Effect of paraxanthine on FFA mobilization after intravenous caffeine administration in humans

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HETZLER, RONALD K., RONALD G. KNOWLTON, SATU M. SOMANI, DALE D. BROWN, AND ROLLIN M. PERKINS III. *Effect of paraxanthine on FFA mobilization after intravenous caffeine administration in humans.* J. Appl. Physiol. 68(1): 44–47, 1990.—Because it has previously been shown that it takes much more caffeine to cause fat mobilization in vitro than in vivo, it has been suggested that there may be an active metabolite working with caffeine causing an increase in lipolysis in vivo. To determine the relationship between the appearance of paraxanthine (caffeine's major dimethylxanthine metabolite) and free fatty acid (FFA) mobilization after intravenous caffeine administration, 10 men were studied at rest after receiving a dose of 4 mg/kg lean body mass. Venous blood samples were obtained before dosing and at *minutes 5, 10, 15, 30, 45, 60, 90, 120, 150*, and *180*. Serum levels of FFA, glycerol, caffeine, and paraxanthine were determined in duplicate. Concentrations of FFA and glycerol were corrected for plasma volume changes. A high negative correlation was seen between decreases in caffeine and increases in FFA ($r = -0.90$) and a high positive correlation was seen between the appearance of paraxanthine and FFA ($r = 0.93$). It was concluded that paraxanthine may play a role in increased lipolysis after caffeine administration in humans.

methylxanthines; lipolysis; caffeine metabolite

IT IS WELL KNOWN that caffeine (1,3,7-trimethylxanthine) is one of the most widely used drugs in the world. Methylxanthines are central nervous system stimulants and effective bronchodilators useful in the treatment of asthma (13). The administration of caffeine (CAF) to fasted humans also causes an increase in free fatty acid (FFA) mobilization (3, 8). Recently it has been proposed that the mechanism of action of CAF for the increased lipolysis is blockage of adenosine receptors (13). It has also been shown that it takes much more CAF to cause fat mobilization in vitro than in vivo (22), which suggests that there may be an active metabolite working with CAF to bring about the increase in lipolysis.

Paraxanthine (PX; 1,7-dimethylxanthine) is the major dimethylxanthine metabolite of CAF in humans (16). There have been a number of recent pharmacokinetic studies on PX describing the half-life, volume of distribution, and clearance in animals and humans (5, 11, 16). However, to our knowledge, the pharmacodynamic actions of PX on lipolysis have not been studied. Therefore, the purpose of the present study was to investigate the relationship between the appearance of PX in the circulatory system and the increase in serum FFA concentrations after the administration of CAF.

PROCEDURES AND METHODS

Ten healthy adult men were recruited as subjects. The subjects had the following physical characteristics (mean ± SD): age, 27.9 ± 6.2 yr; weight, 85.1 ± 15.4 kg; height, 178.9 ± 4.5 cm; percent fat, 20.4 ± 9.5; lean body mass, 66.7 ± 8.0 kg; and CAF dose, 266.9 ± 32.1 mg. They were required to complete a health questionnaire and sign an institutionally approved informed consent form.

The subjects were asked to report to the laboratory on two separate occasions. During the initial session the subjects were hydrostatically weighed to determine body density by using the equation of Keys and Brozek (14). Siri's equation (20) was used to calculate the percent of fat, with lean body mass considered to be the difference between total body weight and the weight of the fat mass. Residual volume was estimated according to the procedure described by Wilmore (21).

The final session served as the treatment condition in which the subjects were asked to report to the laboratory 12 h postprandial after abstinence from CAF-containing products for a minimum of 48 h. A pharmaceutically prepared dose of CAF (4.0 mg/kg lean body wt) was administered intravenously in the form of CAF citrate (Pharma-Serve, Queens Village, NY). One subject (*subject 9*) experienced discomfort and disorientation immediately after administration of the drug. This subject also complained of severe headaches after the session. The infusion of the CAF took <2 min, after which the subjects remained seated throughout the 3-h test session.

Serial samples of blood were obtained from an indwelling Teflon catheter (20 gauge) placed in a superficial arm vein. The catheter was kept patent with an intravenous infusion of isotonic saline. Blood was collected utilizing a four-syringe technique in which the first 2.0 ml were discarded to ensure against the contamination of the blood with the saline solution. Venous blood samples were collected before dosing and at *minutes 5, 15, 30, 45, 60, 90, 120, 150*, and *180* after the dosing. An ~30-ml blood sample was collected at each time period in 10-ml syringes. Approximately 3 ml of blood were transferred to a tube containing EDTA, and the remaining portion of the sample was transferred to silicone-coated tubes.

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The samples collected in tubes containing EDTA were immediately separated into triplicate samples for the determination of hematocrit and duplicate samples for the determination of blood hemoglobin concentrations. Serum was prepared from the silicone-coated tubes and transferred to glass vials, which were stored at −20°C.

The concentration of hemoglobin was determined by the cyanmethemoglobin method (Sigma Chemical, St. Louis, MO). Hemoglobin standard curves were established with a standard supplied by Sigma Chemical. Hematocrit values were corrected for 4% trapped plasma in the packed erythrocytes. Plasma volume shifts were calculated via the method of Dill and Costill (9).

Duplicate determinations of FFA concentrations were made colorimetrically following the procedure described by Falholt et al. (12). Standard curves for FFA were established from dilutions of a stock solution of 2 mM palmitic acid resulting in concentrations that were in the expected physiological range. The coefficients of variation for the assays were 8.3% within day and 4.2% between day. Serum-free glycerol (GLY) concentrations were determined spectrophotometrically by enzymatic assay (Sigma Chemical). Values for the concentrations of FFA and GLY were corrected for changes in plasma volume.

The concentrations of CAF and PX were determined in duplicate through the use of reverse-phase high-pressure liquid chromatography (11). The sample was injected through a 100-μl injection loop connected to a model 7126 syringe-loading sample injector valve (Rheodyne, Cotati, CA) onto a radial compression 5-μm C_{18} reverse-phase column protected with a C_{18} Corasil Bondapak precolumn (Waters, Bedford, MA). The columns were connected to a Waters model 5000 pump. A Waters (model 481) multiwavelength ultraviolet detector set at 214 nm, coupled with a Hewlett-Packard model 3390A integrator/recorder was used to determine the area under each peak.

The ratio of the area under the sample peaks to the area under the internal standard peaks was compared with previously prepared standard curves to determine the concentrations of CAF and PX. Standard curves were prepared (using standards obtained from Research Biochemicals, Wayland, MA) by spiking blank plasma with CAF and PX. Between-day coefficients of variation were determined by injecting duplicate samples onto the column on 5 consecutive days and within-day variations by injecting 10 samples onto the column on the same day. The coefficients of variation for the assays were 9.3% within day and 7.1% between day. The sensitivity of this assay allowed for the detection of samples to a level of 0.2 μg/ml.

Descriptive statistics were generated. The relationships between the appearance of FFA and GLY with CAF and PX were determined through the use of the Pearson product moment correlation coefficient. A one-way analysis of variance was performed to test for concentration changes over time including a Fisher least significant difference test for post hoc comparisons.

RESULTS

The mean concentrations of CAF and PX are reported in Table 1. The corrected values for serum FFA and GLY are presented in Table 2. Only trace amounts of CAF were seen in the serum before dosing. Serum FFA concentrations changed significantly over time ($P < 0.0001$) and demonstrated a transient decrease below resting levels in the initial 5–15 min followed by a continuous elevation throughout the remaining 3-h period. Serum CAF concentrations changed significantly over time ($P < 0.0001$) and systematically decreased throughout the trial. There was a high negative correlation between the changes in CAF concentrations and changes in FFA concentrations ($r = -0.90$, $P < 0.05$).

The mean serum PX concentrations changed significantly over time ($P < 0.0001$) and rose throughout the trial. There was a high correlation between changes in PX and FFA concentrations ($r = 0.93$, $P < 0.05$). The correlation between appearance of PX and serum-free GLY was not significant ($r = 0.57$, $P > 0.05$).

TABLE 1. *CAF and PX values*

Time, min	CAF	PX
0	0.06±0.05	0.08±0.07
5	8.72±1.23	0.24±0.07[a]
15	5.67±0.29[a]	0.43±0.09[a,b]
30	5.30±0.40[a,b]	0.60±0.09[a–c]
45	4.66±0.20[a,b]	0.68±0.07[a–c]
60	4.34±0.20[a–c]	0.71±0.07[a–c]
90	3.82±0.20[a–d]	0.86±0.09[a–f]
120	3.37±0.17[a–d]	1.01±0.06[a–g]
150	3.32±0.22[a–d]	1.05±0.05[a–g]
180	3.03±0.18[a–f]	1.15±0.08[a–h]

Values are means ± SE in μg/ml. CAF, caffeine; PX, paraxanthine. Significantly different ($P < 0.05$) from [a] 0 min, [b] 5 min, [c] 15 min, [d] 30 min, [e] 45 min, [f] 60 min, [g] 90 min, [h] 120 min.

TABLE 2. *FFA and GLY values*

Time, min	FFA	GLY
0	0.47±0.02	0.13±0.01
5	0.42±0.03	0.13±0.02
15	0.43±0.04	0.15±0.01
30	0.57±0.07[a–c]	0.16±0.02
45	0.64±0.04[a–c]	0.15±0.01
60	0.64±0.04[a–c]	0.16±0.01
90	0.65±0.03[a–c]	0.16±0.01
120	0.66±0.03[a–c]	0.17±0.01
150	0.72±0.03[a–d]	0.15±0.01
180	0.71±0.06[a–d]	0.14±0.01

Values are means ± SE in mM. FFA, free fatty acid; GLY, glycerol. Significantly different ($P < 0.05$) from [a] 0 min, [b] 5 min, [c] 15 min, [d] 30 min.

DISCUSSION

The most important finding of the present study was the high correlation observed between the appearance of PX and the increase in serum FFA. CAF increases lipolysis primarily as the result of blocking adenosine receptors on the surface of the adipose cell (13). After administration, CAF undergoes an initial *N*-demethylation in the liver to give dimethylxanthine metabolites, which are subsequently metabolized further (2). It has previously been reported that xanthines are the only major class of adenosine antagonists (6). Prior studies have shown that some xanthines are more potent specific antagonists to adenosine at the receptors on the surface of cells than

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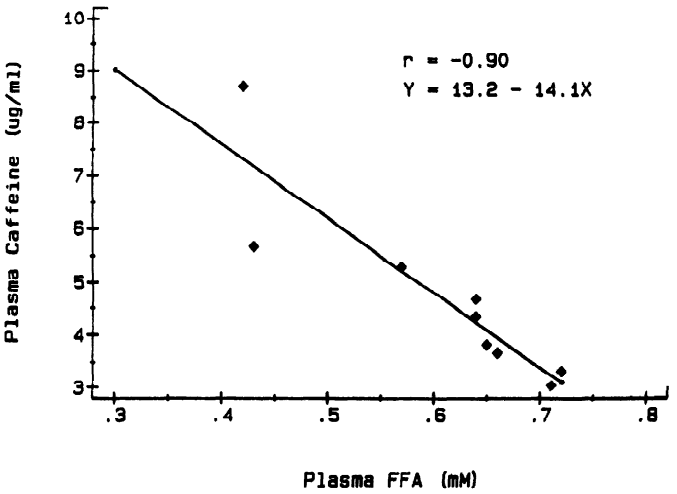

FIG. 1. Relationship between changes in caffeine concentrations and free fatty acid (FFA) concentrations, including line of best fit.

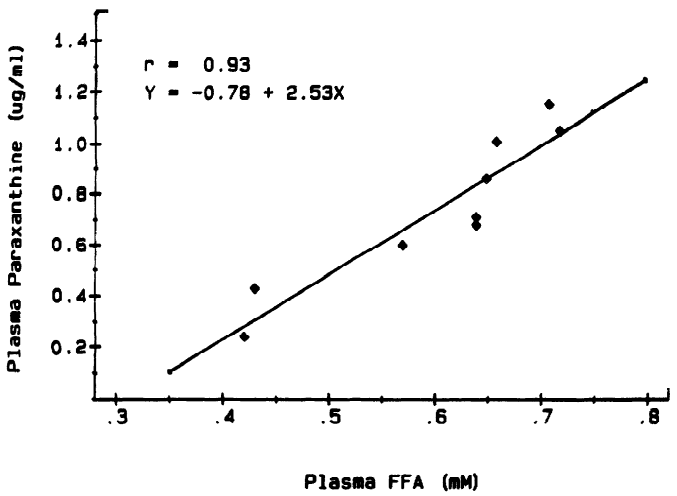

FIG. 2. Relationship between changes in paraxanthine concentrations and free fatty acid (FFA) concentrations, including line of best fit.

others (13, 18) and that xanthines must have a methyl group in position 1 to be pharmacologically active (10, 18). Because PX (1,7-dimethylxanthine) is CAF's major dimethylxanthine metabolite and has previously been shown to be a more potent adenosine receptor blocker than CAF (5, 18), PX may also act as an antagonist of adenosine at the adenosine receptors on the surface of the adipose cell and thus increase lipolysis.

Bonati and co-workers (4) studied CAF deposition after oral doses ranging from 0.22 to 10 mg/kg. They reported that only the three least polar metabolites, the dimethylxanthines (theobromine, theophylline, and PX), could be measured in serum. Therefore, of the ~26 metabolites of CAF, only these compounds would be expected to exert a physiological effect on other organ systems.

After an oral dose of CAF, peak serum levels of the drug are reached within 1–2 h (1, 3, 17). Over a period of several hours the serum concentration of CAF goes down, but the concentration of FFA continues to rise or level off (1, 3). Because the concentration of PX was constantly increasing during the 3 h of rest while CAF is being metabolized, it is possible that PX has a role in lipolysis in vivo. Although drug metabolism usually leads to an accelerated termination of pharmacological activity, there are a number of examples where compounds that cause increased effects are produced by metabolism (15).

Winder (22) reported that much larger concentrations of CAF are necessary to stimulate lipolysis in adipocytes in vitro vs. in vivo and suggested that this may be the result of production of "more effective lipolytic metabolites of CAF" in vivo. Because the only dimethylxanthine with a methyl group in position 1 that is formed in any appreciable amount is PX, it seems logical to suggest that this metabolite is the most likely "effective lipolytic metabolite of CAF." A high negative correlation ($r = -0.90$) was seen between decreases in CAF concentrations and increases in serum FFA concentrations (Fig. 1). There was also a high correlation between the appearance of PX and changes in FFA concentrations ($r = 0.93$; Fig. 2). Therefore, as CAF was metabolized to PX, there was a simultaneous increase in lipolysis. Although correlational relationships should not be interpreted as being causal, data from the present study offer support for the idea that PX may have an important role in the mechanism of action of CAF on lipolysis.

Serum-free GLY is an important marker for lipolysis. During increased fat mobilization, FFA and GLY are released into the blood from the adipose tissue. Simultaneous release of GLY and FFA indicates augmented lipolysis, not diminished esterification (7). Although increase in serum-free GLY cannot be used as a quantitative measure of lipolytic activity, it may be used as an indirect indicator of lipolysis (23). If triglycerides are completely hydrolyzed and if FFA are not simultaneously metabolized, then 3 mol of FFA are released for each mole of GLY. However, this relationship was not seen in the present study, probably because GLY is removed in the liver as a gluconeogenic precursor (19) and GLY may not be detected as serum-free GLY as a result of incomplete hydrolysis of FFA.

In summary, as CAF is metabolized and PX is formed, there is a concomitant rise in FFA. The strong relationship between the appearance of PX and FFA, coupled with the fact that more CAF is needed to elicit the same lipolytic response in vitro compared with in vivo, indicates that PX may be an active lipolytic agent. Therefore, a need exists to further investigate physiological responses of humans to PX, which will lead to a better understanding of the mechanism of action of CAF.

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