for both GIR averaged during the final 30 min of the clamp and resting muscle glycogen concentrations were determined using a one-way ANOVA. A Tukey's *post hoc* test was used to locate differences when necessary. Differences were considered significant at a $P = 0.05$ in two-tailed testing.

Results

Caffeine, insulin and catecholamine concentrations

In the basal state, plasma caffeine concentrations were not detectable in any of the subjects – confirming that all subjects had followed the pre-experimental instruction to abstain from consuming caffeine-containing products (Fig. 1). Ingestion of caffeine capsules resulted in a rapid increase in plasma caffeine concentration, such that a stable concentration of ∼40 μM was achieved within 30 min (Fig. 1). In all other trials plasma caffeine concentrations remained undetectable.

In the basal state, plasma insulin concentrations were similar on all trial days and did not change during the pre-clamp period or during the first 30 min following treatment ingestion and/or infusion ($t = 0$–30 min). With initiation of the clamp, plasma insulin concentrations increased rapidly to ∼400 pM and remained at this level throughout the experiments (Fig. 2).

In the basal state, plasma adrenaline concentrations were similar on all trial days. During the placebo experiment adrenaline concentrations did not change from baseline levels (Fig. 3*A*). Following caffeine ingestion

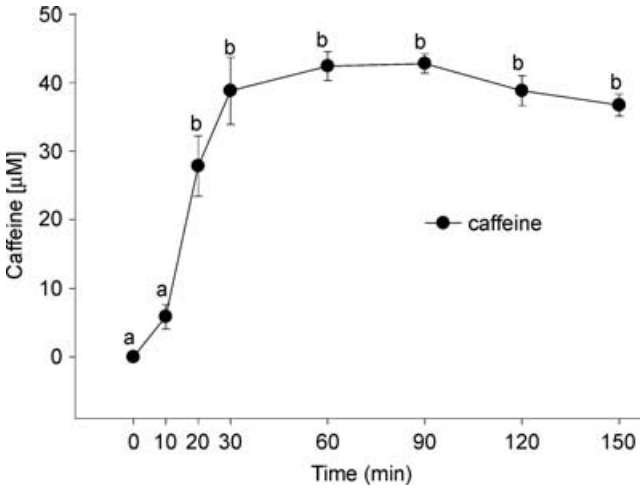

Figure 1. Plasma caffeine concentrations
Results are shown during both the 30 min pre-clamp period ($t = 0$–30 min) and during the 120 min isoglycaemic–hyperinsulinaemic clamp ($t = 30$–150 min) following the ingestion of caffeine capsules (dose = 5 mg kg^{-1}). Time points not sharing a lower case letter are significantly different from each other. Values are means ± S.E.M.

a slightly protracted increase in adrenaline concentrations was observed, achieving a stable level at ∼0.58 ± 0.02 nM (from $t = 30$–150 min). With adrenaline infusions, stable concentrations of 0.75 ± 0.05 (LAdr) and 1.18 ± 0.09 nM (HAdr) were obtained within 10 min of the infusions. The plasma adrenaline concentration during the LAdr trial was significantly higher than during the caffeine trial ($P < 0.05$). In all trials, basal noradrenaline concentrations were similar; however, caffeine ingestion resulted in a significantly higher noradrenaline response compared with both placebo and LAdr treatments (Fig. 3*B*).

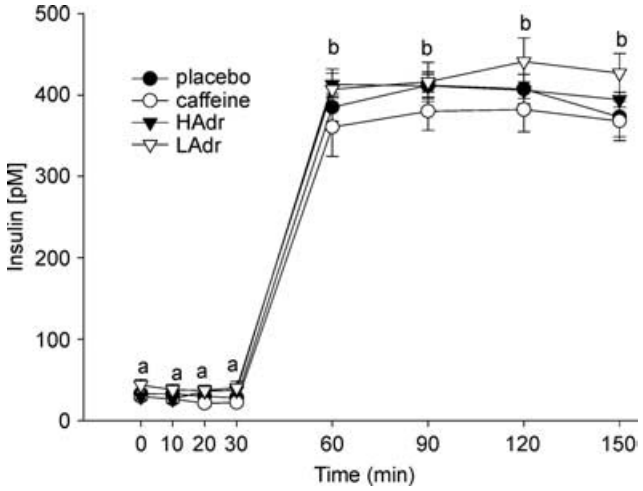

Figure 2. Plasma insulin concentrations
Results are shown for the placebo (●), caffeine (○), HAdr (▼) and LAdr (▽) trials during both the 30 min pre-clamp period ($t = 0$–30 min) and during the 120 min isoglycaemic–hyperinsulinaemic clamp ($t = 30$–150 min). Time points not sharing a lower case letter are significantly different from each other. No treatment effects were observed. Values are presented as means ± S.E.M.

Glucose kinetics

EGP in the basal state, before ingestion of placebo/caffeine capsules, was always similar (∼2 mg min^{-1} kg^{-1}) during the trials (data not shown). Within the first 30 min following the ingestion of capsules containing either gelatin (placebo and HAdr trials) or alkaloid caffeine (caffeine trial) EGP did not change significantly (data not shown). Upon initiation of the clamp, EGP decreased

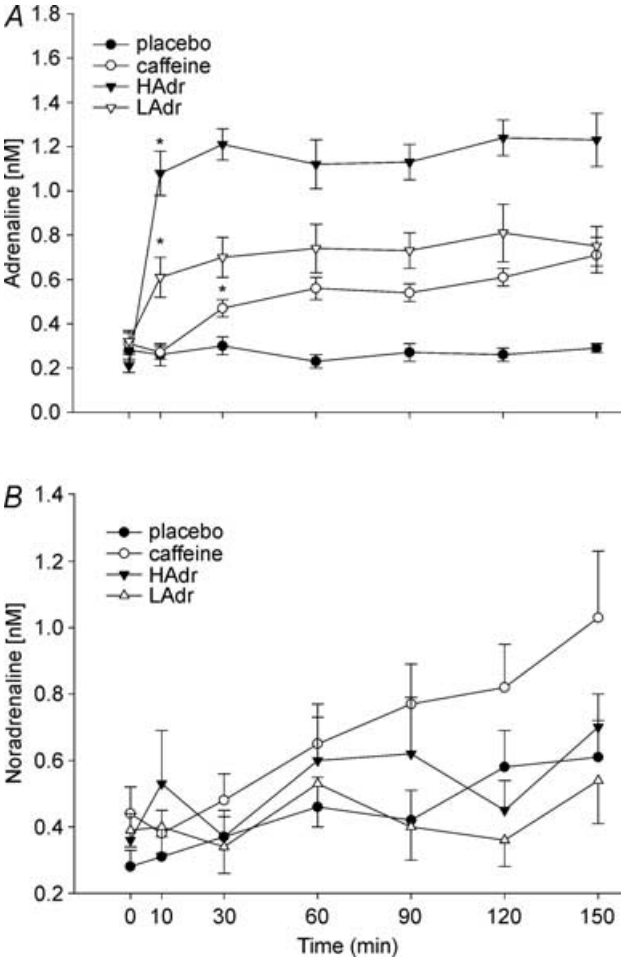

Figure 3. Plasma adrenaline (*A*) and noradrenaline (*B*) concentrations
Results are shown for the placebo (●), caffeine (○), HAdr (▼) and LAdr (▽) trials during both the 30 min pre-clamp period ($t = 0$–30 min) and during the 120 min isoglycaemic–hyperinsulinaemic clamp ($t = 30$–150 min). The asterisk indicates the first time point at which adrenaline concentrations were higher than baseline within a trial. The adrenaline concentrations were different among all treatments and caffeine resulted in a higher noradrenaline concentration compared with all other treatments ($P < 0.05$). Values are presented as means ± S.E.M.

rapidly, reaching a level close to zero within 30 min of the insulin infusion and remained at this level throughout the experiments (data not shown).

The tracer-determined R_d showed the highest values in the control (placebo) trial, followed by the caffeine trial, and finally the lowest R_d was observed with the HAdr infusion (data not shown). In essence the tracer-determined R_d mirrored the clamp-generated glucose infusion rates.

During the 120 min clamp period, plasma glucose concentrations were maintained at each subject's individual fasting levels (~5.2 mM), with no difference between the four trials (Fig. 4). The coefficient of variation during the clamps was 4.2 ± 0.3%.

GIRs during insulin stimulation differed substantially between the trials (Fig. 4). Compared with the placebo trial, ingestion of caffeine resulted in a significant ($P < 0.05$) reduction in GIR (Fig. 4). This reduction in GIR was apparent after only 40 min of insulin infusion and remained at a lower rate throughout the clamp period. On average the reduction was ~13% in the last half-hour of the clamp. Infusion of adrenaline at the high dose (HAdr) resulted in a marked decrease in GIR compared with all other trials, with the largest difference (~30%) observed between HAdr and placebo ($P < 0.05$). Infusion of adrenaline at the low dose (LAdr) did not reduce GIR significantly compared with placebo (Fig. 4).

Although caffeine ingestion resulted in a decrease in GIR, the magnitude was less than previously reported (see Discussion). By closer *post hoc* inspection of the data we observed that three subjects responded to caffeine ingestion with an increase in GIR of 18, 32 and 10% compared with placebo. It is particularly noteworthy that these same three subjects also responded to the adrenaline infusion in an opposite manner to the rest of the subjects, i.e. GIR increased, not decreased, compared with placebo. If these three subjects are excluded from the analysis, caffeine ingestion resulted in the expected 20% decrease in GIR compared with placebo.

Lactate

In the basal state, lactate concentrations were 0.68 ± 0.04, 0.79 ± 0.06, 0.88 ± 0.12 and 0.65 ± 0.03 mM in the placebo, caffeine, LAdr and HAdr trials, respectively, and remained at these levels during the pre-clamp period. During the insulin infusion lactate concentrations increased significantly ($P < 0.05$) to 0.98 ± 0.05, 1.19 ± 0.06, 1.01 ± 0.11 and 1.01 ± 0.09 mM, respectively (Table 2).

FFAs

At baseline plasma FFA concentrations were similar on all trial days. However, with the highest adrenaline infusion, HAdr, FFA concentrations increased ($P < 0.05$) markedly by 20 min (Fig. 5). As the clamp was initiated

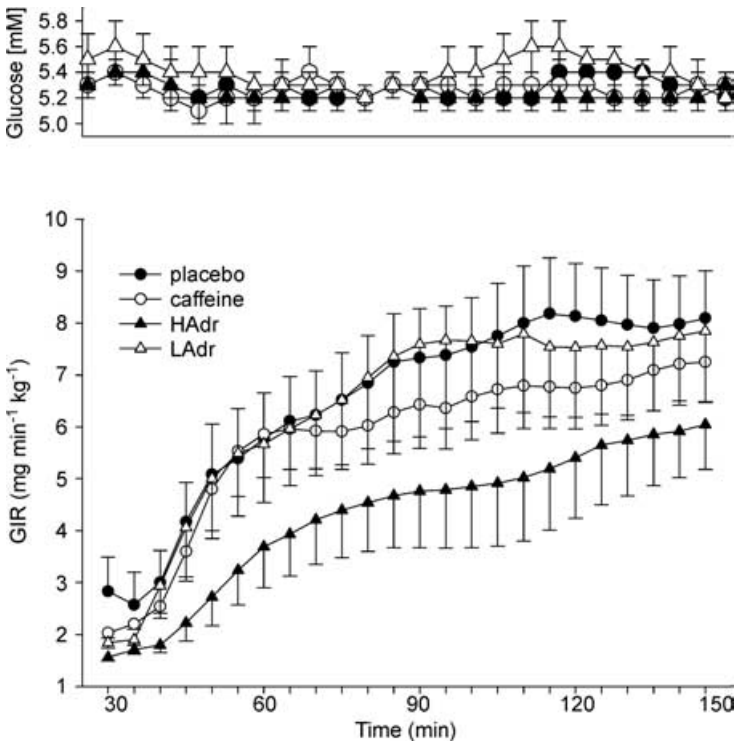

Figure 4. GIRs averaged every 5 min during the 120 min isoglycaemic–hyperinsulinaemic clamp, along with the corresponding glucose concentrations
Compared with the placebo (●), both caffeine (○) and HAdr (▲) resulted in a significant 13 and 30% decrease in GIR, while LAdr (△) decreased GIR only slightly (5%) ($P > 0.05$). Glucose concentrations were not different between treatments. Values are means ± S.E.M.

Table 2. Blood lactate concentrations and heart rate for all trials

Parameter	Placebo	Caffeine	HAdr	LAdr
[Lactate] (mM)				
0 min	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	1.0 ± 0.2
30 min	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
150 min	1.0 ± 0.1*	1.1 ± 0.1*	1.0 ± 0.1*	1.1 ± 0.1*
Heart rate (beats min^{-1})				
0 min	62 ± 4	60 ± 3	56 ± 4	62 ± 5
30 min	59 ± 3	56 ± 3	60 ± 4	58 ± 4
150 min	63 ± 4	61 ± 3	66 ± 5	59 ± 4

Data are means ± S.E.M. Lactate concentrations and heart rate are given at baseline ($t = 0$ min), just prior to the initiation of the isoglycaemic–hyperinsulinaemic clamp ($t = 30$ min) and at the end of the clamp ($t = 150$ min).
*Lactate levels significantly increased above baseline within a trial ($P < 0.05$). At no time were differences observed between treatments with respect to heart rate ($P > 0.05$).

and insulin infused, FFA concentration decreased rapidly to concentrations below the baseline level ($P < 0.05$) (Fig. 5).

Heart rate and blood pressure

At baseline, heart rate and blood pressure were similar in all trials. While caffeine ingestion resulted in no effect on heart rate (Table 2), it did result in an increase in both systolic and diastolic blood pressure ($P < 0.05$) – a response that was not present with adrenaline infusions (Fig. 6).

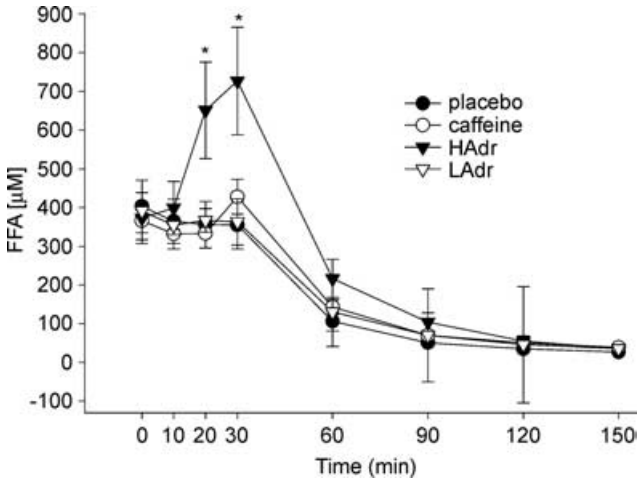

Figure 5. Plasma FFA concentrations
Results are given for the placebo (●), caffeine (○), HAdr (▼) and LAdr (▽) trials during both the 30 min pre-clamp period ($t = 0$–30 min) and during the 120 min isoglycaemic–hyperinsulinaemic clamp ($t = 30$–150 min). HAdr resulted in higher FFA concentrations compared with all other treatments, as indicated by the asterisks. Values are means ± S.E.M.

Glycogen content in muscle biopsies

Muscle biopsies were obtained from eight subjects approximately 1 h prior to each trial. The glycogen content was similar on all days (461 ± 38, 441 ± 39, 467 ± 56, 390 ± 25 nmol kg^{-1} for placebo, caffeine, HAdr and LAdr trials, respectively) confirming that pre-experimental dietary and activity guidelines were followed.

Discussion

While the present findings confirm that acute caffeine ingestion impedes insulin-mediated glucose disposal, we have demonstrated that caffeine at a plasma dose of 40 μM has no effect on endogenous glucose production in humans. In addition, we have demonstrated that the small increase in adrenaline concentration following caffeine ingestion does not alone play a dominant role in caffeine's negative effect on glucose disposal and additional mechanisms must also be involved.

We found that caffeine *per se* did not affect endogenous glucose production, as measured in the first 30 min of the experiment, i.e. before insulin infusion was initiated. In this time period peripheral caffeine concentrations were rapidly increasing (Fig. 1) and although levels did not significantly increase until 20 min following ingestion it is likely that the portal vein concentrations were much higher because caffeine to a large extent is extracted by the liver (Pencek *et al.* 2004). In addition, caffeine at concentrations of 3–9 μM, which we achieved by 15 min post-ingestion, are known to be biologically active (Smits *et al.* 1991). The absence of an accompanying increase in adrenaline concentrations cannot explain the lack of caffeine effect on EGP, as the higher adrenaline concentrations obtained in the HAdr trial also did not increase EGP. The adrenaline infusion rate and the achieved adrenaline concentration during HAdr was chosen to obtain a level that was higher than normally seen during caffeine intake, but not so high that it *per se* would influence EGP during euglycaemia; the threshold being ~1.2 nM (Clutter *et al.* 1980; Galster *et al.* 1981).

Insulin-mediated, whole-body glucose uptake rates, as measured by GIRs, decreased significantly with caffeine intake, in accordance with previous findings (Greer *et al.* 2001; Keijzers *et al.* 2002; Thong *et al.* 2002; Lee *et al.* 2005). The decrease in GIR was evident after only 40 min of insulin infusion (Fig. 4), and it was not due to differences in plasma insulin concentrations, as these were similar in all four clamp experiments (Fig. 2). However, the magnitude of the decrease in GIR with caffeine intake was not as prominent as previously reported in studies using similar doses of caffeine and insulin concentrations (Greer *et al.* 2001; Thong *et al.* 2002). The reason for the diminished effect in the present study is not readily obvious. In the placebo trials (i.e. without caffeine intake)

the absolute level of GIR and the variation were similar to those in the study by Greer *et al.* (2001) and the present study. Furthermore, in the present study the plasma glucose concentrations during the clamps were kept within very narrow limits (Fig. 4) and were very similar between clamps, testifying that the clamp experiments were performed carefully. In addition, the independent measure of glucose uptake – tracer-determined R_d – supports the clamp-derived magnitude of difference (∼13%) between caffeine and placebo trials (data not shown). Finally, the observation of negligible plasma caffeine concentrations at baseline and the similar resting muscle glycogen concentrations prior to each experiment, which is known to affect insulin-mediated glucose uptake (Richter *et al.* 1988), confirms that pre-experimental guidelines were followed and therefore cannot explain the less pronounced caffeine effect on GIR.

Upon closer examination of the data, we observed that three subjects responded in an opposite manner to caffeine, i.e. with an increase in GIR following caffeine ingestion, compared with the rest of the subjects. Interestingly, these same three subjects also responded in this manner to adrenaline, such that both levels of adrenaline infusion resulted in increases in GIR compared with placebo. If the relative change in GIR between caffeine and placebo is compared with the relative change in GIR between placebo and adrenaline (HAdr and LAdr), a significant correlation is observed ($P = 0.037$) (data not shown), suggesting that an individual responds to both caffeine and adrenaline in a similar manner with respect to both magnitude and direction. The reasons for the opposing responses in these three particular individuals are not clear. In all the experiments conducted in these three individuals, experimental conditions – including insulin and glucose, adrenaline and caffeine concentrations – during the clamp were identical to those achieved in experiments with the remaining nine subjects. Furthermore, these subjects display no commonalities with respect to any body composition measure (BMI, percentage body fat), reported fitness level, regular use of caffeinated products, resting glycogen concentrations or heart rate/blood pressure responses. If these three subjects are excluded from the analysis, the observed decreases in GIR become more pronounced (respectively 20%, 31% and 10% for caffeine, HAdr and LAdr *versus* placebo) and thus similar to previously published effects of caffeine (∼20%) (Greer *et al.* 2001; Keijzers *et al.* 2002; Thong *et al.* 2002).

Infusion of adrenaline at the high dose (adrenaline concentration 1.2 nM) resulted in a significant decrease in GIR by 30% compared with placebo. Adrenaline in high concentrations (3–4 nM) is known to inhibit insulin-mediated, whole-body glucose disposal by 40–50% (Deibert & DeFronzo, 1980; Bessey *et al.* 1983; Baron *et al.* 1987; Laurent *et al.* 1998). In the present study we used somewhat lower doses (0.75 and 1.2 nM) of adrenaline, and, accordingly, observed a less inhibitory effect on GIR. Infusion of adrenaline at the low dose (adrenaline concentration 0.75 nM) resulted in no significant decrease in GIR compared with placebo (Fig. 5). In the caffeine trials, adrenaline concentrations reached only 0.58 nM, but even so, inhibition of glucose uptake rates was 13%. Due to the fact that adrenaline

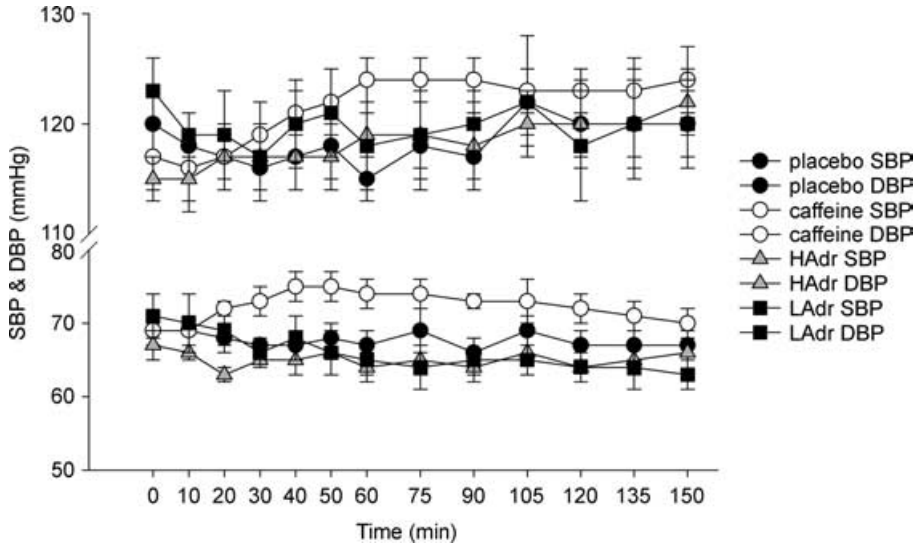

Figure 6. Systolic (SBP) and diastolic (DBP) blood pressure
Results are shown for the placebo (●), caffeine (○), HAdr (△) and LAdr (■) trials during both the 30 min pre-clamp period ($t = 0$–30 min) and during the 120 min isoglycaemic–hyperinsulinaemic clamp ($t = 30$–150 min). Caffeine resulted in a higher SBP and DBP response compared with all other treatments ($P < 0.05$). Values are means ± S.E.M.

dose-dependently attenuates GIR, one would expect the slightly higher adrenaline concentration in the LAdr (0.75 nM) to result in a greater decrease in GIR compared with caffeine. Our data therefore suggest that the inhibitory effect of caffeine is not dependent on the accompanying increase in plasma adrenaline concentrations and are in contrast to the findings of Thong and Graham (2002) who attributed all of caffeine's effects to adrenaline.

The fact that 3 out of 12 subjects displayed opposite responses to both caffeine and adrenaline stimulation, i.e. GIR increased instead of decreased, is interesting. Caffeine is a non-specific adenosine receptor antagonist (Smits *et al.* 1987), and while the positive effects of adenosine on adipose tissue glucose uptake are well documented (Joost & Steinfelder, 1983; Vannucci *et al.* 1992; Akiba *et al.* 2004), the effect of adenosine on skeletal muscle glucose uptake is controversial. Previous studies using adenosine receptor antagonists have demonstrated increases (Espinal *et al.* 1983; Crist *et al.* 1998), decreases (Han *et al.* 1998) and no effects (Vergauwen *et al.* 1994) on insulin-mediated glucose uptake in this tissue. Adrenaline inhibits glucose uptake in skeletal muscle via the β_2-receptor (Lager *et al.* 1986). Adenosine and β-adrenergic receptors both elicit their effects via G-coupled proteins and changes in cAMP (Nambi & Drummond, 1979; Fredholm *et al.* 2000). It is therefore intriguing that the three subjects displayed opposite responses to both adrenaline and caffeine stimulation, and one might speculate if this is due to alterations in common post-receptor proteins.

Elevated plasma FFA concentrations are known to inhibit insulin-mediated glucose uptake in skeletal muscle (Kruszynska *et al.* 2002). In the present study, plasma FFA concentrations were similar between the trials for most of the clamp period (Fig. 5) and can therefore not account for the observed differences in GIR. Adrenaline infusion at the high rate resulted, as expected, in an initial increase in FFA concentration (Divertie *et al.* 1997; Horowitz & Klein, 2000). However, when insulin infusion started a rapid decline below baseline levels was observed.

Heart rate did not change during the experiments (Table 2), which is not surprising given the low concentrations of adrenaline, and furthermore it is in accordance with previous findings (Thong & Graham, 2002). Both systolic and diastolic blood pressure increased with caffeine intake (Figure 6), which has been reported in previous studies using similar doses of caffeine (Graham *et al.* 2000; Keijzers *et al.* 2002; Thong & Graham, 2002). These effects are probably partially due to the accompanying increase in plasma noradrenaline concentrations. While some studies observe no changes in noradrenaline concentrations within mixed venous plasma following caffeine ingestion (Graham *et al.* 2001; Greer *et al.* 2001), Graham *et al.* (2000) observed an increase in noradrenaline spillover (i.e. an increase in arterial plasma concentrations) during moderate-intensity exercise. Furthermore, it is also possible that the increase in both systolic and diastolic blood pressure (Fig. 6) was due to caffeine acting as an adenosine receptor antagonist (Smits *et al.* 1987) inhibiting the vasodilatory effects of adenosine (Smits *et al.* 1990; Abbink-Zandbergen *et al.* 1999).

In conclusion, we have demonstrated that acute caffeine ingestion does not affect EGP. Furthermore, we have demonstrated that caffeine's attenuation of insulin-mediated, whole-body glucose disposal is not attributed to the small increase in adrenaline concentration alone and that additional mechanisms, possibly adenosine receptor antagonism, must be involved.

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