Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers\textsuperscript{1-3}

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ABSTRACT In humans caffeine stimulates thermogenesis by unknown mechanisms and its effect on body weight has not been studied. The effect of placebo and 100, 200, and 400 mg oral caffeine on energy expenditure, plasma concentrations of substrates and hormones, blood pressure, and heart rate was investigated in a double-blind study in healthy subjects who had a moderate habitual caffeine consumption. Caffeine increased energy expenditure dose dependently and the thermogenic response was positively correlated with the response in plasma caffeine \((r = 0.52; p < 0.018)\), plasma lactate \((r = 0.79; p < 0.000001)\), and plasma triglyceride \((r = 0.53; p < 0.02)\). Stepwise regression analysis with the thermogenic response as the dependent variable excluded plasma caffeine and yielded the following equation: thermogenic effect (kcal/3 h) = -0.00459 \times \text{heart rate} + 0.30315 \times \text{triglyceride} + 0.53114 \times \text{lactate} + 15.34 (r = 0.86; p = 0.0001). The results suggest that lactate and triglyceride production and increased vascular smooth muscle tone may be responsible for the major part of the thermogenic effect of caffeine. Am J Clin Nutr 1990;51:759-67.

KEY WORDS Caffeine, energy expenditure, obesity, substrate cycles

Introduction

Caffeine, a methylxanthine derivative, is the most widely used drug, as popular as alcohol and tobacco. It is principally present in coffee, tea, cocoa, chocolate, and many cola-type soft drinks. Pharmacologically, caffeine has bronchodilatory, cardiotonic, and diuretic effects. In addition, caffeine is contained in several over-the-counter preparations used for slimming (1, 2). Indeed, in rodents caffeine promotes weight loss by reducing lipid stores because of increased energy expenditure but without decreasing energy intake (3, 4).

In humans the use of caffeine as a slimming agent has never been justified by clinical documentation of its weight-reducing properties. In three studies caffeine was reported to stimulate energy expenditure and lipolysis in humans (5-7). Although Acheson et al (5) found that a cup of coffee (4 mg caffeine/kg body wt) consumed with a meal produced a significantly greater thermic response than that which followed the intake of the same meal with a cup of decaffeinated coffee, this difference can be almost totally accounted for by the thermic effect of the caffeine. Another study also found an increased energy expenditure by caffeine infused coffee (100 mg caffeinated) compared with decaffeinated coffee (6 mg) (6). The third study found a reduced thermogenic response to caffeine (4 mg/kg ideal body wt) in obese and postobese patients compared with lean control subjects (7). It appears that only a few different doses of caffeine have been studied, and other constituents of coffee than caffeine may influence lipid metabolism (8). The thermogenic profile and the mechanisms behind this action of caffeine are poorly understood.

The present study was undertaken to provide results from normal adults in a double-blind, placebo-controlled dose-response study with respect to the thermogenic, cardiovascular, and metabolic effects of caffeine and to relate the responses to the plasma concentrations of caffeine and its metabolites.

Subjects and methods

Experimental design

The study was designed as a placebo-controlled, double-blind test of caffeine (100, 200, and 400 mg), three different doses of ephedrine, and two similar placebo tests. Only the results from the caffeine and one placebo test are reported here. The order of the tests was not entirely randomized but it was organized by a third party (a statistician) in a sequence that allowed testing for a carry-over effect (an effect of the previous dose on the baseline values of the next test). The study was approved by the Danish National Health Service and by the Municipal Ethical Committee of Copenhagen.

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Subjects

The experimental subjects were recruited from the Medical School, University of Copenhagen. All were medical students except one, who was a laboratory technician. Six healthy, normal-weight subjects of both sexes aged between 20 and 32 y were included. None were engaged in physical training, regular exercise, or sports. They were all habitual coffee drinkers with a moderate coffee consumption of 150-300 mL/d. However, because heavy caffeine intake induces tolerance to at least some of the effects of caffeine (9), subjects with a habitual intake of more caffeine, from coffee, tea, cola beverages, cocoa, and chocolate, than corresponding to 1-2 daily cups of coffee (>100-200 mg caffeine) were not allowed to participate. This intake was assessed by a questionnaire on consumption of coffee, tea, cola beverages, cocoa, and chocolate. None were taking medicine during the study. One subject occasionally smoked tobacco. They were instructed in a weight-maintenance diet containing -250 g carbohydrate/d and 100 mmol Na/d to ensure filled glycogen stores and to avoid the influence of a low-sodium diet on the sympathetic nervous system (10). The physical characteristics of the experimental subjects are given in Table 1.

The percentage of overweight was calculated from the individual body weight by use of the midpoint of the medium frame given in the 1959 Metropolitan Life Insurance Company tables of desirable weights (11). The body fat content was estimated by skinfold-thickness measurements (12). This estimate was obtained from duplicate measurements of the biceps, triceps, subscapular, and suprailiac skinfolds with a Harpenden caliper on 2 experimental days.

Test substances

The test substances were given orally as gelatin capsules, containing either placebo (lactose) or caffeine (100, 200, or 400 mg). All capsules had the same appearance and weight; lactose was used as an inactive additive to fill them. The capsules were swallowed with 300 mL tap water (20 °C).

Experimental protocol

The experimental subjects abstained from food, coffee, tea, cola beverages, cocoa, chocolate, and smoking overnight (>12 h) before each test was started at 0830. The subjects were instructed to adhere strictly to the protocol, in particular regarding abstinence from sweets and beverages containing caffeine, during the study. The intake of the test substance was supervised and compliance was controlled by measuring plasma concentrations of methylxanthine metabolites before and after intake.

Only a 150-mL glass of tap water was allowed in the morning. There was a minimum of 3 d between two consecutive tests. The subjects were instructed not to have any physical activity (bicycling, jogging, etc) in the morning. During the experiments the subjects rested supine and light music was played by a tape recorder to induce relaxation and to avoid hyperventilation, but sleeping was not permitted. No movements or changes in position were allowed, in order to avoid any influence of physical activity on energy expenditure.

At least 60 min before beginning the experiments, a Venflon catheter (Viggo Products, Helsingborg, Sweden) was inserted percutaneously into an antecubital vein for blood sampling. The catheter was kept open during the experiment by flushing with isotonic sodium chloride solution (154 mmol/L) after each sampling. The room temperature was kept constant at 25-27 °C. All blood samples for determining substrate, metabolite, and hormone concentrations were collected from the antecubital vein. Blood was drawn -30, 0, 30, 60, 90, 120, 150, and 180 min relative to capsule intake. The subjects breathed through a low-resistance, SCUBA one-way mouthpiece. After ~10 min of adaptation, expiratory gas was collected in Douglas bags for 10 min. Gas collection was made after each blood sampling. Before the study the experimental subjects were habituated to the experimental procedures to prevent hyperventilation, anxiety, and uneasiness.

Analyses

Energy expenditure was measured by indirect calorimetry. Expiratory gas was continuously analyzed for oxygen and carbon dioxide with a Godart Rapox Oxygenometer (Rapox, Godart NV, Bilthoven, Holland) and a Beckman LB-1 medical gas analyzer. Respiratory steady state was assumed to exist when the end-expiratory carbon dioxide fraction was constant. Expiratory gas was collected in Douglas bags and analyzed for oxygen and carbon dioxide with gas electrodes connected to an acid-base analyzer (PHM 71, Radiometer A/S, Copenhagen) and the volume was measured with a gas meter. Energy expenditure was calculated by use of a formula assuming a fixed protein catabolism (13) because the error of calculating the energy...
expenditure by omitting the exact correction from urinary nitrogen is negligible. The CV on resting energy expenditure at a 1-d interval and a 1-wk interval was found to be ~3%. To obtain this accuracy the gas electrodes were calibrated with standard gases of known composition before every sampling for gas analysis. The standard gases were analyzed by the Scholander microtechnique (14) with a measuring error on the gas fraction of < 0.0005%, ie, an error on measurement of expired gas of ±0.1–0.2%. The apparatus used in this investigation had a CV on repeated hourly measurements of ≤3%.

Through the indwelling antecubital cannula, blood was sampled without stasis in iced syringes. The blood was then centrifuged for 10 min at 3000 × g and 10 °C, and nonesterified fatty acids (NEFAs) were immediately extracted and later determined as previously described (15). Plasma glucose and lactate were analyzed by standard enzymatic methods (16); glycerol, as described by Laurell and Tibbling (17); and triglyceride, as described by Giegel et al (18). Blood for determining methylxanthine metabolites was collected in tubes containing reduced glutathione and EGTA. Samples were immediately centrifuged for 10 min at 3000 × g and 4 °C, and the plasma was stored at −40 °C until determination of methylxanthines by high-pressure liquid chromatography (HPLC) (19). Immunoreactive insulin, pancreatic glucagon, and C-peptide concentrations were measured in plasma with radioimmunoassay kits purchased from Novo, Copenhagen.

All plasma samples for determining pancreatic hormones and methylxanthine metabolites were coded and analyzed in a random order to avoid any systematic error attributable to the order of analysis. Plasma sodium and potassium concentrations were determined by flame spectrophotometry. CVs for measurements were as follows: NEFAs, 3.5%; glucose, 1%; sodium, 1%; potassium, 2%; glycerol, 4.7%; lactate, 4%; triglyceride, 4%; insulin, 9.7%; C-peptide, 11%; and pancreatic glucagon, 42%.

Arterial blood pressure was measured in the right arm by an inflatable cuff attached to a sphygmomanometer and heart rate was determined by palpation of the peripheral pulse in the ipsilateral radial artery. These measurements were performed after each blood sampling.

A Trimline apparatus (PyMaH, Copenhagen) was used to measure arterial blood pressure. A cuff 12–14 cm wide was used. The manometer pressure was slowly and gradually reduced from 200 mm Hg and the first Korotkoff sound was registered as the systolic pressure. The diastolic blood pressure was determined as the manometer pressure when the Korotkoff sound quality changes from tapping to muffled. Subjective feelings of side effects were assessed by questioning the experimental subjects after each test substance.

Statistical analyses

The responses to a test substance were estimated separately for each subject as the difference between the integrated numerical area of the response curve (by a trapezoidal approach) and the rectangular area determined by the basal values. A two-way analysis of variance (ANOVA) for repeated measures was performed to test differences between experimental periods within the same experiment and to test differences between the responses to different doses (20). Two means were compared by post hoc testing (20). A possible carry-over effect was evaluated by comparing data obtained from the two placebo periods by means of a paired t test. p values < 0.05 were considered significant. Linear and step-wise regression analyses and correlation analysis were performed with Statgraphics software (Graphical Software Systems, Inc, Rockville, MD). All results are expressed as X ± SEM. There were no protocol deviations or dropouts.

Results

Caffeine and metabolites

Concentrations of caffeine, theobromine, and paraxanthine are shown in Figure 1. Analyses of plasma caffeine from four experiments were technically unsuccessful, so all statistics involving these analyses are based on data from the complete 20 sets only. The low preintake concentrations of caffeine (≤ 5 μmol/L) verified the abstinence from caffeine in accordance with the protocol. When the baseline values from the two placebo tests were analyzed, no difference could be found with respect to any of the methylxanthine derivates. Plasma concentrations of caffeine increased dose dependently (p = 0.004) whereas the increase in paraxanthine was delayed, and comparison of its course after intake of 200 and 400 mg caffeine points to saturation of the pathway that converts caffeine to paraxanthine. No changes occurred in theobromine after placebo or caffeine intake.

Energy expenditure and respiratory quotient

The changes in energy expenditure and the integrated responses after placebo and the various caffeine doses are shown in Figure 2. The integrated thermogenic response to 100, 200, and 400 mg caffeine were 9.2 ± 5.7, 7.2 ± 6.0, and 32.4 ± 8.2 kcal/h (p < 0.001) above the placebo responses. These values are minimum figures because the energy expenditure had not returned to baseline after the measurements ended, 3 h after the intake (Fig 2). The thermogenic effects of 100 and 400 mg caffeine were significantly above the placebo effect (p < 0.05 and p < 0.001, respectively) whereas the difference between the effect of 200 mg caffeine and placebo was not statistically significant. Linear-regression analysis showed that there was a significant linear relation between caffeine dose and integrated response above baseline of plasma concentration of caffeine (y = 0.0055x − 0.176; n = 20; r = 0.81; p = 0.00015). Linear-regression analysis also showed a significant relation between caffeine dose and thermogenic response (y = 0.064x + 7.1; r = 0.59; p = 0.006). Correlation analysis showed a significant linear relation between plasma caffeine response and thermogenic response (y = 0.0084x + 11.72; r = 0.52; p = 0.018). A multifactor analysis of variance including gender as a covariate did not show any significant influence of sex on the thermogenic response to caffeine (p = 0.85).

The respiratory quotient decreased slightly after all doses, including after the placebo. No differences in the integrated response could be detected between the active compounds and placebo (data not shown). This indicates that the energy expended above that observed after the placebo, ie, the expenditure induced by the active compounds, was caused by an equally increased carbohydrate and lipid oxidation.

Glucose and lactate

Plasma glucose concentration decreased slightly but insignificantly after placebo and 100 and 200 mg caffeine (Fig 3)
Caffeine conc. (µmol/L) 75-
50-
25-
0-

Paraxanthine (µmol/L) 15-
12-
9-
6-

Theobromine (µmol/L) 10-
7-
4-
1-

Glucose (mmol/L) 5.2-
4.8-
4.4-
4.0-
3.6-

Lactate (mmol/L) 1.2-
0.8-
0.4-

FIG 1. Plasma concentrations of caffeine, paraxanthine, and theobromine before and after oral intake of different doses of caffeine or placebo. Mean values (±SEM) of five to six subjects.

FIG 2. Energy expenditure before and after oral intake of different doses of caffeine or placebo. The right-hand figure shows the integrated responses above baseline. Mean values (±SEM) of six subjects.

FIG 3. Plasma glucose and lactate concentrations before and after intake of 0, 100, 200, and 400 mg caffeine. Mean values (±SEM) of six subjects.

whereas a small increase was noted between 60 and 90 min after intake of 400 mg caffeine (p < 0.05). When the responses from baseline were compared with placebo no difference could be detected (Table 2). However, there was a positive correlation between the caffeine dose and both plasma caffeine response and glucose response (Table 3). Caffeine had a significant effect on plasma lactate (p < 0.01). Plasma lactate concentration decreased below baseline after placebo and 200 mg caffeine whereas a small increase was found after 100 mg caffeine, and there was a pronounced increase after 400 mg caffeine (Fig 3 and Table 2). A positive correlation was found between caffeine dose and plasma lactate response (Table 3).

Insulin and C peptide

Plasma insulin concentration decreased significantly below baseline after placebo and 100 and 200 mg caffeine (Fig 4).
THERMOGENIC EFFECT OF CAFFEINE

TABLE 2
Integrated response from baseline over 3 h after caffeine intake*

<table>
<thead>
<tr>
<th>Caffeine dose (mg)</th>
<th>0</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>3.2 ± 2.2</td>
<td>2.0 ± 1.2</td>
<td>1.5 ± 1.8</td>
<td>6.3 ± 1.6‡</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>1.8 ± 2.0</td>
<td>2.7 ± 0.8</td>
<td>-0.2 ± 1.2</td>
<td>6.3 ± 1.5§</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>0.4 ± 1.8</td>
<td>-0.9 ± 2.2</td>
<td>-1.9 ± 1.7</td>
<td>-0.2 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.1 ± 0.0</td>
<td>-0.1 ± 0.1</td>
<td>-0.1 ± 0.0</td>
<td>0.0 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate (μmol/L)</td>
<td>-79 ± 34</td>
<td>6 ± 14</td>
<td>40 ± 31</td>
<td>98 ± 38</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glycerol (μmol/L)</td>
<td>1.7 ± 3.3</td>
<td>11.1 ± 3.9‡</td>
<td>14.4 ± 1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA (μmol/L)</td>
<td>2 ± 24</td>
<td>97 ± 66</td>
<td>85 ± 38</td>
<td>120 ± 34</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (μmol/L)</td>
<td>53 ± 42</td>
<td>6 ± 38</td>
<td>10 ± 64</td>
<td>143 ± 38</td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>-2.7 ± 2.2</td>
<td>-2.7 ± 2.8</td>
<td>-5.1 ± 2.7</td>
<td>0.2 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>C-peptide (pmol/L)</td>
<td>20 ± 0</td>
<td>40 ± 20</td>
<td>0 ± 20</td>
<td>10 ± 20</td>
<td>NS</td>
</tr>
</tbody>
</table>

* x ± SEM.
† These p values indicate statistical difference between doses analyzed by a two-way analysis of variance.
§§ Significantly different from placebo (by comparing x ± SEM of the three caffeine doses with placebo by post hoc testing by use of the mean square of residuals): ‡ p < 0.05, § p < 0.001, ¶ p < 0.005.
†† Nonesterified fatty acids.

whereas no significant change was observed after 400 mg caffeine. The changes, however, were small and no significant difference was found between the integrated responses assessed by ANOVA (Table 2). In contrast, a significant positive correlation was found between plasma caffeine response and the integrated insulin response (r = 0.43; p = 0.04). Interestingly, a positive correlation was found between insulin response and lactate response (r = 0.45; p = 0.02). No changes were found in plasma concentrations of C peptide (Fig 4 and Table 2) or of pancreatic glucagon (data not shown).

Glycerol and nonesterified fatty acids

Plasma glycerol concentration increased slightly after placebo as a result of lipolysis induced by fasting (Fig 5 and Table 2). By contrast, caffeine had a pronounced impact on plasma glycerol concentration, the most potent stimuli being 200- and 400-mg doses. There was also a positive correlation between the caffeine dose and the glycerol response (Table 3). In the placebo test nonesterified fatty acids (NEFA) did not change significantly above baseline (Fig 5 and Table 2). All doses of caffeine had a pronounced effect on NEFA but a considerable variation between subjects was observed.

The plasma triglyceride concentration increased slightly but significantly after placebo and 100 and 200 mg caffeine (p < 0.05) (Fig 5 and Table 2). After 400 mg caffeine a more marked increase was found. After this dose triglyceride increased progres-

TABLE 3
Correlation coefficients between caffeine dose and serum concentrations and integrated responses of thermogenesis, substrates, metabolites, and hormones

<table>
<thead>
<tr>
<th>Caffeine dose (n=24)</th>
<th>Serum caffeine concentration (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermogenesis</td>
<td>0.59*</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.56*</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.52†</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.48†</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.35</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.49†</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.25</td>
</tr>
<tr>
<td>C-peptide</td>
<td>0.19</td>
</tr>
<tr>
<td>Glucagon</td>
<td>-0.13</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.42†</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.27</td>
</tr>
<tr>
<td>Heart rate</td>
<td>-0.10</td>
</tr>
</tbody>
</table>

* p < 0.01.
† p < 0.05.

FIG 4. Plasma concentration of insulin and C peptide before and after oral intake of 0, 100, 200, and 400 mg caffeine. Mean values (±SEM) of six subjects.
caffeine and the changes did not differ from those observed after placebo (Fig 6). In contrast, 400 mg caffeine increased systolic blood pressure, with a peak of 10 mm Hg 60 min after the intake and with an average increase of 6.3 mm Hg (Table 2). A similar increase was observed in diastolic blood pressure. There was a positive correlation between caffeine dose and integrated increase in systolic blood pressure \((p = 0.04)\) whereas the relation between serum caffeine concentration and systolic blood pressure was not statistically significant \((p < 0.09)\). Analysis showed a linear, positive correlation between the integrated responses of systolic and diastolic blood pressures \((r = 0.86; n = 24; p < 0.00001)\).

The heart rate increased, on average, 4 beats/min after placebo \((p < 0.05)\). After all three doses of caffeine a diphasic response was observed (Fig 6). Initially, heart rate decreased by 3–4 beats/min 30–90 min after the intake and subsequently increased again at 90–180 min. Only after 400 mg caffeine was the final increase significantly above baseline.

**Side effects**

Few (0–1) side effects were reported after placebo and 100 and 200 mg caffeine. By contrast, after 400 mg caffeine significantly more subjects reported side effects compared with placebo \((p < 0.01)\): four reported palpitation; three, anxiety; three, headache; two, restlessness; and one, dizziness.

**Analysis of factors determining the thermogenic response**

When all single factors were tested separately against the integrated thermic response to caffeine, only three significant re-

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**FIG 5.** Plasma concentrations of glycerol, nonesterified fatty acids, and triglyceride before and after intake of different doses of caffeine. Mean values of six subjects.

**FIG 6.** Systolic and diastolic blood pressure and heart rate before and after intake of different doses of caffeine. Mean values (±SEM) of six subjects.
The thermogenic effect of caffeine. A highly significant correlation was found between the plasma response of lactate and the thermogenic response \( r = 0.79; p = 0.000001 \); Fig 7). Also, the integrated increase in plasma triglyceride was positively correlated with the thermogenic response \( r = 0.59; p = 0.009 \). To elucidate the possible contribution of these changes and other variables to the thermogenic effect, a stepwise regression analysis was performed with the thermogenic response as the dependent variable and all integrated changes in plasma substrates, hormones, blood pressure, and heart rate as independent variables. The analysis demonstrated that the changes in lactate, triglyceride, and heart rate were significant predictors of the thermogenic response (Table 4). The squared coefficient of correlation \( r^2 = 0.82^2 = 0.67 \) implies that 67% of the variation in thermic response may be accounted for by these three variables.

Although initially well correlated with the thermogenic response, the plasma caffeine response was excluded during the analysis. This points to caffeine as exerting its thermogenic effect not directly but indirectly through energy-consuming processes related to the three variables.

It is likely that substrate cycles involving lactate and triglyceride are responsible for the major part of the thermogenic response. Clearly, the changes in plasma concentrations are not accurate measures of the fluxes through the energy-consuming substrate cycles. Thus determination of the fluxes by isotopes may produce results that will better explain the variation in thermogenic response to caffeine.

The relative importance of the mechanisms by which caffeine exerts its various effects is not fully clarified. Caffeine probably exerts most of its effects through antagonism of adenosine receptors although phosphodiesterase inhibition and calcium mobilization may also be important. The effects on carbohydrate metabolism are generally considered to be small and inconsistent (21), and the increases in plasma glucose and insulin after caffeine in the study by Acheson et al (5) were not statistically significant (see Table 3 in ref 5). By contrast, a small but significant increase was found in blood glucose after a dose of 250 mg caffeine compared with water (22). Also, in the present study the impact of caffeine on plasma glucose, insulin, and C peptide was very modest (Figs 3 and 4 and Table 2). However, in the present study the impact of caffeine on plasma glucose, insulin, and C peptide was very modest (Figs 3 and 4 and Table 2). However, in the present study the impact of caffeine on plasma glucose, insulin, and C peptide was very modest (Figs 3 and 4 and Table 2). However, in the present study the impact of caffeine on plasma glucose, insulin, and C peptide was very modest (Figs 3 and 4 and Table 2). However, in the present study the impact of caffeine on plasma glucose, insulin, and C peptide was very modest (Figs 3 and 4 and Table 2). However, in the present study the impact of caffeine on plasma glucose, insulin, and C peptide was very modest (Figs 3 and 4 and Table 2).

**TABLE 4**

Results of model fitting by use of stepwise regression analysis of the thermogenic response to caffeine as the dependent variable and substrate, hormone, and other measures (see text) as the independent variables.

<table>
<thead>
<tr>
<th></th>
<th>( k )</th>
<th>( SEE )</th>
<th>( t )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>15.341</td>
<td>2.0092</td>
<td>7.6356</td>
<td>0.0000</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.531</td>
<td>0.1176</td>
<td>4.5153</td>
<td>0.0004</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.303</td>
<td>0.0894</td>
<td>3.3878</td>
<td>0.0038</td>
</tr>
<tr>
<td>Heart rate</td>
<td>-0.005</td>
<td>0.0022</td>
<td>-0.2042</td>
<td>0.0580</td>
</tr>
</tbody>
</table>
the association between the lactate increase and thermogenic response suggest the involvement of the thermogenic Cori cycle, ie, the conversion of glycogen and glucose to lactate in muscle and adipose tissue (23) and subsequent hepatic gluconeogenesis and glycogenosis (24). This substrate cycle and other hepatic thermogenic processes triggered by lactate (25) may explain the thermogenic contribution of lactate to the effect of caffeine. Svedmyr (26) also found that the increase in plasma lactate was closely correlated with the thermogenic effect of epinephrine in humans.

The increases in plasma glycerol and NEFAs are due to a well-described lipolytic action of caffeine and other methylxanthine derivates (5, 7). By contrast, the increase in triglyceride is less well recognized (21). In the present study this effect was dose-dependent and correlated with the plasma caffeine concentration (Fig 5 and Tables 2 and 3). The increase in plasma triglyceride was positively correlated with the thermogenic response \((r = 0.53)\) and this variable was not excluded by the stepwise regression analysis (Table 4). Thus lipolysis in adipose tissue and subsequent hepatic reesterification, ie, formation of triglyceride, may explain why the increase in triglyceride correlates with the thermic effect of caffeine. This is supported by the findings that inhibition of lipolysis by nicotinic acid reduces the thermogenic response to norepinephrine (27) and that raising the NEFA concentrations by adding triglyceride exogenously has a pronounced thermic effect and increases hepatic blood flow (28).

Except for 400 mg caffeine, only a weak influence of caffeine was detected on blood pressure and heart rate (Fig 6 and Tables 2 and 3). The cardiovascular effects of caffeine are known in detail because of the studies by Robertson et al (9, 10) and Whitsett et al (29). When 250 mg oral caffeine was given to people who do not drink coffee, systolic blood pressure increased by 10 mm Hg whereas heart rate showed a diphasic decrease after the first hour followed by an increase above baseline after 2 h (10). However, in a subsequent study Robertson et al (9) found that during chronic caffeine intake an essentially complete tolerance developed to these effects after 1–4 d of consumption of 750 mg caffeine/d.

Because a modest daily caffeine intake was allowed in the present study, our subjects may have developed partial tolerance. This could explain why only the highest dose of caffeine had a substantial effect on blood pressure (Fig 6). The diphasic response in heart rate was found after 400 mg caffeine (Fig 6). That the response in heart rate inversely contributes to the thermic effect of caffeine is not immediately intelligible. Caffeine significantly increases cardiac contractility and hence stroke volume and cardiac output (30). In addition, caffeine increases peripheral vascular resistance (29). Consequently, the decrease in heart rate is likely mediated by reflexes secondary to the increase in blood pressure, and the magnitude of the decrease may reflect the energy expenditure associated with increased cardiac contractility and vasoconstriction caused by increased smooth muscle tone.

Caffeine significantly increases plasma concentrations of norepinephrine and epinephrine (9, 10). Conceivably, the thermogenic effect may be mediated by \(\beta\)-adrenergic stimulation. However, this possibility was ruled out by Jung et al (7), who found that a \(\beta\)-adrenergic blockade did not reduce the thermogenic or lipolytic effect of caffeine. In addition, Robertson et al (10) reported that the cardiovascular effects of caffeine are normal in patients who lack a functioning autonomic nervous system.

A significant thermogenic effect was found even after the lowest dose of caffeine (100 mg) despite the fact that our experimental subjects had a habitual caffeine intake of 100–200 mg/d, which was confirmed by the finding of a fasting plasma concentration of caffeine of 2–6 \(\mu\)mol/L (Fig 1). The magnitude of the thermogenic response was clearly underestimated because the energy expenditure had not returned to baseline levels when the measurements were ended 3 h after the intake (Fig 2). Although a certain degree of tolerance to the thermogenic effect of caffeine may develop, these results suggest that a substantial effect remains during a moderate daily caffeine consumption. In a very recent study Dulloo et al (31) found a 3–4% increase in energy expenditure after oral administration of 100 mg caffeine in subjects who consumed 250–500 mg/d, which is twice the daily intake of our subjects. Also, physical activity may enhance the thermogenic response to caffeine (32), which may be important for weight reduction. The thermogenic effect of caffeine may reduce body fat stores in coffee drinkers if energy balance is not maintained by an increased energy intake. Thus the effect of caffeine in the treatment of obesity remains to be determined.

References
13. Garby L, Astrup A. The relationship between the respiratory quo-