THE EFFECTS OF CAFFEINE AND FASTING ON PLASMA FFA, GLYCEROL, AND GLUCOSE LEVELS DURING AEROBIC EXERCISE

by

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DEDICATION

To my husband, Russell, for his patience and understanding when times were not easy, and his continual love, support, and encouragement.

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Chapter 1

Introduction

Jogging as a means of enjoyment, fitness, and weight reduction has increased in popularity in recent years. When participating in aerobic exercise, energy comes from two main sources, carbohydrates and fats (Costill & Miller, 1980; Jones, Heigenhauser, Kukis, Matsos, Sutton, & Toews, 1980). The major function of carbohydrates and fats are to supply energy (Williams, 1983). While there is a limited supply of carbohydrate stores in the body, there is an abundant supply of fat. In fact, the major storage depot of energy in the human body is the fat stored in the adipose tissue. The problem that develops is that people store a large excess of calories in the form of body fat. So when participating in aerobic exercise for the purpose of weight reduction, fat would be the preferred energy source. It would be very beneficial to find a means of greater fat release and utilization during aerobic exercise.

Triglycerides, stored in the adipose tissue, are broken down by the process of hydrolysis into free fatty acids (FFA) and glycerol (Guyton, 1981). The breakdown usually occurs for the purpose of providing energy to the muscle cells. Hydrolysis occurs when there is a decreased availability of glucose, which increases the removal rate of fatty acids from the adipose tissue. Since the plasma FFA level is the controlling factor of the rate of FFA uptake (Armstrong, Steele, Altszuler,

Dunn, Bishop, & DeBodo, 1961), an increase in the removal rate of fatty acids from the adipose tissue will increase the amount of fats utilized by the muscle cell. The plasma FFA are used readily by the muscle cell for energy while glycerol is not as metabolically active (Costill, Dalsky, & Fink, 1978; Havel, Carlson, Ekelund, & Holmgren, 1964). So, the level of plasma FFA and glycerol indicate the availability of plasma FFA during muscular work.

An adequate supply of carbohydrates produces a "fat-sparing" effect (Guyton, 1981). During the first few hours after a meal there is an increase in the glucose and insulin levels and carbohydrates are the preferred energy source. After these first few hours following a meal, there is a drop in insulin secretion associated with a reduction in blood glucose and the rate of glucose utilization. The major energy source thus switches from carbohydrates to fats. This seems to indicate that more FFA and glycerol would be released in the fasted state than right after a meal. So, aerobic exercise in the fasted state may contribute to a greater utilization of FFA.

Caffeine ingestion increases plasma FFA levels (Acheson, Zahorska-Markiewicz, Pittet, Anantharaman, & Jequir, 1980; Bellet, Kershbaum, & Finck, 1968; Bellet, Kershbaum, & Roman, 1968; Van Handel, Burke, Costill, & Cote', 1977). Thus, during aerobic exercise after caffeine ingestion, plasma FFA levels increase and there is an enhanced utilization of FFA by the exercising muscles (Costill et al., 1978; Ivy, Costill, Fink, & Lower, 1979). Since both caffeine and fasting increase plasma FFA levels, a combination of the two may further enhance the release of FFA into the bloodstream.

Statement of the Problem

The purpose of the proposed study is to investigate the effects of caffeine ingestion and fasting on plasma glycerol, FFA, and glucose levels during aerobic exercise.

Significance of the Study

Aerobic exercise used as a means of weight reduction and fat loss is a common practice. Some people jog before breakfast in the fasted condition or the time when fat utilization is already elevated. The ingestion of a meal causes an increased insulin secretion and glucose level which produces a "fat-sparing" effect (Guyton, 1981). If one jogs after breakfast or lunch in the non-fasted condition, carbohydrates would be preferred over fats. If exercise is for the purpose of fat loss it would be important to identify conditions that would maximize fat utilization during exercise.

Several investigators (Armstrong et al., 1961; Hickson, Rennie, Conlee, Winder, & Holloszy, 1977) have shown that if more plasma FFA are available, then more will be utilized by the exercising muscles. If by some means there is an augmented rate of plasma FFA release from the adipose tissue, there will also be a rise in the plasma FFA concentration, increasing their availability to the muscle cells. This increased rate of plasma FFA release would thus tend to increase the utilization of plasma FFA for energy.

Caffeine has been shown to increase the FFA concentration

in the blood. (Acheson et al., 1980; Bellet, Kershbaum, & Finck, 1968; Bellet, Kershbaum, & Roman, 1968; Costill et al., 1978; Ivy et al., 1979; Van Handel et al., 1977). Since caffeine is a substance that is commonly ingested in such forms as coffee, tea, or cola drinks, it would have practical significance if it were shown that moderate amounts of caffeine stimulate the fat mobilization process and enhance fat utilization during aerobic exercise.

Both fasting and caffeine ingestion are thought to enhance the fat mobilization process. A combination of the two may further enhance fat release and utilization. Since an increase in plasma FFA or glycerol concentrations indicates a greater plasma FFA uptake, it would be of great interest to find out when the greatest amount of plasma FFA's are released by the adipose tissue. If more plasma FFA are released in one exercise condition compared to another, the fat loss process could be augmented and encouraging results may be seen sooner.

Many questions remain concerning the best way to lose fat weight, so further investigation into the subject is needed. The intention of this study is to investigate several different conditions, the combinations of aerobic exercise in the fasted and non-fasted states, with and without prior caffeine ingestion and determine which is most advantageous for fat utilization. If one condition shows significantly greater plasma FFA and glycerol levels than another, the results would indicate that there is a particular condition which would enhance the fat utilization during aerobic exercise.

Little data are available concerning the differences of

plasma FFA, glycerol, and glucose levels when exercising in the fasted and non-fasted conditions. This study would contribute to the field of exercise physiology by helping fill this gap in the knowledge. Several non-exercise studies (Acheson et al., 1980; Bellet, Kershbaum, & Finck, 1968; Bellet, Kershbaum, & Roman, 1968; Van Handel et al., 1977) have found that ingesting caffeine increases the level of plasma FFA. It has also been shown that there is an enhanced utilization of plasma FFA if caffeine is ingested prior to aerobic exercise (Costill et al., 1978; Ivy et al., 1979). The studies investigating caffeine and exercise used well-trained cyclists who would already have an increased capacity for fat utilization. This study will be investigating recreational runners, who are not well-trained, so there may be some differences in plasma FFA, glycerol, and glucose levels. There are no studies that combine the effects of caffeine and fasting on plasma FFA, glycerol, and glucose levels during aerobic exercise. This study will investigate the possibility that caffeine ingestion prior to aerobic exercise in the fasted state would enhance plasma FFA and glycerol levels to an even greater extent than either treatment alone.

Hypotheses

The study will attempt to support four hypotheses:

1. Aerobic exercise in the fasted state will show a greater release of FFA and glycerol into the blood than aerobic exercise in the non-fasted state.
2. Caffeine ingestion prior to aerobic exercise will

show a greater release of FFA and glycerol into the blood.

3. Caffeine ingestion prior to aerobic exercise in the fasted state will further enhance the release of FFA and glycerol into the blood.
4. Neither caffeine nor fasting will affect blood glucose levels.

Delimitations of the Study

The subjects included six male and six female recreational joggers between the ages of 19-33 years old. All subjects were capable of sustaining a pace at 60 percent of the maximum oxygen uptake for 30 minutes while running on a treadmill.

Limitations of the Study

The results of this study may be affected by several factors which have failed to be controlled.

1. Subjects were not randomly selected, but were volunteers known to be physically active and capable of running for 30 minutes at 60 percent of their maximum oxygen uptake.
2. The amount and type of food intake was not controlled for the subjects, but diets vary from one person to the next so, by not controlling the food intake, the study will be more realistic.
3. No attempt was made to identify differences, if any, in caffeine sensitivity.

Definition of Terms

Several terms that are used throughout the study are defined to present more clarity to the reader.

Fasted State

Prior to the aerobic exercise, the subjects will not ingest any food for a twelve hour period.

Non-fasted State

Three to four hours before the aerobic exercise, the subjects will consume their regular noon meal.

Plasma Free Fatty Acids (FFA)

Long-chain fatty acids in the blood formed by the hydrolysis of triglycerides.

Plasma Glycerol

Product in the blood which is the backbone of the triglyceride molecule, holding together the three FFA molecules. Glycerol is separated from the FFA during triglyceride hydrolysis.

Glucose

A monosaccharide that is the principle blood sugar.

Caffeine

A bitter crystalline alkaloid occurring in coffee, tea, and chocolate, and added to many other foods.

Chapter 2

Review of Literature

Introduction

The level of plasma FFA and glycerol determine the rate of plasma FFA uptake by the cell (Costill et al., 1978; Hickson et al., 1977). It has been demonstrated that caffeine ingestion (Acheson et al., 1980; Bellet, Kershbaum, and Finck, 1968; Bellet, Kershbaum, & Roman, 1968; Van Handel et al., 1977) and the fasted condition (Dole, 1956; Paul, Issekutz, & Miller, 1966) result in the elevation of plasma FFA concentration. So, it would seem that caffeine and the fasted condition would enhance FFA release and subsequent uptake by the muscle cell during exercise.

The following chapter will describe some of the literature which investigates several topics that are similar to and will help justify the study that is proposed in this paper. The topics that will be covered include FFA release and utilization, hydrolysis of triglycerides, fasted and non-fasted states, caffeine and FFA levels, and glycerol as an indicator of FFA mobilization and utilization.

FFA Release and Utilization

The rate of plasma FFA turnover has been demonstrated to be related to the availability of plasma FFA to the cell by Armstrong et al. (1961). In order to measure the FFA turnover rate, C^{14}-palmitate was infused intravenously into eight

unanesthetized dogs. Blood samples were taken at intervals to determine the concentration and specific activity of plasma FFA. Different agents, which either raise or lower the plasma FFA levels, were injected at different times during the experiment. Results showed that if the level of plasma FFA increased there was an increase in the rate of FFA turnover. If there was a decrease in the FFA level than there was also a decrease in the rate of turnover. The authors concluded that the FFA concentration is the controlling factor in the rate of FFA uptake by the cells.

Exercise will elevate the release of FFA by the adipose tissue (Bulow and Tondevold, 1982; Gollnick, 1967) and increase the utilization of FFA by the working skeletal muscles (Friedburg, Harlan, Trout, & Estes, 1960; Jones & Havel, 1967). In a study by Hickson et al. (1977) it was proposed that the rate at which fatty acids are oxidized by the working muscles is "roughly proportional" to the amount of fatty acids available. Female specific-pathogen free Wistar rats served as subjects and were exercised aerobically for six to seven weeks. The rats were then placed in either a control group or a corn oil-heparin group and were further sub-divided into an exercise or a sedentary group. Corn oil and heparin were used to raise plasma FFA levels. Blood samples were drawn from the abdominal aorta to determine blood glucose, glycerol, and FFA levels. In the sedentary corn oil-heparin group, there was a sixfold increase of plasma FFA levels over the sedentary controls. The plasma FFA values in the exercise corn oil-heparin group were always at least two times greater, with blood glycerol

levels significantly higher and blood glucose lower than in the exercise controls. Animals with increased FFA levels were able to run about one hour longer then the controls. The authors believed that the elevation of the plasma FFA concentration led to a glycogen-sparing effect which was largely responsible for the increased endurance in the corn oil-heparin group. It was concluded that the higher the level of FFA available to the working muscles, the more the muscles would utilize for energy.

Experiments completed on exercising dogs by Paul (1970) and Issekutz, Miller, Paul, & Rodahl (1964) both concluded that the role of FFA utilization by the exercising skeletal muscles is related to the concentration of FFA in the bloodstream. Paul (1970) demonstrated this by having normal dogs run at three different workloads. Expired air was collected and arterial blood samples were taken and analyzed for FFA, glucose, and lactate. The results showed that when the workload increased, the FFA concentration, turnover rate, and percent of fatty acids oxidized also increased. Paul concluded that as long as there was an adequate oxygen supply, then the major source of energy at any level of energy expenditure would be FFA and that the rate of release of FFA controls the plasma FFA concentration. Changes in the rate of removal are a result of a mass action effect.

Issekutz et al. (1964) looked at the interrelationship of plasma FFA concentration and the rate of FFA uptake. Dogs running on a treadmill oxidized 90 percent of the FFA taken up by the muscle fiber to 22 percent in the pre-exercise and 20

percent in the post-exercise periods. Results indicated that these same percents of FFA were oxidized regardless of an increase or a decrease in the plasma FFA levels. The authors concluded that FFA utilization depends on the concentration of FFA in the blood.

Plasma FFA levels were investigated during a rest period before exercise, during exercise, and following exercise on five healthy male volunteers (21-25 years old) (Friedburg, Sher, Bogdonoff, & Estes, 1963). Blood samples were collected at three to five minute intervals and exercise was completed on a bicycle at 72 revolutions per minute. Plasma FFA levels remained stable during the rest period, but the onset of exercise caused an abrupt decline, followed by an increase. At the conclusion of the exercise, plasma FFA levels increased abruptly and then fell after a few minutes. The authors proposed that mobilization factors made available increasing amounts of FFA during exercise along with an increased utilization and turnover rate of FFA. After exercise ceased, the sudden rise in plasma FFA was due to the abrupt decline in utilization by the working muscles. Since both the concentration of plasma FFA and the fractional turnover rate increased with exercise, there was a faster turnover rate of plasma FFA.

The literature investigating plasma FFA release and utilization all support the same conclusion that the rate of plasma FFA uptake depends upon the availability of plasma FFA to the cells. If the rate of release of FFA from the adipose tissue is increased, there will also be an increased plasma FFA concentration. An increased rate of uptake by the active

muscle cells will follow.

Hydrolysis of Triglycerides

Fat is stored in the adipose tissue in the form of triglycerides and is mobilized in the form of FFA which can be utilized for energy (Severson, 1979). The first stage in the utilization of triglycerides for energy is the hydrolysis of the triglycerides in a stepwise fashion with FFA and glycerol as the end products. The rate of release of FFA is accomplished by the activation of hormone sensitive lipase. Changes in cyclic AMP levels activate protein kinase, which leads to the phosphorylation and activation of triglyceride lipase (Schimmel, Buhlinger, & Serio, 1980; Severson, 1979). The final stepwise process of splitting each of the three FFA from the glycerol molecule will then take place.

The level of cyclic AMP depends upon the activities of two enzymes, 1) adenyl cyclase catalyzes the formation of cyclic AMP from ATP; and 2) phosphodiesterase inactivates cyclic AMP (Robinson, Butcher, & Sutherland, 1968). Adenylate cyclase is believed to be activated by several lipolytic hormones, such as epinephrine, ACTH, norepinephrine, and glucagon (Hittelman, Wu, & Butcher, 1973; Rosak & Hittelman, 1977; Severson, 1979; Vaughan & Steinburg, 1963). On the other hand, the activity of cyclic AMP is terminated by phosphodiesterase. Caffeine acts upon phosphodiesterase by inhibiting its activity, so when caffeine is ingested, there is an increased accumulation of cyclic AMP and lipolysis is thus increased (Butcher & Sutherland, 1962; Hittelman, et al., 1973; Rosak & Hittelman, 1977;

Timson, 1977; Vaughan & Steinburg, 1963).

Fasted and Non-Fasted States

Insulin plays an important role in the source of energy in the cells by controlling whether carbohydrate or fats will be utilized (Guyton, 1981). The major product of carbohydrate digestion is glucose and, after consumption of a meal, there is an increase in carbohydrate metabolism. Glucose initiates a rapid secretion of insulin which promotes the utilization of carbohydrate and depresses fat utilization. If there is an adequate supply of carbohydrates, the breakdown and release of triglycerides is greatly depressed and carbohydrates would be the preferred energy source. Thus, during the first few hours after a meal the conditions for the muscle to utilize glucose is increased, therefore, the muscle cell will utilize carbohydrates preferentially over fatty acids. After these first few hours following consumption of a meal, the insulin levels become minimal so the muscle cells change the energy preference to the utilization of fats. Fatty acids then become the prime energy source of the muscles during the time period between meals in the fasted condition.

Mongrel dogs of both sexes were used in order to test the interrelationship between glucose and fatty acid metabolism (Paul et al., 1966). Experiments took place after an 18, 48, and 72 hour fast in order to acquire a large range of FFA levels. Nicotinic acid (NiAc), which inhibits FFA mobilization, was infused during some time of each fasted state. Arterial blood samples were taken to determine FFA and glycerol levels

in the plasma. During fasting, there was an increase in the FFA concentration and a decrease in the respiratory quotient. NiAc caused an increase in the turnover rate of glucose and a shift toward carbohydrate metabolism. When the FFA concentration increased, the oxidation of fat also increased, and a decrease in the FFA level caused an increase in carbohydrate oxidation.

Release of FFA by the adipose tissue is increased during fasting while glucose suppresses FFA release (Dole, 1956; Moorehouse, Roy, & Grahame, 1960). Eight healthy humans were fasted and intravenously given one gram glucose per kilogram body weight (Moorehouse et al., 1960). In the fasted state, mean blood levels for glucose were 77 milligrams (mg) per 100 milliliters (ml) of blood and for FFA, 0.678 mEq./liter. After glucose injection, mean levels for glucose increased to 199 mg/100 ml and FFA decreased by 52 percent to 0.322 mEq./liter. The decrease in FFA levels was attributed to the increase in the glucose concentration.

Dole (1956) investigated the level of FFA in 82 subjects after fasting, a mixed meal, glucose ingestion, insulin injection, corn oil ingestion, and epinephrine injection. A ten to twelve hour fast caused an increase in FFA level and a slow, continuous rise was seen as fasting persisted. One hour after ingestion of a mixed meal, FFA decreased from 850 $\pm$ 330 to 400 $\pm$ 160 uEq./liter. Ingestion of 50 mg of glucose caused a marked decrease in FFA level from 850 $\pm$ 220 to 290 $\pm$ 160 uEq/liter. This depressant effect of glucose over fatty acid

release was predominant when both fat and glucose were given in the mixed meal. Epinephrine caused a sharp increase in FFA while an insulin injection caused a sharp decrease.

After a carbohydrate meal, FFA levels decreased, reaching a minimum at about two to three hours and then FFA slowly began to rise again (Swan, Davidson, & Albrink, 1966). Glucose ingestion decreased FFA levels while it enhanced plasma insulin and glucose levels (Pruett, 1970). Insulin enhances glucose uptake in the muscle while suppressing the mobilization of fatty acids from the adipose tissue (Randle, Hales, Garland, & Newsholme, 1963).

Very little data exists which investigates the differences in plasma FFA release and uptake during exercise in the fasted and non-fasted states. Most of the articles to follow were not designed to examine just the fasted and non-fasted conditions. Nevertheless, these articles simulate what might happen when exercise is performed after the ingestion of a meal.

Six members of a wrestling team in good physical condition walked on a treadmill for two hours at a speed of three to four miles per hour (Havel, Naimark, & Borchgrevink, 1963). The subjects fasted for 12 to 15 hours. Four subjects then ate a fat-free breakfast and were given 25 grams of sucrose every half hour for four hours. Palmitate-1-C^{14} was infused and used as a tracer for FFA. Expired air and blood samples were collected. Results indicated that the fasted subjects had a six times greater FFA concentration and a four times greater turnover rate then the fed subjects. Plasma FFA were the fuel delivered to the working muscles in the fasted subjects. The

fed subjects, on the other hand, had a smaller concentration of circulating FFA, which appeared to be the result of a decreased influx of FFA into the blood. So, fewer FFA were burned in the fed state primarily because the skeletal muscles received less to burn.

The effects of glucose on plasma FFA concentrations were examined in three separate studies (Ahlborg & Felig, 1976; Costill, Coyle, Dalsky, Evans, Fink, & Hoopes, 1977; Ivy et al., 1979). Ivy et al. (1979) used nine trained cyclists, who fasted for 12 hours and then bicycled for two hours at 80 revolutions per minute. In one of the exercise trials, a glucose drink was consumed just prior to exercise and every 15 minutes during exercise for the first 90 minutes. Venous blood samples were taken before the exercise and at 10, 30, 60, 90, and 120 minutes during exercise so plasma FFA, glycerol, glucose, and insulin levels could be determined. Results showed that during exercise control trials, FFA rose significantly above the pre-exercise levels. During the glucose polymer trial, the FFA concentration was 33 percent below and the glycerol concentration 35 percent below the control values. The glucose polymer showed a significant elevation in glucose and insulin levels over the control trial, but carbohydrate utilization during the glucose condition did not exceed control values until the last 10 minutes of exercise.

The study by Costill et al. (1977) used six endurance trained and one untrained male to compare the elevation of FFA with the elevation of glucose and insulin on the rate of muscle glycogen utilization during exercise. Three randomized trials

were used: a glucose trial, a fatty meal/heparin injection trial, and a control trial. Each trial consisted of a 30 minute treadmill run at 60 percent of the subjects maximum oxygen uptake. Venous blood samples were taken before glucose or heparin administration, before exercise, and 10, 20, and 30 minutes during exercise to determine glucose, FFA, glycerol, and insulin levels. The fatty meal/heparin trial elevated plasma FFA and glycerol levels and increased fat utilization by 32 percent and 68 percent over the control and glucose trials, respectively. The increase in FFA concentration also caused a decrease in carbohydrate oxidation and muscle glycogen usage. The glucose trial increased glucose levels by 38 percent before exercise but during exercise glucose concentration dropped. This drop was attributed to the combined affects of insulin and exercise on the uptake of glucose by the active tissues. The authors concluded that an increase in plasma FFA has a "glycogen sparing" effect. An increased availability of fatty acids slows the utilization of carbohydrates in the exercising skeletal muscles. The elevation of plasma glucose and insulin levels has an opposite effect, increasing the rate of carbohydrate metabolism.

The study by Ahlborg and Felig (1976) was undertaken to determine the effects of glucose on the changes in arterial concentrations of glucose, FFA, insulin, and glucagon. Six healthy males bicycled for four hours at 30 percent of their maximum oxygen uptake after fasting 12 to 14 hours. At 90 minutes of exercise, 200 grams of glucose was ingested. Expired air and blood samples were collected in the resting

state and after 40, 90, 140, 180, and 240 minutes of exercise. After glucose ingestion, the arterial glucose levels increased 35 percent above resting values and remained 25 to 30 percent above basal levels. Arterial FFA and glycerol responded in a similar manner. Before glucose administration, glycerol levels increased two to four times and FFA rose 50 percent above basal levels. After glucose ingestion, glycerol levels fell and then stabilized staying 60 to 70 percent lower than control levels. The onset of exercise caused arterial insulin levels to fall, but after glucose ingestion, insulin levels rose fourfold. The authors concluded that glucose ingestion alters the metabolic response to prolonged exercise. The decrease in FFA and glycerol levels after glucose ingestion resulted in a marked inhibition of exercise-stimulated lipolysis, which suggests that fat utilization is decreased following glucose ingestion.

In summary, such things as a meal, infusion of NiAc, or glucose ingestion cause a decrease in plasma FFA and glycerol levels. Insulin enhances glucose uptake while suppressing the release of FFA (Pruett, 1970). So, until excess glucose and insulin disappear from circulation, FFA levels will remain suppressed. Plasma FFA and glycerol levels were found to be increased after fasting or heparin ingestion. This caused an increase in the utilization of FFA while carbohydrate utilization decreased.

When exercising in the fasted or non-fasted conditions, similar conclusions were formed. When exercising in the fasted

state, higher FFA concentrations were found and FFA were the fuel delivered to the working muscles. While exercising in the non-fasted state, such as following a meal or after glucose ingestion, plasma insulin and glucose levels increased and plasma FFA levels decreased. It was concluded that an increase in plasma FFA increases the availability and utilization of FFA while slowing the utilization of carbohydrates. On the other hand, an increase in the plasma glucose and insulin levels inhibit lipolysis and decreases the utilization of FFA.

Caffeine and FFA Levels

Both human and animal subjects were used in order to determine the response of plasma FFA to caffeine (Bellet, Kershbaum, & Finck, 1968). The human subjects included normal male volunteers (21-31 years old). All subjects participated in four randomized treatments. Treatments included ingestion of caffeinated coffee (250 mg caffeine), decaffeinated coffee (20 mg caffeine), hot water (control), and coffee (250 mg caffeine) plus sucrose. Blood samples were collected before and two, three, and four hours after coffee ingestion. Findings indicated that after caffeinated coffee ingestion there was a 92 percent increase in FFA levels above the initial pre-coffee level. The ingestion of decaffeinated coffee showed a moderate but non-significant increase. After the coffee plus sucrose treatment, there was a suppression in FFA levels, dropping the level significantly below the initial value for the first two hours. In both coffee treatments, with and without sucrose, the blood caffeine concentrations reached its maximum value

within one hour. The authors proposed that the difference in the response of FFA to caffeine and caffeine plus sucrose was due to the difference in the mobilization of FFA from the adipose tissue.

In the same study, 26 mongrel dogs were used as subjects; 13 serving as controls and 13 participating in the experimental group. The experimental group was injected with caffeine in the amount of 25 mg/kg body weight. Blood samples were taken 30, 20, 10, and zero minutes prior to caffeine and 30, 60, 120, 180, and 240 minutes after caffeine. The results showed that the experimental groups mean serum FFA value increased 137 percent above the pre-caffeine value and this level was highly significant over the control groups.

Another study by Bellet, Kershbaum, and Roman (1968) used 12 healthy males to compare the effect of diet and regular cola drinks on plasma FFA concentrations. Blood samples were taken from an antecubital vein before, one, two, three, and four hours after the cola ingestion. After ingestion of the 16 ounce diet cola there was a steady and significant increase in FFA concentrations reaching maximum level at four hours. The mean FFA concentration increased 48 percent above the pre-cola ingestion. The ingestion of the regular 16 ounce cola, which contained sucrose, caused a decrease in the mean FFA concentrations for the first two hours followed by an elevation which reached its peak value at four hours.

The effects of caffeine and coffee on metabolism and substrate utilization was studied (Acheson et al., 1980). After

caffeine ingestion, a significant increase in the metabolic rate and plasma FFA were found while there was no significant change in blood glucose and insulin levels. FFA levels rose in both the caffeine and the control groups but the rise was greater in the caffeine group (432 ± 31 to 807 ± 82 uEq/liter) than the placebo group (416 ± 28 to 500 ± 40 uEq/liter). The ingestion of coffee increased the oxidation of fat. It was concluded that caffeine provided an additional supply of substrate energy and the ingestion of caffeine would be supplementary to those in a weight reduction program.

Caffeine ingestion increases the level of plasma FFA (Avogaro, Capri, Pais, & Cazzolato, 1973; Jung, Shetty, James, Barrand, & Callingham, 1981; Oberman, Herzberg, Jaskolka, Harell, Hoerer, & Laurian, 1975; Van Handel et al., 1977). No significant difference was found in the plasma glucose levels. Jung et al. (1981) also found a significant and rapid increase in the metabolic rate after caffeine ingestion. He concluded that the metabolic response to caffeine appears to result from the effect that caffeine has on the inhibition of phosphodiesterase so an increased lioysis can take place. Avogaro et al. (1973) concluded that coffee produces a strong lipolytic effect because of the increase in plasma FFA and glycerol levels.

Ivy et al. (1979) found that caffeine significantly enhanced work production during aerobic exercise. Seven male and two female trained cyclists were used as subjects. Each participated, in random order, in three different threatments, involving a caffeine trial, a glucose trial, and a control trial.

Venous blood samples were taken before and 10, 30, 60, 90, 120 minutes during exercise to determine FFA, glycerol, glucose, and insulin levels. Results showed that plasma FFA levels rose significantly in both the control and caffeine trials, but by the end of the exercise, FFA levels in the caffeine trial were 30 percent greater than in the control trial. Plasma glycerol levels in both the caffeine and control trials were not significantly different. The plasma FFA and glycerol levels in the glucose trial were both below the control values. The respiratory exchange ratio showed that carbohydrate utilization was about the same in all three treatments while fat utilization was significantly higher after caffeine ingestion. The authors proposed that because of increased fat mobilization and utilization after caffeine ingestion, there was an increased work production.

Caffeine was noted to increase work production in a study by Costill et al. (1978). The subjects included seven male and two female competitive cyclists. Results showed that after caffeine ingestion, total time to exhaustion was 19.5 percent greater than the no caffeine trial. Plasma FFA levels were not significantly different between the two trials. The glycerol levels were significantly higher in the caffeine trial, which indicates greater fat utilization. The study showed a marked increase in fat oxidation after caffeine ingestion, while in both the caffeine and no caffeine trials approximately 240 grams of carbohydrates were oxidized.

Further investigation of the effect of caffeine upon the

enhancement of fat utilization during exercise was done by Wilcox (1982). Subjects included 60 mature male Sprague-Dawley rats who were assigned to one of four treatment groups: caffeine/exercise, caffeine/sedentary, no caffeine/exercise, and no caffeine/sedentary. Wilcox found both a significant caffeine and exercise effect in reducing body weight, food intake, fat-pad weight, and epididymal fat cell size. He proposed that these caffeine and exercise effects were the result of an increased mobilization and utilization of body fat and an increased metabolic rate.

These studies support the idea that caffeine has an effect of increasing the FFA concentration. This would indicate that there would be an increased mobilization from the adipose tissue and an increased uptake of FFA by the muscle cells due to the ingestion of caffeine.

Glycerol as an Indicator of FFA Mobilization and Utilization

Three long chain fatty acids bound to one glycerol molecule make up the basic chemical structure of triglycerides (Guyton, 1981). For triglycerides to be utilized for energy, hydrolysis into fatty acids and glycerol must first take place. These substances can then be transported through the bloodstream to the active tissues and oxidized to give energy. The FFA are readily used by the cell while glycerol is not as metabolically active (Costill et al., 1978; Havel et al., 1964; Horstman, Mendez, Buskirk, Boileau, & Nicholas, 1971). Since glycerol is not used as extensively, it remains in the bloodstream longer and can be used as an indicator of lipid

mobilization and oxidation.

Blood glycerol significantly increased when FFA levels rose (Avogaro et al., 1973; Havel et al., 1963; Hickson et al., 1977). Exercise increased blood glycerol concentrations markedly and to a greater extent than plasma FFA levels (Havel et al., 1964; Larson, Myhre, Vik-Mo, & Mjos, 1981). The increased glycerol concentration thus suggests an increase in lipolysis (Carlson, 1967) and glycerol appears to be a more direct index of lipolysis then plasma FFA (Avogaro et al., 1973).

Summary

The literature demonstrated that an increase in plasma FFA and glycerol levels indicates an increase in their mobilization and thus an increase in the availability of FFA to the working muscle cells. If the rate of plasma FFA release increases, then a rise in the FFA concentration and rate of uptake will follow.

Triglycerides are stored in the adipose tissue and hydrolyzed into three long chain fatty acids and a glycerol molecule. The release of FFA is accomplished by the activation of hormone sensitive lipase. Changes in cyclic AMP levels activate protein kinase. These steps lead to phosphorylation of triglyceride lipase, which leads to the final stepwise process of splitting the triglyceride molecule into FFA and glycerol.

Phosphodiesterase inactivates cyclic AMP, thus slowing lipolysis. Since caffeine inhibits phosphodiesterase, in-

gestion of caffeine will increase cyclic AMP accumulation and lipolysis.

The fasted condition produces a greater plasma FFA level, which is associated with an increased release and uptake of FFA. The fed state, on the other hand, increases insulin and glucose levels and decreases FFA levels. This has the effect of inhibiting the utilization of fat and increasing glucose oxidation. Caffeine was also shown to increase plasma FFA and glycerol levels, again indicating an increase in the rate of release from the adipose tissue and rate of uptake by the muscle cells.

When plasma FFA concentrations rise, there is also an increase in plasma glycerol levels. During exercise, glycerol levels are usually increased to a greater extent than FFA levels. Since glycerol is not as metabolically active, it remains in the bloodstream longer than FFA, which can be readily taken up by contracting muscle fibers. This makes blood glycerol levels a better indicator of fat mobilization.

Since many studies have shown that fat utilization is greater during exercise when serum FFA levels are elevated, the present investigation will study the effects of caffeine and fasting on serum FFA, glycerol, and glucose during exercise. The literature indicates that caffeine ingestion and prior fasting can increase fat mobilization and elevate serum FFA and glycerol.

Chapter 3

Procedures

This study will investigate the effects of caffeine and fasting on plasma FFA, glycerol, and glucose levels of recreational joggers performing endurance treadmill running. It was conducted at Kansas State University during the Spring semester of the 1983-84 school year. The following chapter is a description of the procedures used in the study.

Subjects

The subjects used for the study were six male and six female volunteers between the ages of 19-33 years old. Each subject was a recreational jogger capable of sustaining a pace at 60 percent of their maximum oxygen uptake for a 30 minute period.

Equipment

To determine percent body fat, the subjects were underwater weighed, using a Chatillon scale. Residual volume was determined by the nitrogen wash-out technique, using a Hewlett-Packard Nitrogen Analyzer. The running tests were conducted on a Quinton Treadmill. Blood was sampled using blood collection tubes and separated with an Aloe Conical Centrifuge. A Gilford Micro-Sample Spectrophotometer (300-N) was used for the glycerol determinations and a Beckman DU Spectrophotometer was used for the glucose and FFA determinations.

Running Speed Determinations

Each subject completed a maximal performance test while running on the treadmill. The maximum oxygen uptake was determined and the subjects running speed at 60 percent maximum oxygen uptake was found. The protocol for the maximum oxygen uptake test included starting the treadmill at five or six miles per hour. The exercise intensity was increased gradually so it would be apparent when the subjects were running at 60 percent maximum oxygen uptake. Every two minutes the speed was increased by one mile per hour or the treadmill was elevated two and a half percent until the subject reached exhaustion.

Data Collection

The study was a repeated measures design consisting of two independent variables which were caffeine and the fasting state. Each subject underwent each of the four treatments:

1. Caffeine/Morning (CM)
2. No Caffeine/Morning (NCM)
3. Caffeine/Afternoon (CA)
4. No Caffeine/Afternoon (NCA)

The subjects were randomly assigned to the order in which they took part in each of the four treatments.

Each exercise session included a 30 minute running period with the subjects running at 60 percent of their maximum oxygen uptake. The treatments in the morning, both with and without caffeine, required the subjects to be in a fasted state. Food

ingestion was not allowed for 12 hours prior to the run. Subjects arrived between 7:00 a.m. and 8:30 a.m. and participated in the run.

The treatments in the afternoon, both with and without caffeine, required the subjects to be in a non-fasted state. The subjects consumed their regular noon meal three to four hours before the run. Subjects arrived in between 3:30 p.m. and 5:00 p.m. and participated in the afternoon run. No food or drink was allowed between the noon meal and the exercise.

For the caffeine treatments, 45 minutes before the exercise the subjects ingested four milligrams of caffeine per kilogram body weight. To allow for accurate measurement, the caffeine was in a powdered form and for easier ingestion was mixed in a glass of an artificially sweetened lemonade drink containing Nutra-Sweet™. After the drink was ingested, subjects were instructed to sit quietly for 45 minutes until exercise began.

In each of the four treatments, blood samples were taken from an antecubital vein in order to determine plasma FFA, glycerol, and glucose levels. For the treatments with no caffeine, three blood samples were taken at 15 minute intervals. The times were just prior to exercise, 15 minutes into the exercise, and promptly after the exercise. For the treatments with caffeine, four blood samples were taken. The sampling times were before caffeine ingestion, directly before the exercise began, 15 minutes into the exercise, and directly after exercise.

Plasma Glucose, Glycerol, and FFA Determinations

Blood samples were taken using blood collection tubes, and then allowed to clot. The blood was centrifuged at 2500 rpm for approximately ten minutes. The serum was drawn from the collection tubes and placed into three different test tubes so glucose, glycerol, and FFA levels could be determined for each sample taken. The blood was then frozen until the blood assays could take place. A method in the SIGMA Technical Bulletin No. 510 (Appendix D) was used for the determination of plasma glucose levels. The plasma glycerol level was determined by a method in the SIGMA Technical Bulletin No. 320-UV (Appendix E). Plasma FFA levels were determined by a method developed by Fink (Appendix F).

Analysis of Data

The design in the study was a repeated measures design. The data was analyzed using a one way analysis of variance method. An alpha level at .05 was used for determining statistical significance.

Chapter 4

Results and Discussion

Six male and six female recreational joggers ranging in age from 19 to 33 years were used as subjects. A repeated measures design was used with each subject participating in each of four conditions while exercising aerobically. Conditions were caffeine/morning (CM), no caffeine/morning (NCM), caffeine/afternoon (CA), and no caffeine/afternoon (NCA). In the following chapter the results of the study will be described and discussed.

Results

Subject Information. Before testing began, the participants' maximum oxygen uptake and percent body fat were assessed. The means and the standard deviations of the results are listed in Table 1.

Table 1. Descriptive Data on Subjects (Means and Standard Deviations)

	Age	% Fat	Max VO_2 (1/min)
Male	25.67 ± 5.5	12.5 ± 5.39	4.49 ± 0.59
Female	25.83 ± 5.0	21.6 ± 5.39	2.98 ± 0.59

The subjects participated in a 30 minute run at 60 percent of their maximum oxygen uptake. All but one subject completed each treatment; one female subject did not participate in the CA trial. Blood samples were collected during each of the

trials in order to determine plasma glucose, FFA, and glycerol levels. The time intervals for the no caffeine trials included before exercise (BE), at the midpoint of exercise (ME), and directly after exercise (AE). Intervals for the caffeine trials included the same three plus one before caffeine ingestion (BC).

Plasma Glucose Results. No significant difference was found in the glucose levels as a result of ingesting caffeine. During exercise, the glucose levels remained constant, except for a slight but significant elevation in the CM condition. A listing of the glucose levels treatment means and standard deviations appears in Table 2. The glucose levels for each treatment at each of the sampling intervals appears in Figure 1. The tables following present the statistical analysis of the glucose values in the four treatment conditions as they progressed across time. The tables for the ANOVA for the glucose values include in Table 3 the CM trial, Table 4 the NCM trial, Table 5 the CA trial, and Table 6 the NCA trial.

Table 2. Treatment Means and Standard Deviations for Serum Glucose Concentrations (mg/100 ml blood)

	BC	BE	ME	AE
CM	85.3 ± 11.8	77.6 ± 7.5	85.4 ± 10.8	90.8 ± 10.9
NCM		82.3 ± 7.4	81.0 ± 9.8	84.6 ± 11.9
CA	86.0 ± 8.4	81.7 ± 9.7	83.9 ± 9.1	90.5 ± 12.2
NCA		83.4 ± 14.0	86.7 ± 11.7	88.4 ± 18.2

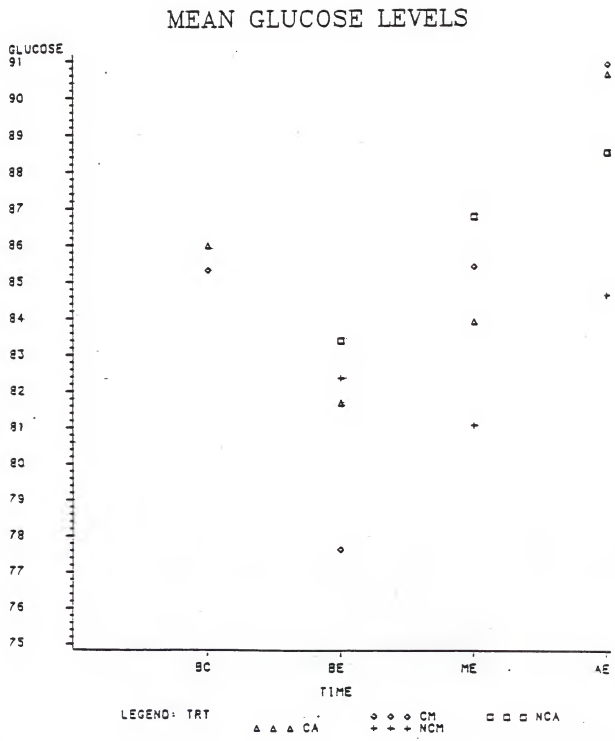

Figure 1. Glucose Levels (mg/100 ml blood) for the Four Treatments at Each of the Sampling Intervals.

Table 3. ANOVA for Glucose Values For Caffeine Morning

Source	DF	SS	MS	F	PR>F
Model	14	2078.61	148.47	1.36	0.25
Error	23	2510.73	109.16		
C. Total	37	4589.34			

Source	DF	SS	F	PR>F
Time	3	1050.42	3.21	0.042
ID	11	1162.67	0.97	0.50

LS Means Comparison Probabilities Over Time

	BE	ME	AE	
BC	0.080	0.956	0.203	
BE		0.164	0.005*	
ME			0.273	*p < .05

Table 4. ANOVA for Glucose Values for No Caffeine Morning

Source	DF	SS	MS	F	PR>F
Model	13	1859.69	143.05	2.87	0.022
Error	17	848.83	49.93		
C. Total	30	2708.52			

Source	DF	SS	F	PR>F
Time	2	63.86	0.64	0.54
ID	11	1798.10	3.27	0.014

Table 5. ANOVA for Glucose Values for Caffeine Afternoon

Source	DF	SS	MS	F	PR>F
Model	13	1550.25	119.25	1.32	0.28
Error	19	1718.26	90.43		
C. Total	32	3268.51			

Source	DF	SS	F	PR>F
Time	3	356.64	1.31	0.299
ID	10	1185.27	1.31	0.293

Table 6. ANOVA for Glucose Values for No Caffeine Afternoon

Source	DF	SS	MS	F	PR>F
Model	13	4923.37	378.72	4.04	0.004
Error	17	1595.47	93.85		
C. Total	30	6518.84			

Source	DF	SS	F	PR>F
Time	2	274.22	1.46	0.26
ID	11	4776.79	4.63	0.003

The following tables present the ANOVA for the glucose values at the four different blood sampling intervals. Table 7 analyzes the glucose values at the time interval before caffeine ingestion. Table 8 analyzes the glucose values at the time interval before exercise. Table 9 analyzes the glucose values at the time interval at the midpoint of the run.

Table 10 analyzes the glucose values at the time interval after exercise. At each sampling interval, there were no differences in the glucose values among any of the four treatments.

Table 7. ANOVA for Glucose Values Before Caffeine Ingestion

Source	DF	SS	MS	F	PR>F
Model	12	1386.88	115.57	1.43	0.33
Error	7	563.79	80.54		
C. Total	19	1950.68			
Source	DF	SS		F	PR>F
TRT	1	29.78		0.37	0.56
ID	11	1384.55		1.56	0.28

Table 8. ANOVA for Glucose Values Before Exercise

Source	DF	SS	MS	F	PR>F
Model	14	1155.74	82.55	0.78	0.68
Error	26	2759.22	106.12		
C. Total	40	3914.96			
Source	DF	SS		F	PR>F
TRT	3	117.64		0.37	0.78
ID	11	960.86		0.82	0.62

Table 9. ANOVA for Glucose Values at the Midpoint of the Run

Source	DF	SS	MS	F	PR>F
Model	12	2256.01	188.00	3.93	0.005
Error	18	860.49	47.81		
C. Total	30	3116.50			

Source	DF	SS	F	PR>F
TRT	3	124.20	0.87	0.48
ID	9	2098.68	4.88	0.002

Table 10. ANOVA for Glucose Values After Exercise

Source	DF	SS	MS	F	PR>F
Model	14	3570.51	255.04	1.82	0.09
Error	26	3647.80	140.30		
C. Total	40	7218.31			

Source	DF	SS	F	PR>F
TRT	3	268.81	0.64	0.60
ID	11	3320.72	2.15	0.05

Plasma FFA Results. There was no difference in FFA levels before and after caffeine ingestion. Each treatment

responded to exercise in a similar manner. From the beginning to the midpoint of exercise no difference was found in plasma FFA levels. As exercise continued, FFA levels rose. By the last sampling period, directly following exercise, FFA concentrations were significantly increased above all other sampling periods. Table 11 is a listing of FFA means and standard deviations. Figure 2 presents FFA levels for each treatment at each of the sampling intervals. The tables following present the statistical analysis of FFA values in the four treatment conditions as they progressed across sampling times. The tables for the ANOVA for FFA values include in Table 12 the CM trial, Table 13 the NCM trial, Table 14 the CA trial, and Table 15 the NCA trial.

Table 11. Treatment Means and Standard Deviations for Serum FFA Concentrations (mM)

	BC	BE	ME	AE
CM	0.34 ± 0.17	0.38 ± 0.21	0.26 ± 0.08	0.64 ± 0.22
NCM		0.34 ± 0.11	0.31 ± 0.14	0.59 ± 0.19
CA	0.25 ± 0.17	0.28 ± 0.19	0.35 ± 0.18	0.60 ± 0.34
NCA		0.29 ± 0.13	0.25 ± 0.13	0.51 ± 0.22

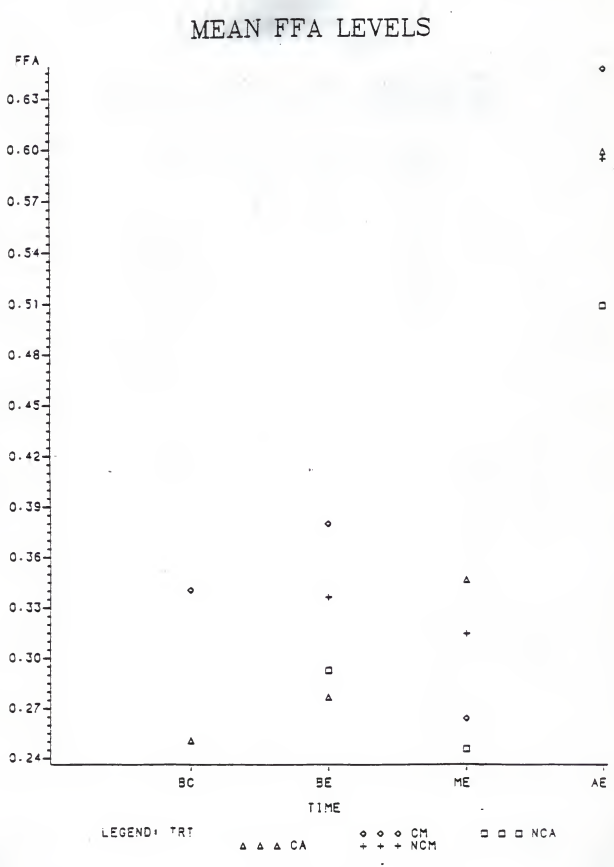

Figure 2. FFA Levels (mM) for the Four Treatments at Each of the Sampling Intervals.

Table 12. ANOVA for FFA Values for Caffeine Morning

Source	DF	SS	MS	F	PR>F
Model	14	1.413	0.101	3.94	0.001
Error	25	0.640	0.026		
C. Total	39	2.053			

Source	DF	SS	F	PR>F
Time	3	0.747	9.72	0.0002
ID	11	0.643	2.28	0.042

	BE	ME	AE
BC	0.0553	0.821	0.0001*
BE		0.515	0.0003*
ME			0.001*

*p < .05

Table 13. ANOVA for FFA Values for No Caffeine Morning

Source	DF	SS	MS	F	PR>F
Model	13	0.900	0.069	4.40	0.002
Error	19	0.299	0.016		
C. Total	32	1.199			

Source	DF	SS	F	PR>F
Time	2	0.431	13.69	0.0002
ID	11	0.377	2.18	0.065

LS Means Comparison Probabilities Over Time

	ME	AE
BE	0.994	0.0002*
ME		0.0003*

*p < .05

Table 14. ANOVA for FFA Values for Caffeine Afternoon

Source	DF	SS	MS	F	PR>F
Model	13	2.138	0.164	12.83	0.0001
Error	23	0.295	0.013		
C. Total	36	2.433			

Source	DF	SS	F	PR>F
Time	3	0.700	18.19	0.0001
ID	10	1.432	11.17	0.0001

LS Means Comparison Probabilities Over Time

	BE	ME	AE
BC	0.632	0.388	0.0001*
BE		0.655	0.0001*
ME			0.0001*

*p < .05

Table 15. ANOVA for FFA Values for No Caffeine Afternoon

Source	DF	SS	MS	F	PR>F
Model	13	1.076	0.028	9.05	0.0001
Error	18	0.165	0.009		
C. Total	31	1.241			

Source	DF	SS	F	PR>F
Time	2	0.414	22.66	0.0001
ID	11	0.668	6.64	0.0002

LS Means Comparison Probabilities Over Time

	ME	AE
BE	0.356	0.0001*
ME		0.0001*

*p <.05

There was no significant change found in the FFA levels between treatments at any of the blood sampling intervals. This suggests that caffeine or fasting did not affect FFA concentrations. The following tables present the ANOVA for the FFA values of the four different sampling intervals. Table 16 analyzes FFA values at the time interval before caffeine. Table 17 analyzes FFA values at the time interval before exercise. Table 18 analyzes FFA values at the time interval at the midpoint of the run. Table 19 analyzes FFA values at the time interval after exercise.

Table 16. ANOVA for FFA Values Before Caffeine Ingestion

Source	DF	SS	MS	F	PR>F
Model	12	0.502	0.042	2.75	0.060
Error	10	0.152	0.015		
C. Total	22	0.654			
Source	DF	SS		F	PR>F
TRT	1	0.032		2.11	0.177
ID	11	0.455		2.72	0.063

Table 17. ANOVA for FFA Values Before Exercise

Source	DF	SS	MS	F	PR>F
Model	14	0.529	0.038	1.68	0.113
Error	31	0.698	0.023		
C. Total	45	1.228			
Source	DF	SS		F	PR>F
TRT	3	0.059		0.87	0.469
ID	11	0.456		1.84	0.089

Table 18. ANOVA for FFA Values at the Midpoint of the Run

Source	DF	SS	MS	F	PR>F
Model	12	0.239	0.020	1.12	0.405
Error	18	0.321	0.018		
C. Total	30	0.599			
Source	DF	SS		F	PR>F
TRT	3	0.046		0.85	0.483
ID	9	0.190		1.18	0.362

Table 19. ANOVA for FFA Values After Exercise

Source	DF	SS	MS	F	PR>F
Model	14	1.382	0.099	2.74	0.012
Error	27	0.972	0.036		
C. Total	41	2.354			
Source	DF	SS		F	PR>F
TRT	3	0.115		1.06	0.382
ID	11	1.275		3.22	0.007

Plasma Glycerol Results. Glycerol levels did not change due to the ingestion of caffeine before or during exercise. Exercise caused a steady and significant rise in the glycerol concentrations. By the end of exercise, glycerol levels were significantly increased in all of the treatments over the pre-exercise levels. A listing of the glycerol means and standard deviations appears in Table 20. The glycerol levels for each treatment at each of the sampling intervals appear in

Figure 3. The tables for the ANOVA for the glycerol values include in Table 21 the CM trial, Table 22 the NCM trial, Table 23 the CA trial, and Table 24 the NCA trial.

Table 20. Treatment Means and Standard Deviations for Serum Glycerol Concentration (mg/100 ml blood)

	BC	BE	ME	AE
CM	1.36 ± 0.66	1.24 ± 0.46	1.94 ± 0.43	2.76 ± 1.01
NCM		1.29 ± 0.41	1.84 ± 0.41	2.51 ± 0.41
CA	0.97 ± 0.38	0.96 ± 0.26	1.46 ± 0.64	1.92 ± 0.73
NCA		1.16 ± 0.22	1.37 ± 0.24	1.84 ± 0.73

Table 21. ANOVA for Glycerol Values for Caffeine Morning

Source	DF	SS	MS	F	PR>F
Model	14	22.34	1.596	3.36	0.005
Error	22	10.44	0.474		
C. Total	36	32.78			

Source	DF	SS	F	PR>F
Time	3	15.30	10.75	0.0001
ID	11	6.77	1.30	0.289

LS Means Comparison Probabilities Over Time

	BE	ME	AE
BC	0.672	0.080	0.0001*
BE		0.048*	0.0001*
ME			0.970

*p < .05

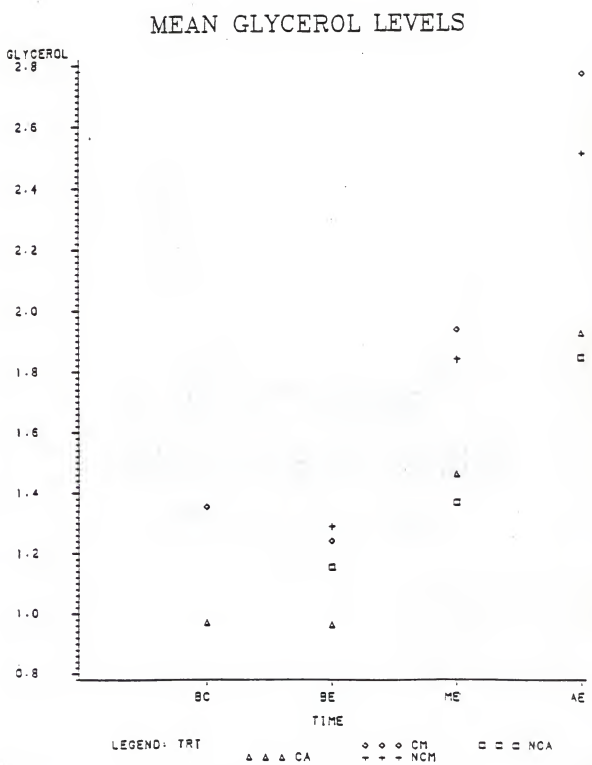

Figure 3. Glycerol Levels (mg/100 ml blood) for the Four Treatments at Each of the Sampling Intervals.

Table 22. ANOVA for Glycerol Values for No Caffeine Morning

Source	DF	SS	MS	F	PR>F
Model	13	11.13	0.856	6.97	0.0001
Error	19	2.33	0.123		
C. Total	32	13.46			

Source	DF	SS	F	PR>F
Time	2	8.22	33.46	0.0001
ID	11	2.64	1.96	0.096

LS Means Comparison Probabilities Over Time

	ME	AE
BE	0.0008*	0.00001*
ME		0.0013*

*p < .05

Table 23. ANOVA for Glycerol Values for Caffeine Afternoon

Source	DF	SS	MS	F	PR>F
Time	13	12.29	0.945	7.05	0.0001
Error	23	3.08	0.134		
C. Total	36	15.37			

Source	DF	SS	F	PR>F
Time	3	6.34	15.76	0.0001
ID	10	6.05	4.51	0.0014

LS Means Comparison Probabilities Over Time

	BE	ME	AE
BC	0.894	0.016*	0.0001*
BE		0.012*	0.0153*

*p < .05

Table 24. ANOVA for Glycerol Values for No Caffeine Afternoon

Source	DF	SS	MS	F	PR>F
Model	13	6.32	0.486	3.14	0.012
Error	19	2.94	0.155		
C. Total	32	9.26			

Source	DF	SS	F	PR>F
Time	2	2.72	8.79	0.002
ID	11	3.53	2.07	0.079

LS Means Comparison Probabilities Over Time

	ME	AE
BE	0.157	0.0005*
ME		0.020*

*p < .05

No significant change was found in the glycerol levels between the treatments in the before caffeine and the before exercise blood sampling periods. The midpoint of exercise showed that both the CM trial and the NCM trial were significantly higher than the NCA trial. By the end of the exercise the CM trial had risen significantly above the CA trial, and both the CM and NCM trials were higher than the NCA trial. Since the CM trial was significantly increased over the CA trial and the NCM trial was significantly elevated over the NCA trial, then the glycerol levels showed a fasting effect by the last sampling interval. The following tables compare the different treatments by presenting the ANOVA for the

glycerol values at the four different sampling intervals. Table 25 analyzes the glycerol values at the time interval before caffeine ingestion. Table 26 analyzes the glycerol values at the time interval before exercise. Table 27 analyzes the glycerol values at the time interval at the midpoint of the run. Table 28 analyzes the glycerol values at the time interval after exercise.

Table 25. ANOVA for Glycerol Values Before Caffeine Ingestion

Source	DF	SS	MS	F	PR>F
Model	12	5.93	0.494	8.72	0.002
Error	8	0.45	0.057		
C. Total	20	6.39			
Source	DF	SS		F	PR>F
TRT	1	0.13		2.35	0.164
ID	11	5.16		8.28	0.003

Table 26. ANOVA for Glycerol Values Before Exercise

Source	DF	SS	MS	F	PR>F
Model	14	2.01	0.144	1.18	0.339
Error	29	3.52	0.121		
C. Total	43	5.53			
Source	DF	SS		F	PR>F
TRT	3	0.63		1.74	0.181
ID	11	1.36		1.02	0.457

Table 27. ANOVA for Glycerol Values at the Midpoint of the Run

Source	DF	SS	MS	F	PR>F
Model	12	4.29	0.357	2.41	0.036
Error	19	2.71	0.142		
C. Total	31	6.99			

Source	DF	SS	F	PR>F
TRT	3	1.46	3.41	0.039
ID	9	2.50	1.95	0.106

LS Means Comparison Probabilities Over Treatments

	NCA	CA	NCM
CM	0.032*	0.149	0.870
NCA		0.431	0.012*
CA			0.119

*p<.05

Table 28. ANOVA for Glycerol Values After Exercise

Source	DF	SS	MS	F	PR>F
Model	14	18.08	1.291	3.43	0.003
Error	28	10.55	0.377		
C. Total	42	28.62			

Source	DF	SS	F	PR>F
TRT	3	5.11	4.52	0.011
ID	11	11.54	2.79	0.014

LS Means Comparison Probabilities Over Treatments

	NCA	CA	NCM
CM	0.004*	0.008*	0.332
NCA		0.836	0.035*
CA			0.062

*p<.05

The results for plasma glucose, FFA, and glycerol levels for all four treatments have been presented. The glycerol levels in the CM and NCM trials were significantly increased above the glycerol levels in the CA and NCA trials, respectively. Since glycerol can be used as an indicator of fat mobilization and utilization then the fasted state showed an increased FFA release and uptake over the non-fasted state. The hypothesis, aerobic exercise in the fasted state would show a greater release of FFA and glycerol into the blood than aerobic exercise in the non-fasted state is thus supported. Caffeine ingestion did not effect glucose, FFA, or glycerol levels. Neither of the hypotheses, caffeine ingestion prior to aerobic exercise would show a greater release of FFA and glycerol into the blood or, caffeine ingestion prior to aerobic exercise in the fasted state would further enhance the release of FFA and glycerol into the blood, were supported. Glucose levels showed no change in any of the four treatments except for a slight but a significant increase during aerobic exercise in the CM trial. This increase was probably of little physiological significance, so the hypothesis, neither caffeine nor fasting would affect blood glucose levels, can be supported. The following section will discuss and interpret the results.

Discussion

Exercise increases plasma FFA and glycerol levels (Friedburg et al., 1963; Gollnick, 1967; Havel et al., 1964; Havel et al., 1963; Horstman et al., 1971). During the first

few minutes of exercise FFA levels first fall and then begin to increase. The initial fall occurs because of an increase in FFA uptake by the working muscles, and the rise is due to an increased influx of FFA from the adipose tissue. So, during the first part of exercise, FFA mobilization lags behind FFA utilization (Carlson, 1967; Havel et al., 1963; Horstman et al., 1977; Issekutz et al., 1965). Glycerol, the backbone of the tryglyceride molecule, is not as metabolically active, so no initial fall is seen at the beginning of exercise. Glycerol levels, therefore, rise steadily throughout the entire exercise (Carlson, 1967; Horstman et al., 1977).

In the present study, it was again demonstrated that both glycerol and FFA levels increase significantly with exercise. FFA levels showed a trend towards declining during the first 15 minutes of exercise while glycerol levels increase throughout the exercise. It can be assumed that at the beginning of exercise, FFA utilized by the working muscle cells exceeded FFA mobilized from the adipose tissue. The glycerol levels increased steadily throughout exercise. Since glycerol can be used as an indicator of lipid mobilization and utilization (Costill et al., 1978; Havel et al., 1964; Horstman et al., 1971), then it can be concluded that FFA were being mobilized and used during the first half of the run.

Caffeine ingestion has been found to increase the level of plasma FFA (Acheson et al., 1980; Bellet, Kershbaum, & Finck, 1968; Bellet, Kershbaum & Roman, 1968; Jung et al., 1981). Both Bellet studies noted that after caffeine ingestion,

FFA levels rose steadily reaching maximum values at four hours. Jung et al. (1981) found that caffeine ingestion increased FFA levels significantly after 60 minutes. Following ingestion of caffeine (8 mg/kg body weight), FFA levels were increased after just 30 minutes (Acheson et al., 1980).

In the present investigation, subjects ingested caffeine (4 mg/kg body weight) and, 45 minutes later, exercised aerobically for 30 minutes. FFA levels were not significantly different from the pre-caffeine to the pre-exercise sampling period. The reason that FFA levels did not change may have been because the dose of caffeine was not large enough to elicit a response in such a short time period. The time period between caffeine ingestion and exercise may not have been long enough to cause a significant rise in FFA levels. The FFA concentrations were similar before and after the caffeine ingestion, so there was little indication that FFA levels were rising during this period. Another reason a change did not occur in FFA levels may have been because individuals react differently after ingesting caffeine. Thus, after caffeine ingestion, response of FFA levels to caffeine may vary from one individual to another. Only four out of the 12 subjects showed an increase in FFA levels during the 45 minute period after caffeine ingestion. The subjects in this study were, generally, non-responders to caffeine.

During two hours of isokinetic cycling, following caffeine ingestion, FFA levels increased over the last 90 minutes of exercise (Ivy et al., 1979). Neither the respiratory exchange ratio nor the blood data indicated a shift towards fat

metabolism during the first 50 minutes of exercise. Costill et al. (1978) found no significant difference in FFA levels during an exercise to exhaustion at 80 percent maximum oxygen uptake, following caffeine ingestion. There was a significant increase in the glycerol levels during the caffeine trial, but it was unclear whether this increase occurred during the first 30 minutes of exercise. Temples and Haymes (1982) had subjects cycle for 90 minutes after caffeine ingestion. Exercise increased FFA levels but no significant difference was found between the caffeine and control trials.

The present study had the subjects exercise for 30 minutes at 60 percent of their maximum oxygen uptake. Both the Ivy et al. (1979) and Costill et al. (1978) studies had subjects exercise for an extended length of time and at a much higher intensity. Temples and Haymes' (1982) investigation, as in the present study, used a lower intensity of exercise but the duration was longer. The absence of a caffeine effect may have been because the exercise intensity was too low or the exercise duration was too short. But again, there was nothing to indicate that caffeine treatments enhanced fat mobilization, because the caffeine treatments were not different from the no caffeine treatments. Temples and Haymes' (1982) study also did not elicit a caffeine effect. Therefore, there may be some individuals who are non-responsive to caffeine and thus will not elicit an increase in FFA or glycerol levels.

The current investigation did not find a fasting effect in either FFA or glycerol levels before exercise began. After

consumption of a meal, FFA levels decrease, reaching a minimum about two to three hours later, and this is then followed by a slow increase (Swan et al., 1966). The meal also causes a sharp increase in insulin levels, which begin to decline about an hour later. In the present study, the first blood sample was not taken until three to four hours after the noon meal. By this time, insulin levels have started to decrease and FFA levels have begun to increase. The first sample was also a non-exercise value, so the metabolic rate would be low. These could serve as reasons why no morning or fasting effect was seen in FFA and glycerol levels before exercise.

Exercise increases the metabolic rate (Paul, 1970). This increase causes a rise in the sympathetic nervous system (Gollnick, 1967; Havel et al., 1963), which stimulates the release of FFA into the bloodstream. The alteration of metabolism during exercise, in the present research, may be the reason why a fasting effect was not seen until the exercise was underway. When the subjects exercised in the afternoon, they were not yet in the completely fasted state, so the hormones that regulate fuel sources (insulin and glucagon) would be at different levels than when they are fasted. The onset of exercise caused an increase in plasma glycerol levels and by the end of exercise both plasma FFA and glycerol levels were significantly increased over the pre-exercise values. Plasma glycerol levels also showed a definite fasting effect by the end of exercise. CM glycerol levels were significantly

higher than CA glycerol levels, and NCM glycerol levels were significantly increased over the NCA glycerol levels.

FFA values did not exhibit this fasting effect. FFA are used readily by the working skeletal muscles. By contrast, glycerol is not used as extensively by the active muscles and remains in the bloodstream (Costill et al., 1978; Havel et al., 1964; Horstman et al., 1971). An increased glycerol concentration thus suggests an increase in lipolysis, so glycerol can be used as an indicator of lipid mobilization and utilization. If this is true, then the current investigation can assume that since there was a significant increase in the glycerol levels during exercise in the morning compared to the afternoon, then there were more plasma FFA released and utilized during the morning runs.

The concentration of plasma glucose remains relatively stable during exercise (Friedburg et al., 1960; Gollnick, 1967; Paul et al., 1966). Caffeine also does not cause a considerable change in the plasma glucose levels (Acheson, 1980). No differences were found between any of the four treatments in plasma glucose levels, in the present study. Glucose levels remained constant over the four sampling periods in all trials, except a slight elevation was seen in the caffeine/morning trial, which is probably of little physiological significance.

Chapter 5

Summary, Conclusions, and Recommendations

The following chapter will first summarize the study. Conclusions will then be drawn by reviewing the outcome of the results and finally, recommendations for further research will be expressed.

Summary

The higher the level of FFA available to the working muscles, the more the muscles will utilize for energy (Armstrong et al., 1961; Hickson et al., 1977). If more plasma FFA are released in one exercise condition compared to another, the utilization of fat may be augmented. The purpose of this study was to investigate the effects of caffeine ingestion and fasting on plasma glycerol, FFA, and glucose levels during aerobic exercise.

Four aerobic exercise conditions were used: 1.) caffeine/morning, 2.) no caffeine/morning, 3.) caffeine/afternoon, and 4.) no caffeine/afternoon. Subjects included six male and six female recreational runners (19-33 years old) and each participated in all four treatments. Each exercise included a 30 minute run at 60 percent of the subject's maximal oxygen uptake. The fasted state trials took place in the morning after a 12 hour fast. The non-fasted state trials took place in the afternoon, three to four hours after the consumption

of the subject's regular noon meal. During the caffeine trials, caffeine was ingested (4 mg/kg body weight) by drinking an artificially sweetened lemonade drink 45 minutes before exercise. Blood samples were taken during each run. The sampling intervals were before exercise, the midpoint of exercise, and immediately following exercise in the no caffeine trials. For the caffeine trials, blood samples were taken at the same three intervals, plus before caffeine ingestion. From each sample, plasma FFA, glycerol, and glucose levels were determined.

Plasma glucose showed no change throughout the study except a slight elevation in the caffeine/morning trial during exercise. No significant differences were found in FFA, glycerol, and glucose levels as a result of caffeine ingestion. Reasons for these results may be because the caffeine dose was not large enough or the time after caffeine ingestion to exercise was not long enough to elicit a plasma FFA or glycerol response to caffeine. During exercise caffeine also showed no change in the plasma FFA, glycerol, and glucose levels. This may have occurred because the exercise intensity was not high enough or the exercise duration was too short. Some individuals may also be non-responders to caffeine and this may be another reason why a caffeine effect was not seen.

By the end of exercise, both plasma FFA and glycerol levels were increased significantly above the other sampling periods. The glycerol levels increased steadily throughout the entire exercise while FFA levels increased significantly

during only the last 15 minutes. It was assumed that during the first half of the run, plasma utilization exceeded the mobilization. By the end of exercise, the plasma FFA mobilization had increased significantly. Since glycerol increased throughout the exercise it was assumed that plasma FFA were also being released and utilized throughout the exercise.

The plasma glycerol levels in the morning trials were significantly higher than the afternoon trials. Plasma glycerol levels thus exhibited a fasting effect. Since an increased glycerol concentration suggests an increase in lipolysis, the results of this study indicate that more plasma FFA were released and utilized during the morning runs.

Conclusions

The present investigation found that an ingestion of four mg/kg body weight of caffeine did not cause any change in plasma FFA, glycerol, and glucose levels during a 30 minute run. Plasma glucose levels did not change in the study except a slight increase was seen during exercise in the caffeine/morning trial. Plasma FFA and glycerol levels did increase with exercise, indicating that aerobic exercise increases the release and utilization of plasma FFA. Fasting caused a significant difference in glycerol levels during the 30 minutes of running. Glycerol can be used as an indicator of lipolysis, so it was assumed that the fasted state caused an increased FFA release over the non-fasted state.

Recommendations

This study did not find that caffeine ingestion before exercise increased mobilization and utilization of FFA. More research needs to be completed in the area of caffeine and exercise. It is recommended that research be completed by using different doses of caffeine before exercise. It also would be beneficial to find out if a longer waiting period between caffeine ingestion and exercise would help to elicit a caffeine response in such a short exercise bout as was used in this study. The final recommendation is to see if different durations and intensities of exercise would have an effect on finding a caffeine response.

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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 ingestion 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 at 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 4 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 the study include the acquisition 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 acknowledge 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

APPENDIX B

KSU
KANSAS
STATE
UNIVERSITY

Department of Physical Education, Dance, and Leisure Studies

Ahearn Gymnasium
Manhattan, Kansas 66506
913-532-6765

Dear Dr.

_____, a patient of yours, is interested in participating in a research study being conducted by Dr. Anthony Wilcox, Regine Harford, and Deb Wedel, of the Physical Education, Dance, and Leisure Studies Department of Kansas State University. Subjects in this study must be regular joggers, and the testing will involve running at a normal training pace on a treadmill for 30 minutes on 4 different occasions.

Three or four blood samples will be taken from a forearm vein on these test days. The subjects will also have one maximal stress test on the treadmill, where they run for 8-12 minutes while the speed or inclination of the treadmill is increased every minute until the person is too fatigued to continue. The intensity of the test is similar to running in a half-mile race. During the test, the subjects' electrocardiogram will be continually monitored.

The experiment is designed to study fat utilization during running and has received the approval of the Human Subjects Review Committee at Kansas State University. Please indicate below whether you do or do not give clearance for your patient to participate as a subject in this study. If you have any questions, please call me.

Sincerely,

Anthony Wilcox, Ph.D.

AW/lg

Please return the form below:

_____ has does not have my clearance to participate in the above-described study in the Physical Education, Dance, and Leisure Studies Department.

_____

Date _____

APPENDIX C

MAXIMAL OXYGEN UPTAKE TEST

Name _____

Time _____ Date _____

Test Condition _____ P_{Bar} _____

Min.	Speed	Vol.	O_2	CO_2	Temp	STPD

APPENDIX D

GLUCOSE DETERMINATION
(Sigma Technical Bulletin No. 510)

Use of Serum Directly:

1. Label the tubes as follows: BLANK, STANDARD, TEST 1, TEST 2, etc.

2. To BLANK, add: 0.5 ml water.

   To STANDARD add: 0.025 ml of Glucose Standard Solution, Stock No. 635-100, plus 0.5 ml water.

   To each TEST add: 0.025 ml sample plus 0.5 ml water.

3. To each tube add: 5.0 ml of Combined Enzyme-Color Reagent Solution (Reagent A"). Mix each tube thouroughly.

4. Incubate all tubes for 45 minutes at room temperature.

5. At the end of incubation period, read A of STANDARD and TEST, using BLANK as a reference at 425-475 nm. Readings should be completed within 30 minutes.

6. Calculate the TEST values as follows:

$$\text{Serum Glucose (mg/100 ml)} = \frac{A_{TEST}}{A_{STANDARD}} \times 100$$

Note: All assays were completed in duplicate.

REAGENTS

A. PGO ENZYMES, Stock No. 510-6

   Each capsule contains 500 Unites of Glucose Oxidase (Aspergillus niger), 100 Purpurogallin units of Peroxidase (horseradish) and buffer salts.

   Enzyme Solution (Reagent A') - Prepare solution by adding the contents of one capsule to 100 ml distilled water in an amber bottle. Invert bottle several times with gentle shaking to dissolve.

   Combined Enzyme-Color Reagent Solution (Reagent A") - To 100 ml of Enzyme Solution add 1.6 ml of Color Reagent solution (Reagent B'). Mix by inverting several times or with mild shaking.

B. o-DIANISDINE DIHYDROCHLORIDE, Stock No. 510-50

Preweighed vial containing 50 mg.

Color Reagent Solution (Reagent B') - Reconstitute vial with 20 ml water.

C. GLUCOSE STANDARD SOLUTION, Stock No. 635-100

Consists of a solution of beta-Glucose, 100 mg/dL (5.56 mmol/l), in benzoic acid, 0.1%.

APPENDIX E

GLYCEROL DETERMINATION
Sigma Technical Bulletin No. 320-UV

A narrow-bandwidth spectrophotometer is required.

1. To a cuvet, add: 0.3 ml water, 0.2 ml serum, and 2.5 ml Triglyceride Assay Solution (Reagent A'). Cover cuvet with parafilm and invert several times to mix. Wait about 10 minutes for equilibration.

2. Read and record Absorbance at 340 nm vs water. This is the A_0.

3. Immediately add 0.01 ml Glycerokinase Solution, Stock No. 320-20. Mix by Inversion.

4. Exactly five minutes after the addition of the Glycerokinase Solution, read and record the Absorbance at 340 nm vs water. This is A_5.

5. Calculate the values as follows:

$$A = A_0 - A_5$$

Serum Free Glycerol (mg/100 ml) = A x 22.3

6. Explanation of Calculations:

Serum Free Glycerol (mg/100 ml) =

$$\frac{A \times 3.01 \times 92 \times 100}{6.22 \times 10^3 \times 0.2} = A \times 22.3$$

Where 3.01 = volume of liquid in cuvet
92 = molecular weight of glycerol
100 = conversion of mg/ml to mg/100 ml
6.22×10^3 = molar extinction coefficient for NADH at 340 nm
0.2 = sample volume

REAGENTS

A. TRIGLYCERIDE REAGENT, Stock No. 320-10

Reconstituted vial contains the following active ingredients.

Adenosine Triphosphate	0.14 mmol/l
Phosphoenolpyruvate	1.21 mmol/l

Nicotinamide Adenine Dinucleotide, Reduced	0.20 mmol/l
Pyruvate Kinase (rabbit muscle)	1600 units/l
Lactic Dehydrogenase (beef heart)	3400 units/l

Also contains buffer salts and stabilizers

Triglyceride Assay Solution (Reagent A') - Reconstitute Triglyceride Reagent, Stock No. 320-10, with 26 ml water. Swirl gently to dissolve contents.

B. GLYCEROKINASE SOLUTION, Stock No. 320-20

Glycerokinase (Candida myoderma), 150 units/ml, suspension in ammonium sulfate with 1% ethylene glycol.

APPENDIX F

FFA DETERMINATION
Method by Fink

Make some serial dilutions of the standard. At the beginning the standards should be done with each batch of samples, and cover the range .0625 mM to 1 mM.

1. Place .2 ml of serum, working serial standards, and water (for samples, atandards, and blanks, respectively) into the bottom of 16 x 125 screw capped tubes. Cap standards to prevent evaporation.

2. Add 3 ml of extraction reagent. Give the samples a "jiggle" to break up the emulsion that forms. Cap tubes.

3. Add 1 ml of the copper reagent. Cap tubes.

4. Shake the tubes mechanically for 10 minutes.

5. Centrifuge for 20 minutes at about 3000 rpm.

6. Carefully remove tubes from centrifuge. Transfer 2 ml of the upper phase to a clean culture tube. Lay a cap on top of culture tubes to prevent evaporation.

7. To each of the sample, standard, and blank tubes - the 2 ml of the upper phase - add .5 ml of the sodium diethyldithiocarbonate-butanol color reagent. Mix immediately. The color reaction will be immediate, but let the tubes sit for 10 minutes. Cap tubes.

8. Read the tubes on a spectrophotometer at 436 nm. Read the tubes within a half an hour or so. The spectrophotometer is zeroed and spanned with the blank.

9. Calculations: Run the standards several times and run a regression for a parabolic curve. Use this formula for the calculations.

Note: All assays were completed in duplicate.

REAGENTS

A. Extraction Reagent - The extraction reagent consists of one part chloroform, one part heptane, and 2 percent methanol. Place in an automatic dispenser.

B. Copper Reagent - The copper reagent consists of .05 molor of Cupric Nitrate, .1 molor Triethanolamine (TEA), and

.04 to .05 molor Sodium Hydroxide or enough to adjust the pH to 8.3. All of this is brought to the required volume with a saturated Sodium Chloride.

C. Color Reagent - The color reagent consists of sodium diethyldithiocarbonate and butanol (for 10 ml, mix 22 mg of sodium diethyldithiocarbonate in 10 ml of butanol).

D. Standards - Mix as accurately as possible 1 mM palmitic acid in the extraction reagent. This will be the stock standard.

APPENDIX G

SUBJECT INFORMATION SHEET

Males	Age	Height (inches)	Weight (kg)	%BF	$MaxVO_2$	m/week
1	32	69	80.94	15.9	3.88	15
2	22	72	73.04	9.9	3.97	25
3	33	70.5	76.65	19.0	5.07	10
4	25	70	75.91	13.5	4.08	25
5	21	71	65.46	4.0	4.70	--
6	21	71	75.65	13.1	5.23	20
Females						
1	19	64	52.80	13.0	2.80	10
2	30	65	59.83	22.9	2.45	20
3	32	--	59.54	29.3	2.47	--
4	22	71	60.85	19.3	2.71	10
5	28	65	64.66	23.6	3.83	15
6	24	67	61.59	22.4	3.59	35

%BF = percent body fat

$MaxVO_2$ = maximum oxygen uptake

m/week = average miles ran per week

THE EFFECTS OF CAFFEINE AND FASTING ON PLASMA FFA, GLYCEROL, AND GLUCOSE LEVELS DURING AEROBIC EXERCISE

by

DEBRA JO WEDEL

M. S., Kansas State University, 1984

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
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ABSTRACT

The purpose of this study was to investigate the effects of caffeine and fasting on plasma FFA, glycerol, and glucose levels during aerobic exercise. Six male and six female recreational joggers (19-33 years old) ran on a treadmill for 30 minutes at a speed of 60 percent of their maximum oxygen uptake. Each subject participated under four different conditions: following caffeine ingestion in the fasted state (morning) (CM), following caffeine ingestion in the non-fasted state (afternoon) (CA), without caffeine ingestion in the fasted state (NCM), and without caffeine ingestion in the non-fasted state (NCA). In the caffeine trials, 45 minutes before exercise, four milligrams per kilogram body weight of caffeine was ingested in an artificially sweetened lemonade drink. The fasted state trials took place in the morning after a 12 hour overnight fast. The non-fasted state trials took place in the afternoon, three to four hours after consumption of the subjects regular noon meal. Blood samples were collected from an antecubital vein at 15 minute intervals for the no caffeine trials (before, at the midpoint, and directly after exercise). For the caffeine trials, blood samples were collected before caffeine ingestion, plus the same 15 minute intervals during exercise as in the no caffeine trials. The data was analyzed utilizing a repeated measures ANOVA design. There were no significant differences in FFA, glycerol, or glucose levels as a result of ingesting the caffeine. At

the start of exercise FFA, glycerol, and glucose concentrations were the same for all treatments. The 30 minutes of running caused a significant increase in FFA and glycerol levels under all treatment conditions. Glucose levels remained the same during the exercise, except for in the CM condition, which had a slight elevation (from 77.6 $\pm$ 7.5 to 90.8 $\pm$ 10.9 mg/100 ml). There was no caffeine or fasting effect on serum FFA or glucose. At each sampling interval, there was no significant difference between any of the four treatment conditions. There was a significant fasting effect upon the glycerol levels during exercise. At the completion of the run, glycerol levels were significantly higher in the fasted conditions (2.76 $\pm$ 1.01 and 2.51 $\pm$ 0.41 mg/100 ml for the CM and NCM conditions, respectively, as compared to 1.92 $\pm$ 0.73 and 1.84 $\pm$ 0.73 mg/100 ml in the CA and NCA conditions, respectively). Glycerol levels during the runs were unaffected by prior caffeine ingestion. The changes in serum glycerol levels indicates that fat mobilization was greater in the fasted conditions. Caffeine ingestion did not appear to cause any enhancement in the release of fat from the adipose tissue before or during the exercise.