THE EFFECT OF CAFFEINE INGESTION ON FAT METABOLISM DURING EXERCISE IN THE FASTED AND NON-FASTED STATE

by

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Major Professor

To My

MOTHER and FATHER

Ist nicht die Kindheit der verborgene
Keim, aus welchem nach und nach der
reiche Baum des Lebens mit allen seinen
Leiden und Freuden sich auseinanderschlägt?

Johann Peter Hebel

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Chapter 1

Introduction

The most important objective of any weight reduction program is the loss of body fat. Fat loss may be enhanced by any activity, drug, or condition causing a higher rate of fat metabolism. The fact that fat is the preferred energy source utilized during aerobic exercise is well established (Friedberg, Sher, Bogdonoff, & Estes, 1963; Issekutz, Miller, Paul, & Rodahl, 1964; Jones & Havel, 1967; Carlson, 1967; Paul & Holmes, 1975; Ivy, Costill, Fink, & Lower, 1979). Caffeine seems to be another factor capable of stimulating the breakdown of adipose tissue fat and its release into the blood (Bellet, Kershbaum, & Finck, 1968a; Bellet, Kershbaum, & Roman, 1968b; Jung, Shetty, James, Barrand, & Callingham, 1981). Some studies suggest the possibility of an additive fat oxidative effect of caffeine to high intensity bicycling (Costill, Dalsky, & Fink, 1978) and aerobic exercise in rats (Wilcox, 1982). Another factor altering the degree to which fat is utilized for energy production is the time elapsed after food intake. Immediately after a meal, carbohydrate is the main energy source for the body, but with time, a shift to greater fat usage takes place. Thus, in the fasted state (12 hours after the last meal) fat oxidation is higher than right after food intake (Wertheimer, Hamosh, & Shafrir, 1960; Guyton, 1981). It has not been clarified whether this relationship holds true in conjunction with aerobic exercise.

To verify fat utilization, one can measure and analyze air

volumes breathed by a subject and calculate a respiratory exchange ratio (R). R represents the ratio between the carbon dioxide produced and the oxygen taken up by the body cells. It is a valid indicator of substrate utilization, because the chemical composition of fat differs from that of carbohydrates. Carbohydrate molecules are constructed of equal numbers of carbon and oxygen atoms which makes the amount of carbon dioxide produced during their breakdown always identical to the amount of oxygen used. This results in an R of 1.0, showing that only carbohydrate is used as the energy source (Guyton, 1981). Fat molecules, on the other hand, consist of fewer oxygen than carbon atoms. Thus, fat oxidation requires greater cellular oxygen uptake than carbon dioxide production, depressing R values close to 0.7 when only fat is utilized for energy production.

This study is designed to clarify first, whether caffeine and the fasted condition further increase the already high fat oxidation during aerobic exercise and, second, which combination of factors most effectively enhances fat utilization during submaximal activity.

Statement of the Problem

The purpose of this investigation is to study the following questions: is fat metabolism during aerobic exercise enhanced by prior caffeine ingestion, does prior fasting increase the amount of fat oxidized during exercise and, are the effects of caffeine and fasting on fat oxidation additive?

Significance of the Study

Todays lifestyle and concern about maintaining an ideal body weight have popularized weight reduction programs. Although weight loss programs are part of an overwhelming number of people's lives, not all of these reduction programs effectively fight excess body fat. It has been found, for example, that diet alone does not optimally improve body composition (McArdle, Katch, & Katch, 1981). Also, exercise regimens could possibly become more potent fat weight reduction tools through the addition of caffeine and fasted condition effects. Fat metabolizing agents such as caffeine are of special practical value, since they are usually ingested in sufficient amounts as part of the daily food intake. Little is known about the interaction between aerobic exercise, caffeine, and food consumption on the utilization of fat as the major source for energy production. This study will try to clarify these interrelationships and to develop the most effective sequence and combination of these three factors for fat oxidative purposes.

Hypotheses

1. Caffeine ingestion increases fat metabolism during aerobic exercise as manifested in the reduction of the respiratory exchange ratio.
2. Aerobic exercise in the fasted state will produce greater total fat oxidation than in the non-fasted state.
3. Caffeine ingestion prior to aerobic exercise in fasted subjects decreases respiratory exchange ratio values.

Delimitations

1. The subjects of this study were six male and six female volunteers, 19 to 33 years old.
2. All subjects were regular, recreational runners who could run on the treadmill for 30 minutes at an intensity of 60% of their maximal oxygen uptake.

Limitations

1. The R values during data collection time could have been influenced by central nervous system stimulation by caffeine, which would have then, in part, been responsible for lower R values.
2. The lunch meal taken prior to the non-fasted test trials was not restricted (except for caffeine intake) and may have differed substantially between subjects. This was to approximate a "real life" situation as closely as possible.

Definition of Terms

Respiratory Exchange Ratio (R): the ratio between the amount of carbon dioxide produced and oxygen used by the body cells during exercise.

Respiratory Quotient (RQ): the ratio between the amount of carbon dioxide produced and oxygen used by the body cells at rest.

Fasted State: twelve or more hours without caloric intake.

Non-Fasted State: the condition two to three hours after the consumption of the lunch meal each subject was accustomed to.

Maximal Oxygen Uptake (VO_2max): the maximal amount of oxygen that can be utilized by the body cells.

Chapter 2

Review of Literature

The mechanism of fat oxidation and the validity of the respiratory exchange ratio as an indicator of fat substrate utilization will be dicussed. Literature pertaining to fat metabolism during aerobic exercise, after caffeine ingestion, and in the fasted state will be reviewed.

The Mechanism of Fat Substrate Utilization

Most fat not used for energy production immediately after food intake is stored in the body's adipose tissue in the form of triglyceride. When these triglyceride depots need to be tapped, hormones such as epinephrine (Wertheimer et al., 1960) and norepinephrine (Armstrong et al., 1961) stimulate the enzyme adenylate cyclase, which catalyzes an endergonic reaction resulting in the increased production of cyclic AMP (cAMP). This molecule, in turn, causes the conversion of protein kinase from its inactive into its active form which now can catalyze the phosphorylation of the enzyme triglyceride lipase. Triglyceride lipase initiates the hydrolysis of triglycerides to glycerol and free fatty acids (FFA) which are then able to move from the adipocyte into the bloodstream (Severson, 1979). From there, FFA enter the working cells, where they are further catabolized to yield energy. The rate of FFA uptake by active cells is regulated by a mass action effect; the higher the blood FFA concentration, the greater the uptake by the working tissues (Jones & Havel, 1967; Paul, 1970). Thus, high plasma FFA levels

directly cause high fat metabolism (Armstrong et al., 1961).

The Respiratory Exchange Ratio

R is defined as the ratio between the carbon dioxide produced and the oxygen used by the body cells. This ratio is an indicator of substrate utilization because the chemical composition of fats differs from that of carbohydrates. Since the ratio of oxygen to carbon is 1:1 in all carbohydrate molecules, the amount of carbon dioxide produced is always equal to the amount of oxygen used for the breakdown of carbohydrates, resulting in an RQ of 1.0 (Guyton, 1981). Fat molecules, on the other hand, consist of fewer oxygen atoms than carbon atoms. Therefore, the catabolism of fat requires more oxygen consumed than carbon dioxide released, which depresses the RQ below 1.0 and close to 0.7 (McArdle et al., 1981).

The calculations for R are based on the expired total air volume, the partial pressures of carbon dioxide and oxygen in the expired air, and the knowledge of room air composition. Through the use of several equations, raw data can be converted to R values. Once the ratio is obtained, which can range between 0.7 and 1.0, the exact percent contribution of fat and carbohydrate to energy production can be determined from available tables. Proteins do not significantly contribute to energy metabolism during exercise in healthy individuals (Sinning, 1975), so only non-protein R values will be considered in this study.

The validity of RQs as a measure of fat metabolism in conjunction with caffeine ingestion has been of concern (Haldi,

Bachmann, Ensor, and Wynn, 1941; 1944). Haldi et al. found in both their studies that caffeine does stimulate the respiratory centers in the brain and does cause a significant increase in tidal volume 15 to 30 minutes after the ingestion of 3mg/kg or 6mg/kg doses of caffeine, resulting in a marked increase in RQs. They conclude that the subsequent fall in RQ values, with the lowest values occurring at approximately one hour after caffeine intake, is a compensatory effect by which blood carbon dioxide levels are normalized. Newer research has shown,however, that this low dip in the RQ curve, appearing at about one hour after the ingestion of comparable caffeine doses, is associated with high concentrations of FFA in the blood (Bellet. et al. 1968b; Bellet, Roman, DeCastro, Kim, and Kershbaum, 1969). Paul et al. (1966) as well as Pruett (1970) measured both plasma FFA and RQ during resting and exercising conditions and found a high negative correlation between the two measures. The RQ, therefore, is a valid method for the assessment of substrate utilization, even in experiments investigating caffeine effects.

Exercise and Fat Metabolism

At the beginning of exercise, predominantly anaerobic pathways are utilized for energy production, with carbohydrate as the major substrate source. When exercising at very high intensities, glycolysis is continuously responsible for supplying new ATP to the working cells (McArdle et al., 1981). With increasing work intensities more lactate is produced during this process which, in turn, has been found to depress plasma FFA

(Issekutz et al., 1966; Pruett, 1970) and their oxidation (Issekutz et al., 1966). Thus, carbohydrate remains the major energy source throughout this type of exercise. During submaximal activities, however, there is a shift towards greater fat utilization within the first minutes of exercise until a "steady state" is reached (McArdle et al., 1981). Biochemical data indicates that during this time an initial drop in plasma FFA, due to sudden increases in demand, takes place, which is followed by a gradual increase resulting from their enhanced release from adipose tissue (Friedberg et al., 1963; Havel et al., 1964; Issekutz et al., 1965; Carlson, 1967). Then, most energy comes from aerobic fat metabolism (McArdle et al., 1981) as evidenced through greater plasma FFA turnover (Armstrong et al., 1961; Havel et al., 1964; Paul & Holmes, 1975) and increased FFA oxidation (Issekutz et al., 1964; Jones & Havel, 1967). At rest, only 20-22% of the FFA taken up by the muscle cells are oxidized; this value climbs to 80-100% during submaximal exercise in dogs (Issekutz et al., 1964; Paul, 1970). Therefore, during submaximal exercise, R values fall significantly, as has been shown in numerous investigations (Friedberg, Sher, Bogdonoff, & Estes, 1963; Carlson, 1967; Paul, 1970; Froberg, & Mossfeldt, 1971).

The degree of fat oxidation can be altered by changing the availability of FFA. Hickson et al. (1977) found that rats injected with heparin, which causes an elevation of blood FFA, could exercise longer than control groups before reaching exhaustion. They attribute this finding to enhanced fat oxidation coupled with a slower rate of glycogen depletion.

Consistent results were reported with human subjects (Costill et al., 1977) who exercised on the treadmill at 68%$V0_2$ for 30 minutes after ingesting either a high fat meal or glucose. Exercising in the "fat" condition resulted in a significantly higher total fat metabolism when compared to the "glucose" trials. Accordingly, R calculations showed significantly lower values for exercise after the fatty meal. A similar study by Jansson (1982) compares the R for the exercising leg muscles to the total body R during submaximal peddling on a bicycle ergometer after a fat rich or carbohydrate rich diet. Both values were higher for the latter test group, thus confirming the validity of the R, even under extreme dietary conditions.

The Fasted State and Fat Metabolism

During the first few hours after food intake, blood glucose concentrations are very high and the pancreas secretes large quantities of insulin, which causes rapid uptake of glucose by the muscle cells (Guyton, 1981). In addition, insulin increases the activity of phosphofructokinase, the enzyme catalyzing the complete phosphorylation of glucose in the muscle cell, therewith enhancing glycolysis (Guyton, 1981). At the same time, insulin inhibits the action of triglyceride lipase and, thus, hydrolysis of triglyceride to FFA and glycerol (Guyton, 1981). Immediately after a meal, therefore, carbohydrate represents the major energy source.

With time, a shift towards greater fat utilization takes place. The slow decrease in blood glucose concentration results

in greater secretion of glucagon, an antagonist to insulin, from the pancreas (Guyton, 1981). Glucagon activates adenylcyclase in the adipocyte, initiating the reaction chain, as discussed above, ending with the breakdown of triglcyeride. Thus, approximately eight to ten hours after a meal, fat is the predominant substrate source (Guyton, 1981). In the fasted state, blood FFA concentrations are very high (Wertheimer et al., 1960; Paul et al., 1966), causing increased fat oxidation and an RQ very close to 0.7 (Pruett, 1970; Guyton, 1981). This relationship was confirmed at three miles per hour walking speeds either in the postabsorptive state or after a breakfast followed by continuous glucose injections during exrecise (Havel et al., 1963).

Caffeine and Fat Metabolism

Caffeine is known to inhibit the enzyme phosphodiesterase within the fat cell (Severson, 1979; Jung et al., 1981). This inhibitory effect of caffeine prevents the conversion of cAMP to 5'AMP and thus, through increasing cAMP levels, leads to the enhanced breakdown of triglycerides to FFA and glycerol which then are transported into the blood. High plasma FFA concentrations, in turn, are responsible for an increased uptake rate by the working cells and, thus, for increased fat metabolism (Issekutz et al., 1965; Jones & Havel, 1967; Paul, 1970). Another caffeine effect is the enhanced new production of cAMP through its stimulation of the central nervous system and the consequential increased release of catecholamines (Bellet et al., 1969; Levi, 1967). However, the catecholamine response to

caffeine has been found to be highly variable between subjects (Jung et al., 1981). Since adrenergic blockage prevented an increase in plasma FFA in response to exercise in untrained rats but not in trained animals (Gollnick, 1967), the possibility exists that this second caffeine initiated mechanism may be much more pronounced in untrained than in trained individuals.

The enhancing effect of caffeine on fat utilization has been shown by Bellet et al. (1968a). They investigated FFA responses to coffee (with 250 mg caffeine) ingestion during four hours in healthy male subjects and found significantly increased plasma FFA levels, with a peak value of 92% over pre-coffee levels. Sugar-free cola containing 46 mg caffeine had similar effects on FFA, with a maximal increase of 48% over pre-caffeine values (Bellet et al., 1968b). Male and female subjects showed significantly increased plasma FFA levels 15 to 75 minutes after ingestion of 150 mg caffeine in a cola drink (VanHandel, Berke, Costill, & Cote, 1977). A dose of 4mg/kg caffeine was administered during an investigation by Acheson, Zahorska-Markiewiez, Anantharaman, & Jequier (1980). Within the three hour duration of the tests, fat oxidation increased significantly when compared to the contol group.

Since most investigations of caffeine effects are conducted with fasted subjects, the results published by Acheson et al. (1980) are of special value. With a provided breakfast, test persons ingested either coffee containing 4mg/kg caffeine doses or decaffeinated coffee. During the first hour after the meal, fat oxidation decreased in both conditions. Following this initial drop, a significant rise over baseline levels in fat

utilization occurred only in the caffeine trial. It also has been found that sugar ingested in caffeinated drinks depresses the enhancing effect of caffeine on fat metabolism (Bellet et al., 1968a; 1968b). Thus, the caffeine effect can be expected to be much more pronounced in fasted individuals than in non-fasted subjects.

During aerobic exercise, fat metabolism is already increased, which may limit the potential for enhanced fat metabolism following caffeine ingestion. To clarify this question, Ivy et al. (1979) monitored trained cyclists during a two hour isokinetic cycling work-out and found FFA levels in those subjects exercising under influence of a 500 mg total caffeine dose to be significantly higher than in control subjects. At the same time, total fat oxidation was elevated in the caffeine trials. Similar data were collected during bicycle ergometer exercise to exhaustion at 80% VO_2max, where a significant drop in Rs was found after the ingestion of 330 mg of caffeine. Total fat oxidation with caffeine reached 118 g as compared to 57 g in the control trials (Costill. et al. 1978). For both studies (Ivy et al., 1979; Costill et al., 1978), all subjects were tested in the fasted state so that the "caffeine" effect really was the combined effect of caffeine and the fasted condition. A longitudinal study in which rats swam for 90 minutes, five times weekly, for nine weeks showed that the combined caffeine (5mg/kg) and exercise treatment most effectively decreased body weight and fat-pad weight (Wilcox, 1982). The "exercise only" and "caffeine only" proved to be the

second and third potent treatments for body fat and total body weight loss. Thus, it can be concluded that there maybe an "add-on" effect of caffeine to exercise, i.e. caffeine may increase the already high fat oxidation levels present during exercise.

Summary

Even though the mechanisms by which exercise, caffeine, and the fasted state enhance fat metabolism are well understood, very little research has investigated the caffeine and fasting/non-fasting effects during exercise. The question which, caffeine or the fasted condition, is a more potent enhancer of fat metabolism during exercise is yet to be answered. It is the intent of this study to clarify the above relationships.

Chapter 3

Methods

The selection of subjects, the equipment utilized, as well as the independent and dependent variables and their analysis will be outlined in this section.

Selection of Subjects

6 male and 6 female subjects, ages 19 to 33 years, volunteered for this study. All were regular, recreational joggers, able to run on the treadmill at 60% of their maximal oxygen uptake for 30 minutes. Each participant signed an informed consent form (Appendix A) explaining the intent and procedures of the study, and the discomforts and potential hazards involved. Questions, if any, were answered.

Equipment

The subjects exercised on a Quinton treadmill. Expired gases were continuously analyzed by a Beckman OM 11 oxygen analyzer and a Beckman LB 2 carbon dioxide analyzer. Total ventilation was measured with an Alpha Technologies Ventilation Meter. All instruments were carefully calibrated before each trial. The collected per minute gas data were entered into a computer for the calculation of R values.

Independent and Dependent Variables

The independent variable consisted of 4 treatment levels:

CM (caffeine/morning): 45 minutes after the ingestion of a caffeine dose of 4mg/kg body weight, dissolved in an artificially sweetened lemonade drink, the subject exercised on a level treadmill at 60% VO_2max for 30 minutes. The test was administered in the morning, following a 12 hour fast.

NCM (no caffeine/morning): the subject exercised on a level treadmill at 60% VO_2max for 30 minutes, following a 12 hour fast.

CA (caffeine/afternoon): 45 minutes after the ingestion of a caffeine dose of 4mg/kg body weight, dissolved in an artificially sweetened lemonade drink, the subject exercised on a level treadmill at 60% VO_2max for 30 minutes. This test was conducted in the afternoon, 3 to 4 hours after the subject's last meal.

NCA (no caffeine/afternoon): the subject exercised on a level treadmill at 60% VO_2max for 30 minutes, in the afternoon, 3 to 4 hours after the subject's last meal.

In all experiments, the minute values of expired oxygen and carbon dioxide were recorded. Each participant underwent all four treatments in a randomized order. Maximal oxygen uptake was measured with a maximal running test on the treadmill. The test was initiated at a speed of 5 or 6 miles per hour, on a level treadmill. Running speed was increased every 2 minutes, in 1mph increments, until the subject obviously had passed through 60% of their maximal oxygen uptake. At this point, the treadmill was

inclined by 2.5% every 2 minutes, with no change in speed, until exhaustion. By this protocol, the treadmill speed at which each subject reached 60% VO_2max was established. To increase experimental control, each subject's percent body fat, based on underwater weighing procedures, was calculated. The nitrogen dilution method was employed for residual volume computations.

The dependent variable was the exercise R value as a measure of fat substrate utilization.

Analysis of Data

A 2-way treatment structure, radomized complete block design was used and analyzed through a general linear model procedure. For single degree comparisons between caffeine/non-caffeine and fasted/non-fasted conditions least square mean analysis was employed.

Chapter 4

Results & Discussion

Some important subject characteristics will be summarized. The results pertaining to the respiratory exchange ratio, total caloric expenditure, and fat oxidation will be presented. A discussion of caffeine and fasting effects follows.

Results

Descriptive Data

Six male and six female recreational runners volunteered for this study. Their fitness status is summarized in table 1 and 2.

Table 1. Means and standard deviations for some subject characteristics (N=12)

	Means		Stand. Dev.	
	female	male	female	male
age (yrs)	25.83	25.67	5.000	5.501
weight (kg)	59.87	74.57	3.932	5.169
body fat (%)	21.70	12.72	5.297	4.887
max VO_2 ($ml/kg^{-1}min^{-1}$)	49.61	60.52	8.338	9.738

Table 2. Average VO_2 values during each treatment ($l/kg\ min^{-1}$)

	Means		Stand. Dev.	
	female	male	female	male
CM	30.81	36.26	4.0092	3.6188
NCM	33.87	36.59	3.7004	4.1740
CA	29.94	35.12	4.2221	1.6093
NCA	33.33	36.84	5.6351	2.7966

Respiratory Exchange Ratio

The general linear models procedure revealed significant differences ($p < 0.06$) in average respiratory exchange ratios (R_{avg}) between subjects and treatments. Table 3 presents a summary of R values and their statistical significance.

According to least square means analysis, caffeine ingestion prior to exercise resulted in a R_{avg} of 0.89, whereas non-caffeine exercise trials showed a significantly lower value of 0.84 ($p < 0.005$). R_{avg} levels for fasted and non-fasted groups were calculated as 0.84 and 0.89, respectively, and showed a significant difference at the alpha = 0.05 level.

No interaction between caffeine and fasted treatment levels was found. Figure 1 presents R values through exercise time for all treatment combinations.

Table 3. Respiratory exchange ratios and their significance

source	df	SS	F	P
subject	11	0.04558	2.12	0.0561
caffeine	1	0.02044	10.47	0.0033
fasting	1	0.01747	8.95	0.0060
caff*fast	1	0.00020	0.10	0.7536

	caffeine		no caffeine		total	
	LSMean	Std.Dev.	LSMean	Std.Dev.	LSMean	Std.Dev.
fasted	0.868	0.0135	0.818	0.0128	0.843	0.0093
non-fasted	0.907	0.0166	0.866	0.1444	0.887	0.0111
total	0.888	0.0106	0.842	0.0096		

Total Caloric Expenditure

Between-subject differences in total caloric expenditure for the 30 minute aerobic exercise bout was significant at the alpha =0.0001 level.

A total of 303 kilocalories (kcal) was expended during the "with caffeine" runs, whereas 309 kcal were utilized during the non-caffeine trials. This effect showed no statistical significance. Exercise in the fasted and non-fasted states

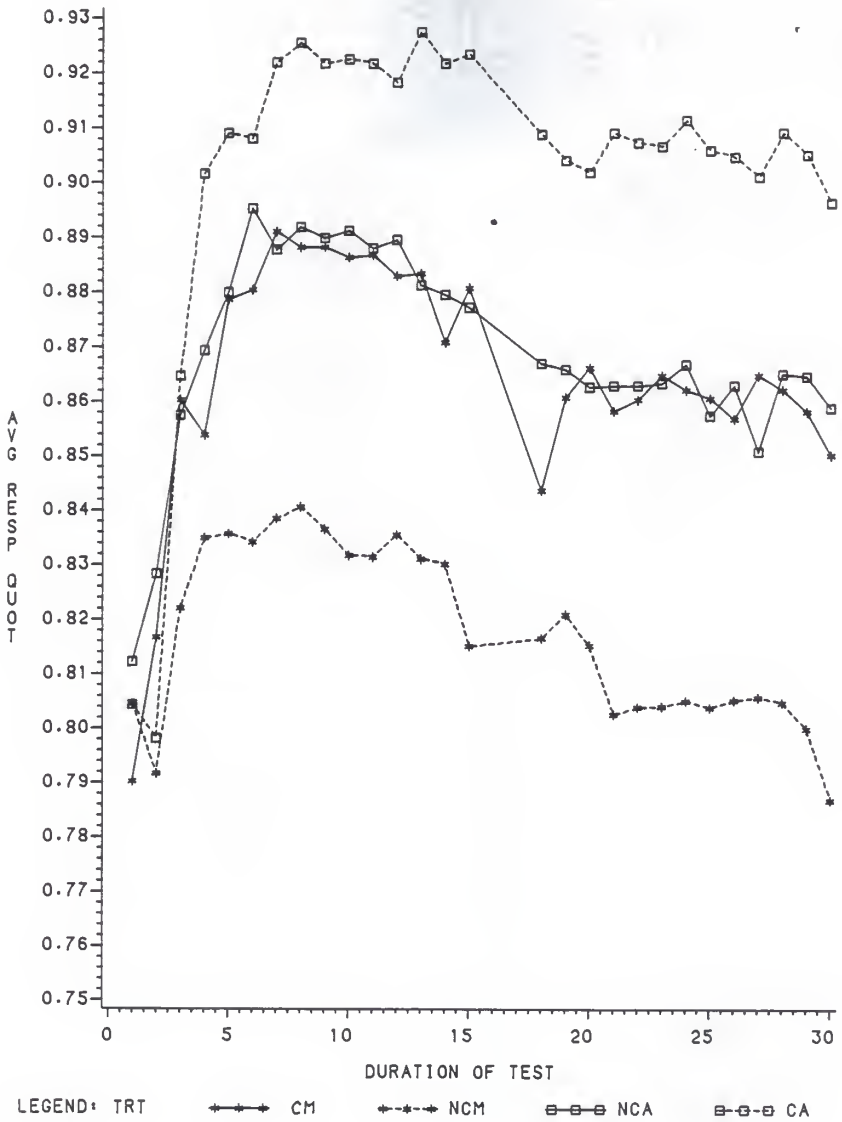

Figure 1. Fat utilization across testing time

resulted in a consumption of 301 and 311 kcal, respectively, a non-significant difference.

No interaction between treatments could be assessed. Table 4 summarizes all findings pertaining to caloric expenditure.

Table 4. Total caloric expenditure and statistical significance.

source	df	SS	F	P
subject	11	139776.7	26.56	0.0001
caffeine	1	284.3	0.59	0.4477
fasting	1	864.3	1.81	0.1905
caff*fast	1	320.4	0.67	0.4206

	caffeine		no caffeine		total	
	LSMean	Std.Dev.	LSMean	Std.Dev.	LSMean	Std.Dev.
fasted	301.4	6.6887	301.1	6.3142	301.27	4.5991
non-fasted	305.3	8.2035	316.6	7.1525	310.96	5.4945
total	303.4	5.2513	308.8	4.7704		

Fat Oxidation

The average calories (cal_{avg}) of fat utilized differed between subjects ($p < 0.02$) and treatments. Table 5 contains a summary of the statistical analysis.

During the non-caffeine trials, an average of 5.84 calories of fat was oxidized per minute of exercise, as compared to 4.09 cal_{avg} in the caffeine conditions ($p < 0.01$). Exercise in the fasted (5.78 cal_{avg}) and non-fasted (4.15 cal_{avg}) states differed significantly from each other ($p < 0.01$).

Interaction between caffeine and fasted treatment levels was not significant.

Table 5. Calories produced through fat oxidation per minute and statistical data

source	df	SS	F	P
subject	11	105.4036	2.86	0.0135
caffeine	1	29.2790	8.74	0.0066
fasting	1	24.4649	7.30	0.0120
caff*fast	1	0.31096	0.09	0.7631

	caffeine		no caffeine		total	
	LSMean	Std.Dev.	LSMean	Std.Dev.	LSMean	Std.Dev.
fasted	4.819	0.5598	6.748	0.5284	5.783	0.3849
non-fasted	3.370	0.6866	4.937	0.5986	4.153	0.4598
total	4.094	0.4394	5.843	0.3992		

Discussion

Caffeine Effect

In resting subjects, caffeine has been found to increase plasma FFA (Bellet et al., 1968a; Bellet et al., 1968b; VanHandel et al., 1977; Acheson et al., 1980). A 500 mg caffeine dose caused an increase in FFA concentration and elevated total fat oxidation during isokinetic bicycling (Ivy et al., 1979). Bicycle ergometer exercise to exhaustion at 80% VO_{max} after ingestion of 330 mg caffeine resulted in a significant drop in R values as well as greater total fat oxidation (Costill et al., 1978).

The results of this investigation do not agree with previous findings. Ingestion of a caffeine dose of 4 mg/kg caused an average R of 0.89 during 30 minutes of submaximal treadmill running. A significantly lower R was calculated for non-caffeine trials (R=0.84). Accordingly, a smaller number of fat calories was expended per minute of exercise in the caffeine (4.09 kcal) than in the non-caffeine condition (5.84 kcal). Caffeine did not stimulate overall metabolism, as expressed through non-significant differences in total caloric expenditure between caffeine and non-caffeine trials.

This discrepancy between previous and present findings cannot be attributed to subject characteristics. All participants described their daily caffeine ingestion in coffee and soft drinks as "average" and had never observed "abnormal" reactions to caffeine. Only two of the twelve subjects showed the hypothesized response, greater fat oxidation during exercise, to

caffeine. Since the maximal oxygen uptake of these two individuals was almost at either extreme of the male sample included in this study, training status could not have caused their "different" caffeine response. It should be taken into consideration, however, that these subjects' percent body fat showed, with 16% and 19%, the highest two values within the group of males tested.

The conflicting findings between the studies cited above and this investigation may be due, in part, to the application of different testing protocols. Costill et al. (1978) investigated fat utilization during bicycling to exhaustion at an intensity of 80% VO_2max, a much higher workload than the one employed in the present study. Since carbohydrate is the preferred energy source during strenuous exercise (McArdle et al., 1981; Issekutz et al., 1966), caffeine may exert an additive effect only in near maximal conditions. No obvious caffeine effect would be seen then during submaximal exercise, when fat oxidative levels are already elevated.

Ivy et al. (1979) tested their subjects after ingestion of a caffeine dose of 500mg which was, in part, administered during the isokinetic bicycle work-out. The amount of caffeine used in Ivy's et al. (1979) tests greatly exeeded the dose utilized in this study; the 4mg/kg dose ranged from 212 to 324 mg of caffeine. In compliance with Ivy et al. (1979), an artificially sweetened lemonade drink was used in this investigation to dissolve the caffeine given. Costill et al. (1978) "caffeinated" decaffeinated coffee for this purpose. A change in the marketed

artificial sweetener from saccharin, contained in the lemonade drink utilized by the earlier investigators (Ivy et al., 1979), to NutraSweet™, ingested as part of the caffeinated lemonade in this study, had taken place. Possible "side effects" of this new sweetener may have suppressed fat oxidation.

Exercise duration may have been another factor responsible for conflicting findings. Two hours of continuous bicycling, starting 60 minutes after caffeine ingestion, with additional, smaller caffeine doses given during the first 90 minutes of exercise, did not result in significant changes in R values or blood data until after the first 60 exercise minutes (Ivy et al., 1979). Thus, even though caffeine effects have been evidenced under resting conditions (Bellet et al., 1968b; Bellet et al., 1969; Paul et al., 1966; Pruett, 1970), and at high intensity exercise conditons (Costill et al., 1978) at approximately one hour after intake, caffeine may not significantly affect fat oxidation until two hours after its ingestion when coupled with aerobic activities.

The potential importance of these differences in protocol are emphasized by findings presented by Casal & Leon (1982) who could not report differences in Rs during 45 minute treadmill runs at 75% VO_2max in male marathon runners in 400mg dose of caffeine dissolved in decaffeinated coffee, decaffeinated coffee only, and exercise only conditions. "Caffeinated" or decaffeinated coffee was ingested 60 minutes prior to exercise in the appropriate trials.

Fasting Effect

During the first few hours after a meal, primarily carbohydrates are used for energy production (Guyton, 1981), whereas in the fasted state, blood FFA concentrations are very high (Wertheimer et al., 1960; Paul et al., 1966) and RQ values close to 0.7 (Pruett, 1970; Guyton, 1981). During walking with continuous glucose injections, these relationships were found to hold true (Havel et al., 1963).

R values of 0.84 and 0.87, calculated for exercise in the fasted and non-fasted state, respectively, confirm the above results. As was to be expected, based on this result, more fat calories per minute were expended during exercise in the fasted trials (5.78 kcal) than in the afternoon conditions (4.15 kcal). Total caloric expenditure did not significantly differ between the two treatments.

To supplement dietary restrictions, aerobic exercise is frequently employed for weight loss purposes, specifically for the reduction of body fat. The results of this investigation clearly indicate further enhancement of fat oxidation during exercise in the fasted condition. During the exercise duration of 30 minutes, which is generally recommended for deriving cardiovascular benefits (McArdle et al., 1981), 173 kcal would be produced through fat oxidation in the fasted state. The comparable value in the non-fasted condition is only 124 kcal. Thus, if caloric intake was restricted to prevent replenishment of the fat stores through triglyceride synthesis from excess carbohydrate, one would have to engage in 30 exercise sessions in

the afternoon, but only in 21 sessions in the morning before breakfast, to lose one pound of body fat. Based on this investigation, therefore, aerobic activity in the fasted state is clearly more advantageous for weight control purposes than exercise in the non-fasted condition.

Chapter 5

Summary, Conclusions, & Recommendations for Future Research

A summary of this investigation will be presented and conclusions will be drawn. Recommendations for future research follow.

Summary

Weight loss programs often employ aerobic exercise to supplement dietary restriction. Submaximal activity is known to enhance fat utilization for energy production (Friedberg et al., 1963; Issekutz et al., 1964; Carlson, 1967; Paul & Holmes, 1975; Ivy et al., 1979). Caffeine seems to increase breakdown of adipose tissue fat in resting subjects (Bellet et al.,1968a; Bellet et al., 1968b; Jung et al., 1981). Some studies indicate the possibility of an additive fat oxidative effect of caffeine to exercise (Costill et al., 1978; Ivy et al., 1979; Wilcox, 1982). Also, in the fasted state, defined as 12 or more hours after the last caloric intake, fat oxidative levels are much higher than during the first few hours after a meal (Wertheimer et al., 1960; Guyton, 1981). Fat utilization seems to be greater during walking exercise in the fasted than in the fed conditon (Havel et al., 1963). Due to the difference in chemical composition of fat and carbohydrate molecules, the respiratory exchange ratio, which represents the ratio of carbon dioxide produced to oxygen used by the body cells, is a valid measure of substrate utilization (Guyton, 1981). Its value ranges from 0.7

to 1.0, indicating that fats or carbohydrates, respectively, are the sole energy source.

The purpose of this investigation was to study the following questions: is fat metabolism during aerobic exercise enhanced by prior caffeine ingestion, does the fasted condition increase the amount of fat oxidized during exercise and, are the effects of caffeine and fasting on fat oxidation additive?

Six male and six female recreational runners, ages 19 to 33, volunteered to participate in each of four test trials. These trials consisted of exercising 1) after caffeine ingestion in the fasted condition, 2) without caffeine in the fasted state, 3) after caffeine ingestion in the non-fasted state, and 4) without caffeine in the non-fasted condition. For the caffeine trials, the subjects ingested a dose of 4mg/kg caffeine. Fasted and non-fasted states were defined as 12 and 3 to 4 hours after the last food intake, respectively. For all treatments, the subjects exercised for 30 minutes on a treadmill at an intensity of 60% VO_2max. The appropriate running speeds were established during a maximal oxygen uptake test. To increase experimental control, body composition was calculated through underwater weighing procedures. Respiratory exchange ratios, calories derived from fat oxidation, and total caloric expenditure were computed and analyzed through a 2-way treatment structure, randomized complete block, general linear models procedure. For single degree of freedom comparisons between the caffeine/no caffeine and fasted/non-fasted treatments least, square mean calculations were employed.

R values showed that fat oxidation was significantly ($p < 0.003$) higher in the non-caffeine trials (R=0.84) than in the caffeine conditions (R=0.89). The number of fat calories expended per minute of exercise differed significantly ($p < 0.01$) between caffeine (4.09 kcal) and non-caffeine (5.84 kcal) conditions. Fasting and non-fasting prior to exercise resulted in R values of 0.84 and 0.89, respectively, a difference significant at the alpha = 0.006 level. Calories produced through fat oxidation differed ($p < 0.01$) between fasted (5.78 kcal) and non-fasted (4.15) trials. Total caloric expenditure values were very similar for each condition. No interaction was found between caffeine/non-caffeine and fasted/non-fasted conditions. The non-caffeine/ fasted treatment combination resulted in an R of 0.82, which was significantly lower ($p < 0.02$) than the values computed for both the non-caffeine/non-fasted (R=0.87) and the caffeine/fasted (R=0.87) trials. An even higher R (0.91) was found during exercise in the caffeine/nonfasted condition. Accordingly, the number of calories of fat expended per minute was greatest during the non-caffeine/fasted condition (6.75 kcal) and lowest in the caffeine/non-fasted trial (3.37 kcal), with medium values of 4.94 and 4.82 kcals in for the non-caffeine/non-fasted and caffeine/fasted groups, respectively. Only the difference between the highest and medium, and the highest to lowest fat caloric expenditure levels reached significance at the alpha =0.03 and alpha = 0.0006 levels, respectively.

Caffeine ingestion prior to aerobic exercise did not enhance fat oxidation. This result conflicts with previous findings (Costill et al., 1978; Ivy et al., 1979), possibly due to

differences in exercise intensity, in the caffeine doses utilized, in the ingestion medium of the caffeine dose, and in the onset of exercise after caffeine intake as well as its duration. The fasted condition, on the other hand, did significantly increase fat oxidation. Thus, in conjunction with dietary restrictions, body fat weight can be reduced considerably faster with aerobic exercise in the morning before breakfast than in the afternoon, several hours after lunch.

Conclusions

The ingestion of a 4mg/kg dose of caffeine 45 minutes prior to a 30 minute treadmill run at an intensity of 60% VO_2max did not result in enhanced fat utilization for energy production. Exercise in the fasted state, however, showed lower R values and greater utilization of calories from fat than the same activity in the non-fasted condition. Exercise in the morning before breakfast will, in conjunction with reduced caloric intake, facilitate fat loss at a significantly faster rate than in the afternoon several hours after lunch. Neither caffeine nor fasting altered total caloric expenditure. The non-caffeine/fasted condition was most advantageous for fat oxidation; the caffeine/non-fasted trials proved to be least effective.

Recommendations for Future Research

Since caffeine is a common part of almost everyone's daily food consumption and, therefore, could be utilized very easily to enhance fat oxidation in conjunction with exercise, further research investigating caffeine effects on fat utilization during aerobic activities is needed. As discussed above, one or more of the differences in testing protocols used in this and previous studies (Costill et al., 1978; Ivy et al., 1979) may have accounted for conflicting findings. Research investigating the effect of various caffeine doses combined with different exercise intensities and durations seems to be indicated. The possibility of a counteraction of the newly marketed sweetening agent NutraSweet™ to the potential enhancement of fat oxidation by caffeine should be explored.

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APPENDIX A

Informed Consent Document

Effects of Caffeine and Fasting on Fat Utilization during Exercise

Investigators: Dr. Anthony Wilcox, Regine Harford, Debra Wedel.

I _____ voluntarily consent to participate in this study which is designed to investigate the effects of caffeine and fasting on fat utilization during running.

I understand that participation in this study involves six (6) visits to the Exercise Physiology lab (Natatorium, Room 5) for testing purposes. My participation will consist of:

1. Assessment of body composition (percent body fat). This will entail being weighted while fully submerged in water.

2. Assessment of maximal oxygen uptake. This will entail running on a motor-driven treadmill while the speed or inclination is increased on one minute intervals. The test starts at a slow speed, gradually increasing until the effort causes fatigue, which occurs within 8 to 12 minutes. The subject indicates to the investigators when he/she wants the test terminated. The effort is similar to the effort of competing in a half-mile race.

3. The other four test days will be 30 minute runs on the treadmill at an intensity of 60% of the person's maximal oxygen

uptake. This intensity is commonly the speed at which people run during a normal exercise session. Two of these runs will be in the morning following an overnight fast. The other two runs will be in the afternoon, 3-5 hours after lunch. Prior to one morning and one afternoon run, the subject will ingest an artificially sweetened beverage containing caffeine. The caffeine will be at a concentration of 4 mg per kg body weight. Thus, a person weighing 70 kg would receive 280 mg caffeine; a person weighing 50 kg would receive 200 mg caffeine. For the purpose of comparison, a cup of coffee contains 100-150 mg caffeine.

Blood samples will be taken from the subject's forearm vein 3 times during the two trials when no caffeine is ingested and 4 times during the two trials when caffeine is taken. Approximately 5 ml of blood will be extracted by standard blood sampling techniques.

I understand that the maximal oxygen uptake test will produce feelings of fatigue that may take several minutes to subside. I also understand that the blood sampling will produce some local discomfort upon insertion of the needle and sometimes leaves a small bruise. I understand that standard laboratory procedures will be employed and represent the least invasive means of gathering the information.

The benefits of participation in this study include the aquisition of knowledge concerning my physical condition (accurate determination of percent body fat and aerobic condition). I will also be participating in a scientific research project that will contribute to our understanding of the effects of caffeine ingestion and the time of day one exercises

on fat utilization during the exercise. The results may help to describe the optimal conditions under which fat may be reduced through exercise.

I understand that the data derived from my participation in the project shall remain confidential. I will be informed of the results of my tests, but I will not be identified in any way in any subsequent presentation or publication of the results of the study.

I understand that the investigators will answer any questions I may have concerning the procedures or the purpose of the study. I also understand that I may withdraw my consent and discontinue my participation in the experiment at any time.

I understand that in the event of physical injury resulting from the research procedure involved in the experiment, no financial compensation will be available, since the regulations of the State prohibit Kansas State University from carrying such insurance.

In signing below I acknowlwedge that I have read and understand the procedures and potential risks and that I voluntarily give my consent to participate in the above-described investigation.

_____ _____

Signature Date

THE EFFECT OF CAFFEINE INGESTION ON FAT METABOLISM DURING EXERCISE IN THE FASTED AND NON-FASTED STATE

by

REGINE HARFORD

B.S., Kansas State University, 1982

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Physical Education, Dance, and Leisure Studies

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1984

The purpose of this investigation was to study the effects of caffeine and fasting on fat metabolism during exercise. Twelve recreational runners exercised in each of four conditions: 1) after caffeine ingestion in the fasted state (CM), 2) without caffeine in the fasted state (NCM), 3) after caffeine ingestion in the non-fasted state (CA), and 4) without caffeine in the non-fasted condition (NCA). A caffeine dose of 4 mg/kg was utilized. Exercise consisted of a 30 minute run on the treadmill at an intensity of 60% VO_2max. For analysis, a 2-way treatment structure, randomized complete block, general linear models procedure and least square mean calculations were employed. Respiratory exchange ratios (R) were significantly ($p < 0.01$) increased by caffeine ingestion and depressed by fasting prior to exercise. Accordingly, the number of fat calories expended per minute of exercise was higher ($p < 0.01$) in the non-caffeine and fasted trials than in the caffeine and non-fasted conditions. The NCM combination resulted in Rs closest to 0.7 and in greatest utilization of fat. Total caloric expenditure was not significantly altered by any treatment. There was no interaction between treatment levels. Caffeine ingestion prior to exercise did not enhance fat oxidation. Exercise in the fasted state, however, resulted in significantly greater fat utilization than in the non-fasted condition.