Dissociation of augmented physiological, hormonal and cognitive responses to hypoglycaemia with sustained caffeine use

J. M. WATSON*, R. S. SHERWIN†, I. J. DEARY‡, L. SCOTT* and D. KERR*

*Bournemouth Diabetes and Endocrine Centre, Royal Bournemouth Hospital, Castle Lane East, Bournemouth BH7 7DW, U.K., †Division of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520-8020, U.S.A., and ‡Department of Psychology, University of Edinburgh, Edinburgh EH8 9JZ, Scotland, U.K.

ABSTRACT

In patients with Type I diabetes and healthy volunteers, ingestion of modest amounts of caffeine augments the usual symptomatic and counter-regulatory responses to hypoglycaemia. The aim of the present study was to determine whether these are lost with sustained caffeine use, i.e. does tolerance develop? Eleven healthy caffeine consumers underwent two identical hyperinsulinaemic glucose clamp procedures. For 7 days prior to each clamp, subjects consumed a caffeine-free diet supplemented with 200 mg of caffeine capsules twice daily (caffeine-replete) or placebo (caffeine-withdrawn). During each clamp, blood glucose was held for 80 min at 4.5 mmol/l and then 2.5 mmol/l. At 85 min, subjects were given a 200 mg caffeine capsule. Measurements were taken of symptoms, plasma catecholamine, middle cerebral artery blood velocity (VMCA) and cognition. Following the acute caffeine challenge and during hypoglycaemia, VMCA fell only in the caffeine-withdrawn condition [5.1 (–7.3, 3.0) cm/s compared with 1.9 (–4.0, +0.2) cm/s in caffeine-replete condition; P < 0.04; values are differences (95% confidence intervals)]. Plasma catecholamine levels and global cognitive performance were unaffected by caffeine status, whereas tests of executive intellectual function were better preserved during hypoglycaemia in the caffeine-replete condition (P < 0.05). The influence of caffeine on hypoglycaemic symptomatic awareness depended upon the duration of the hypoglycaemic stimulus. At onset, symptoms were more intense in caffeine-withdrawn state (P < 0.01); however, with increasing duration of hypoglycaemia, symptom intensity was greater in caffeine-replete condition (P < 0.05). Thus previous caffeine consumption influences the physiological and symptomatic responses to acute hypoglycaemia, but complete tolerance does not develop with sustained use.

INTRODUCTION

Caffeine is the most ubiquitous psychoactive drug, with world-wide consumption averaging 76 mg/person per day [1], rising to 238 mg/person per day in the United States and more than 400 mg/person per day in the United Kingdom and Scandinavia [2]. In everyday life, the consumption of caffeine present in tea, coffee, soft drinks and other foodstuffs produces effects that are difficult to detect or so subtle as to go unnoticed. However, when continuous caffeine use is stopped abruptly, characteristic symptoms (e.g. headache, lethargy and anxiety) appear quickly as a consequence of physical dependence [3].

After abstinence for 24–48 h, acute caffeine ingestion is associated with an approx. 15% reduction in brain blood
Among healthy volunteers and patients with Type 1 diabetes who use caffeine regularly, prior ingestion of modest amounts of caffeine markedly augments the symptomatic and hormonal counter-regulatory responses to hypoglycaemia [6,7]. For example, ingestion of 250–400 mg caffeine at the onset of hypoglycaemia is associated with a more than 2-fold greater rise in plasma adrenaline levels compared with individuals who have not been exposed to caffeine [6,7]. However, these studies were performed in caffeine users who had abstained from the drug for only 72 h when symptoms of caffeine-withdrawal are near maximal. Thus the observed effects could have represented relief from the syndrome of caffeine withdrawal, rather than acute effect of caffeine [8]. The symptoms have subsided by 7 days [9] and are generally related to caffeine consumption [10].

The aim of the present study was to determine whether the acute effects of caffeine ingestion on the perception of, and physiological responses to, hypoglycaemia are attenuated with controlled sustained caffeine use compared with a state of prolonged caffeine-withdrawal.

This work was presented at the 58th American Diabetes Association Meeting, held in Chicago, on 11–16 June 1998, and at the British Diabetes Association Scientific Meeting, held in Glasgow, on 28 April 1999, and subsequently published in abstract form [10a,10b].

**METHODS**

**Subjects and experimental design**

Eleven healthy left-hemisphere dominant and regular caffeine consumers (180–500 mg per day; 5 males, and aged 24–36 years) gave written informed consent for the study, after approval was obtained from the local hospital ethics committee. This research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

None of the subjects had any relevant previous medical history nor were they taking any regular medication. In addition, subjects had to fulfil the following criteria for inclusion in the study: body mass index < 25 kg/m², blood pressure < 140/85 mmHg, non-smoker and < 20 units of alcohol consumption per week. Each subject was informed that they would be required to attend the Department on three separate occasions. On two of these occasions they underwent a hyperinsulinaemic glucose clamp. Each study was performed at least 2 weeks apart to avoid any carry-over effect.

![Graph](image)

**Figure 1** Schematic representation of the study

Each subject completed two studies, which were arranged in a counterbalanced manner. The following were measured during the test battery in the order: blood pressure, heart rate, V_{HA}, cognitive function (as ordered in the Methods section), blood pressure, heart rate and V_{HA}. Asterisk (*) indicates the challenge with 200 mg of caffeine.

**Procedure**

Subjects were studied at the Metabolism Research Unit at Bournemouth Diabetes and Endocrine Centre. The first visit was to familiarize the subjects with the test battery and the order of testing that would be used during the experimental condition. Familiarization helped to minimize practice effects. The results from this session were discarded.

In the two subsequent visits to the laboratory, subjects underwent identical glucose clamp procedures (Figure 1). For 7 days prior to each clamp, subjects consumed a caffeine-free diet supplemented, in a double-blinded randomized cross-over design, with either 200 mg of caffeine twice daily or matched placebo. The final capsule was taken on the morning of the clamp study, 1 h before attending the research unit. Thus the subjects were either caffeine-replete or caffeine-withdrawn at the start of each glucose clamp study.

On the morning of a study, subjects were admitted at 09.00 h, having fasted overnight. A Teflon catheter was inserted into an ante-cubital vein of the non-dominant arm to infuse insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) and a variable infusion of 20% (w/v) dextrose. A second retrograde cannula was inserted into the dorsum of the non-dominant hand and was kept patent by an infusion of 154 mmol/L NaCl. The hand was placed in a ‘hot-box’ (60 °C) to ‘arterialize’ venous blood. Potential distractions, such as conversation and other background noise, were minimized. After insertion of the cannulae, subjects rested supine for 20 min before starting the glucose clamp procedure.

A modified hyperinsulinaemic glucose clamp technique was used to maintain the blood glucose at predetermined levels [10c]. Insulin was infused at a constant rate of 2 m-units/min per kg of body weight with the rate...
Chronic caffeine ingestion and hypoglycaemia

Figure 2  Achieved glucose profile (a) and serum caffeine levels (b) during the hyperinsulinaemic glucose clamp

C-withdrawn, caffeine-withdrawn; C-replete, caffeine-replete. Values are means ± S.E.M.

of glucose infusion adjusted according to the blood glucose concentration measured at the bedside (YSI, Yellow Springs, OH, U.S.A.). Arterialized venous blood samples were obtained every 3–5 min.

The two glucose clamp sessions were identical with euglycaemia maintained for 80 min (4.5 mmol/l) at the end of which a capsule containing 200 mg of caffeine was ingested. Thereafter blood glucose was lowered to 2.5 mmol/l over 20 min and held there for a further 80 min. Subjects were not informed of their blood glucose level at anytime during the laboratory sessions. At 20 (during euglycaemia) and 120 (during hypoglycaemia) min, the test battery began and took 60 min to complete. The individual tests were administered in the order described below with approximate time allowed shown.

Plasma catecholamine and caffeine levels
Blood was taken from the heated hand vein for measurement of catecholamines and caffeine. Catecholamines were measured by HPLC using electrochemical detection (ESA, North Chelmsford, MA, U.S.A.). Plasma caffeine levels were measured by enzyme multiplication immunoassay (EMIT®; Behring Diagnostics, Milton Keynes, U.K.) on an Olympus AU560 autoanalyzer (Olympus Optical, Eastleigh, Hants., U.K.).

Blood pressure and heart rate (4 min)
Blood pressure and heart rate was measured using an automated method (Dinamap; Critikon Corp, Tampa, FL, U.S.A.).

Left- and right-middle cerebral artery blood velocity ($V_{MCA}$; 6 min)
$V_{MCA}$ was measured using a transcranial Doppler technique (SciMed, Bristol, U.K.), a surrogate measure of brain blood flow [11]. Three consecutive readings were taken each time on each side with the maximum velocity recorded for analysis.

Symptom questionnaire (2 min)
A symptom questionnaire was also completed at each stage. Eleven common symptoms of hypoglycaemia were divided into three sub-groups: autonomic (palpitations, sweating, hunger and shaking), neuroglycopenic (confusion, drowsiness, odd behaviour, speech difficulty and incoordination), and non-specific (headache and nausea). The subject graded each symptom on a scale of 1–7 (1, not
present and 7, very intense), with the total for each subgroup calculated [12]. The above tests were repeated after the psychometric tests had been performed.

Psychometric tests of general cognitive function (10 min)
Trail-making type B [13] is a divided attention task in which the subject has to connect correctly an alternating series of numbers (1–13) with letters (A–L) as quickly as possible.

Digit symbol task [14] is as a coding performance test in which 1–9 digits are represented by a specific symbol. The subjects have 1 min in which to write down as many corresponding symbols for each digit in a given array of numbers.

Four choice reaction time test [15] is as a psychomotor performance test in which the subject is presented with a square divided equally into four and, as each of these quarters lights up randomly in turn, the subject had to move the light on by pressing a corresponding button on the control panel. Accuracy and speed of reaction are recorded over 5 min.

Psychometric tests of the hemispheres (10 min)
Semantic processing and line orientation tests were used to discriminate between verbal-logical (left hemisphere) and spatial (right hemisphere) processing. Controlled word association is a test of executive function involving the frontal lobes in particular.

Semantic processing [16] assesses the ability to utilize stored information (semantic memory). This test contains 50 subject-verb questions of which half are sensible (e.g. do caterpillars crawl?) and half are nonsensical (e.g. do dishes yodel?). The time taken to respond to these sentences is recorded. Different statements are used for each administration of the test to the same subject.

Line orientation [17] examines the ability to estimate the angular relationships between line segments by visually matching angled line pairs to 11 numbered radii forming a semi-circle. The number of correct matchings was recorded, as was the time taken to complete the test.

Controlled oral word association [18] is an oral fluency test consisting of three word-naming trials. The subject is instructed to say as many words as possible beginning with the given letter in 1 min. In each set of three letters, words beginning with the first letter have a relatively higher frequency, the second letter has a lower frequency and the third letter still lower.

Visual information processing tests (15 min)
Visual change detection (VCD) [19] assesses the speed of early visual processing by measuring the brain’s ability to identify the locus of change in a stimulus array. The stimulus display consists of an array of 49 rectangles on a computer-monitor screen to which, after a variable interval, a single identical rectangle is added. The subject’s task is to identify this addition. The different time intervals between the presentation of the array and the onset of the change are 14, 28, 42, 56, 70 and 84 ms. The whole test involved ten trials of the six different stimulus duration. A total accuracy score is obtained.

Visual movement detection [19] resembles the VCD test in all respects, except that the target rectangle, rather than appearing after the rest of the array, appears with the array. After a variable interval, it moves horizontally by a distance identical with its width (3 mm). This creates the subjective sensation of sudden movement. The test is generated in the same format as VCD test. The interval between the onset of the array and the target rectangle appearing to move are also identical (i.e. 14–84 ms). A random block of 60 presentations (ten trials of six different stimulus duration) is also employed in this test and the total number of correct responses is obtained.

Statistical analysis
Overall differences between serial measurements were examined by summary measures [20]. The area under the curve (‘AUC’), by the trapezoid method, and maximum response were calculated for the responses of each individual. Group means were compared by the paired Student’s t test. Where data were not normally distributed, comparisons were made after logarithmic transformation. Results are expressed as individual means with point estimate of differences between means and 95% confidence intervals (CI). Otherwise data are shown as means ± S.E.M.

RESULTS
Blood glucose levels were indistinguishable in caffeine-withdrawn and caffeine-replete conditions, as demonstrated by achieved average blood glucose levels of 4.49±0.01 and 2.61±0.01 mmol/l during euglycaemia and hypoglycaemia respectively, in caffeine-withdrawn studies compared with 4.57±0.01 and 2.62±0.01 mmol/l during euglycaemia and hypoglycaemia respectively, in caffeine-replete studies (values are means ± S.E.M.; Figure 2a). At the start of the caffeine-withdrawn studies, caffeine levels were 0.17±0.03 mmol/l compared with 2.2±0.3 mmol/l for the caffeine-replete studies (P < 0.001; Figure 2b). Peak caffeine levels were reached at 120 min in 21 out of the 22 studies (2.79±0.2 mmol/l for caffeine-withdrawn compared with 4.39±0.5 mmol/l for caffeine-replete; P < 0.01).

Haemodynamics
Euglycaemia $V_{MCA}$, heart rate and blood pressure were similar in both studies. After the caffeine challenge, $V_{MCA}$ fell significantly only in the caffeine-withdrawn condition $[-5.1 (-7.3, -3.0)]$ cm/s; $P < 0.001$, $V_{MCA}$ during euglycaemia versus $V_{MCA}$ at 120 min; values are means
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Figure 3  Changes in \( V_{MCA} \) during the two study conditions from euglycaemia to hypoglycaemia
C-withdrawn, caffeine-withdrawn; C-replete, caffeine-replete. Values are means ± S.E.M. Glc, glucose.

(95% CI for the difference) compared with caffeine-replete \([-1.9 \pm 4.0, +0.2] \text{ cm/s}; P = 0.1\), euglycaemia \( V_{MCA} \) versus C-replete at 120 min. A significant difference \((P < 0.04)\) was also observed between the change in caffeine-withdrawn \( V_{MCA} \) with that in the caffeine-replete state. The decrease in \( V_{MCA} \) was sustained for the duration of the study (Figure 3), although the area under the curves were not significantly different \((P = 0.17)\).

When blood glucose was lowered to 2.5 mmol/l, caffeine status did not affect the rise in systolic, or the fall in diastolic, blood pressure (Table 1). Heart rate was unaffected by prevailing blood glucose and caffeine status.

Catecholamines
Baseline values for catecholamines were similar at the start and did not alter significantly during the euglycaemic phase of either study. During hypoglycaemia, the hormonal counter-regulatory response was not significantly affected by prevailing caffeine status (Figure 4).

Symptom score
Overall, the symptoms experienced in each condition were similar, except for neuroglycopenic symptoms, which were more intense in the caffeine-withdrawn condition \((P < 0.04)\). However, the pattern of responses generated differed in that the caffeine-withdrawn condition was associated with an increase in symptoms after approx. 30 min of hypoglycaemia. In contrast, by the end of the hypoglycaemic phase (after 80 min), symptoms were experienced more intensely in the caffeine-replete condition (Figure 5).

Table 1  Measurements of mean blood pressure (systolic and diastolic) and heart rate during euglycaemic and hypoglycaemic periods for the two study conditions
Values are expressed as means ± S.E.M., or difference (95% CI). bpm, beats per min.

| Caffeine-replete | | Caffeine-withdrawn | | |
|------------------|--|------------------|--|
| \( V_{MCA} \) cm/s | | | |
| Systolic (mmHg) | 112 ± 4 | 121 ± 4* | 9 ( +1, +18) | 109 ± 3.0 | 123 ± 4.0* | 13 ( +7, +19) |
| Diastolic (mmHg) | 59 ± 2 | 57 ± 2 | -2 (-5, +1) | 61 ± 1.5 | 57 ± 1.4* | -4 (-6, -1) |
| Heart rate (bpm) | 64 ± 2 | 66 ± 2 | 2 (-2, +7) | 69 ± 1.9 | 69 ± 2.7 | 1 (-5, +6) |

* \( P < 0.01\), euglycaemia versus hypoglycaemia.
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Figure 5 Difference between individual symptom scores experienced during the two study conditions at (a) 124 min and (b) at the end of hypoglycaemia (174 min)

C-withdrawn, caffeine-withdrawn; C-replete, caffeine-replete. Values represent the mean scores with the arrows indicating the 95% CI for the mean difference.

P < 0.01, total symptom score between caffeine-withdrawn and caffeine-replete at 124 min; P < 0.05 total symptom score between caffeine-withdrawn and caffeine-replete at 174 min.

Psychometric tests

Global cognitive performance (trail-making type B, digit symbol substitution and four choice reaction time) and information processing were similarly affected during hypoglycaemia in both studies (Table 2). Verbal tests were more adversely affected in the caffeine-replete state (P < 0.05 compared with caffeine-withdrawn; Figure 6).

DISCUSSION

After a period of abstinence, acute caffeine ingestion simultaneously decreases cerebral blood flow [21] and increases brain glucose utilization [22]. The clinical consequences of these effects of caffeine are markedly augmented catecholamine and growth hormone responses, as well as symptomatic responses to hypoglycaemia [6]. In the present study, where prolonged withdrawal was avoided, ingestion of caffeine was associated with less suppression of V_MCA (a marker of cerebral blood flow), but virtually identical sympathoadrenal and global cognitive responses to hypoglycaemia. In addition, caffeine status also influenced the intensity of associated warning symptoms depending on the duration of the hypoglycaemic stimulus.

In humans, tolerance to the peripheral effects of caffeine is recognized [23] but controversial [24]. Studies in young and middle-aged normotensive individuals suggest a minimum period of 12 h abstinence is needed to avoid developing tolerance to the peripheral haemo-

Table 2 Measurements of the cognitive function tests (general, right hemisphere and visual perception threshold tests) during the two study conditions from euglycaemia and hypoglycaemia

<table>
<thead>
<tr>
<th>Tests</th>
<th>Caffeine-replete</th>
<th>Caffeine-withdrawn</th>
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<tbody>
<tr>
<td></td>
<td>Euglycaemia</td>
<td>Hypoglycaemia</td>
</tr>
<tr>
<td></td>
<td>Euglycaemia</td>
<td>Hypoglycaemia</td>
</tr>
<tr>
<td>Trail-making B (s)</td>
<td>48 ± 7.2</td>
<td>62 ± 9.7</td>
</tr>
<tr>
<td>Digit symbol substitution (total score)</td>
<td>47 ± 1.5</td>
<td>44 ± 1.8</td>
</tr>
<tr>
<td>Four choice reaction time (s)</td>
<td>0.53 ± 0.02</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td>Line matching (wrong score)</td>
<td>5 ± 1.2</td>
<td>5 ± 1.1</td>
</tr>
<tr>
<td>VCD (total score)</td>
<td>42 ± 1.3</td>
<td>40 ± 1.6</td>
</tr>
<tr>
<td>Visual movement detection (total score)</td>
<td>54 ± 0.9</td>
<td>54 ± 2.0</td>
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dynamic effects of acute caffeine ingestion [25]. In animals, tolerance to the effect of caffeine on cerebral energy metabolism does not appear to develop [22]. In the present study, sustained caffeine use was associated with a smaller change in middle cerebral artery velocity, suggesting a degree of central tolerance to caffeine, although the blood pressure and catecholamine responses were not influenced by caffeine status. In an earlier study [5] using orthostasis as a stimulus to sympatho-adrenal activation, dissociation between central and peripheral tolerance to caffeine also developed. In the present study, however, the effect of tolerance to caffeine was demonstrated in the former. This may be due to the different stimulus used or the degree of stimulation; orthostasis leads to a peak adrenaline of only 600 pmol/l. Such a profound stimulus as hypoglycaemia to the sympatho-adrenal axis may also explain the similar effects of caffeine on blood pressure in the two caffeine states.

During euglycaemia, caffeine directly stimulates adrenomedullary catecholamine release, with plasma adrenaline being more sensitive to caffeine than nor-adrenaline [26]. The mechanism of action is unclear, although in tetraplegic individuals, in whom sympatho-adrenal responses are blunted, caffeine ingestion is not associated with a rise in adrenaline [27]. In the present study, there was a marked rise in plasma adrenaline levels during hypoglycaemia, which was greater than that seen in healthy subjects who had not been challenged with caffeine at the onset of hypoglycaemia [5,28]. Tolerance to the caffeine-augmented adrenaline rise during hypoglycaemia did not develop. The stimulus to a rise in plasma adrenaline levels in association with a low blood glucose level is mediated through the ventromedial hypothalamus with modulation by other higher centres [29]. Our data would suggest a similar degree of caffeine-associated neuroglycopenia at the onset of hypoglycaemia, which is independent of caffeine exposure. This may be clinically relevant if caffeine proves to be a useful adjuvant treatment for diabetic patients who have abnormal counter-regulatory responses to hypoglycaemia, although regional cerebral blood is altered by Type I diabetes mellitus with increased blood flow to the frontal cortex [30]. In previous studies [6,7] similar, but different, effects were demonstrated with a single caffeine stimulus after a short period of abstinence in both normal volunteers and patients with Type I diabetes. Further support that a similar result may be obtained with a diabetic patient population was demonstrated in a trial of free-living diabetic patients consuming a caffeine-free diet, where supplementation with 200 mg caffeine twice daily was associated with an increase in symptomatic hypoglycaemic episodes [31].

During the early phase of hypoglycaemia, warning symptoms were more intense in the caffeine-withdrawn state. Interestingly, this was observed with all the hypoglycaemic symptoms measured. However, with a more prolonged period of hypoglycaemia, symptom intensity was greater in the caffeine-replete state, again demonstrated across the range of hypoglycaemic symptoms. The mechanisms involved are unknown, but could reflect differences in baseline rates of cerebral glucose metabolism according to recent caffeine exposure.

Although it is assumed that non-diabetic subjects always have symptoms when their blood glucose levels are low, during sustained hypoglycaemia warning symptoms and abnormalities in cognitive function wane with time, despite sustained increments in counter-regulatory hormone levels [32]. This may be due to the increase in cerebral blood flow, which has been recorded during hyperinsulinaemic glucose clamps (euglycaemia [33] or hypoglycaemia [33,34]). This rise was prevented in the present study by acute caffeine ingestion. Cerebral metabolism is changed by chronic caffeine consumption in that lactate levels do not rise as compared with caffeine consumption in caffeine-withdrawn subjects [35].

In the present study, tests of global cognitive performance deteriorated to the same extent in both the caffeine-replete and caffeine-withdrawn state. Previously, Kerr et al. [33] reported that individual tests of right and left hemisphere function deteriorate to the same extent during hypoglycaemia in healthy caffeine-withdrawn volunteers. In the present study, chronic caffeine use was associated with greater deterioration in executive function, as illustrated by the verbal fluency tests. Although there is a large literature of formal studies examining the effect of caffeine on a variety of intellectual tasks, the results are often conflicting and inconclusive and may relate to increased arousal and suppression of boredom in repetitive tasks than a direct effect on intellectual performance [4].

In summary, after a short period of abstinence, caffeine ingestion at the onset of hypoglycaemia is associated with markedly augmented hormonal and symptomatic responses. With regular caffeine use, the effects on $V_{MCA}$ and early warning symptoms are attenuated, but the hormonal responses are similar to the caffeine-withdrawn state. Although, in general, deterioration in intellectual performance during hypoglycaemia is not influenced by caffeine status, specific tests of verbal processing are negatively affected by recent caffeine exposure. Although this finding could represent a type 2 error, the long-term consequences of this are unknown, warranting further investigation before caffeine can be suggested as an adjuvant treatment for patients who have difficulty in recognizing the onset of hypoglycaemia.

**ACKNOWLEDGMENTS**

We thank Dr Michael Lunt for help with middle cerebral artery analysis, Aida Grosman for catecholamine
measurements, Dr David Hussey for caffeine measurements, and Dr Peter Thomas for statistical advice. Melanie Weiss and Julia Ingleby were invaluable in their support for the project. J.M.W. was supported by a generous research fellowship from Novo Nordisk (UK) Research Foundation. This work was supported, in part, by National Institute of Health grants (DK20495 and RR00125).

REFERENCES