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The effects of caffeine and training on body composition

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THE EFFECTS OF CAFFEINE AND TRAINING ON BODY COMPOSITION

by

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An Abstract

of a thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Science in the School

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ABSTRACT

The effect of caffeine intake, when administered alone or in combination with training, on carcass composition was investigated. Male rats (n = 24) were divided into training and sedentary groups and fed either a caffeine or a no-caffeine diet. Training consisted of running 5 days per week for 9 weeks on a treadmill. Caffeine ingestion consisted of voluntary ingestion of a coffee solution with a caffeine concentration of approximately 7.5 mg/kg/day, and forced intake of caffeine solution containing 10 mg/kg/day. Body weights were measured weekly, and at the end of treatment the percentage of carcass fat and carcass water were determined. The caffeine-trained group had the lowest mean body weight of all groups on Week 9. The no-caffeine-trained group had a significantly lower mean percentage of carcass fat than either the caffeine-sedentary or no-caffeine-sedentary groups. The caffeine-trained group had a significantly larger mean percentage of carcass water than the caffeine-sedentary and no-caffeine-sedentary groups. These results indicate that the combination of training and caffeine decreases body weight, however the influence on carcass fat and carcass water has not been resolved.

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A Thesis Presented to the Faculty of
the School of Health, Physical
Education, and Recreation
Ithaca College

In Partial Fulfillment of the
Requirements for the Degree
Master of Science

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by
Helaine Mary Alessio
May 1983

Ithaca College
School of Health, Physical Education, and Recreation
Ithaca, New York

CERTIFICATE OF APPROVAL

MASTER OF SCIENCE THESIS

This is to certify that the Master of Science Thesis of

Helaine Mary Alessio

submitted in partial fulfillment of the requirements for the degree of Master of Science in the School of Health, Physical Education, and Recreation at Ithaca College has been approved.

Thesis Advisors:

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DEDICATION

I dedicate my thesis to my parents, Salvatore and June Alessio for their unconditional love and support throughout my life and for teaching me that a ship is safe in its harbor, but that is not why ships were built.

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Chapter 1

INTRODUCTION

The North American population is suffering an obesity epidemic, which can be attributed to a lifestyle that promotes overeating and sedentary activities. Obesity involves having an above average amount of stored calories, for if people take in more calories than they expend, there will be an increase in body fat (Insel & Roth, 1977). Fortunately, the increase in body fat creates a cosmetic problem which instills in many people a desire to try various approaches to combat obesity in order to enhance their physical appearance.

Two general approaches to dealing with obesity include dieting and training. The common goal of each approach is to decrease fat storage. Dieting attacks obesity by decreasing the number of calories that are ingested with food intake, while training decreases fat stores by increasing the caloric expenditure, usually by physical exercise, so that the body rids itself of extra calories. The essential factors that determine the number of calories expended have been shown to include the type, intensity, and duration of exercise (ACSM, 1980), as well as the turnover rate of free fatty acids (FFA) and the rate of lipid oxidation (Simko, 1970). A

combination of diet and training may be the best way to confront the problem of obesity.

Beverages containing caffeine are consumed by a large percentage of Americans. Graham (1978) listed several popular caffeinated drinks such as coffee, tea, cola beverages, and cocoa that are ingested by a broad spectrum of the population. These popular drinks all contain methylxanthines, substances that act as stimulants and smooth muscle relaxants (Acheson, Zahorska-Markiewicz, Anantharaman, & Jequier, 1980). Caffeine is an example of a methylxanthine, and has been shown to enhance lipolysis and the FFA release in response to an increased metabolism (Acheson et al., 1980; Bellet, Kershbaum, & Finck, 1968; Van Handel, Burke, Costill, & Cote, 1977).

The addition of caffeine to an exercise program for weight loss may produce a more favorable alteration in body composition by augmenting fat loss. Although there have been many investigations related to the effects of caffeine and training, there is a paucity of information related to the combined effect of caffeine and training on body composition. This study was designed to investigate that combined influence.

Scope of the Problem

The purpose of this study was to determine the effect of caffeine on the body composition of rats when it was administered alone or in conjunction with daily training. The subjects were 24 male Sprague-Dawley derived rats from Blue Spruce Farms. A 2 X 2 factorial experiment was designed to include the variables of training (training or sedentary) and diet (caffeine or no caffeine). Four groups, each consisting of six rats, were organized so that one group ingested caffeine and received training, a second group ingested caffeine only, a third group received training only, and a fourth group neither ingested caffeine nor received training.

Training consisted of running 5 days per week for 9 weeks on a motor-driven treadmill. The duration of the run was increased progressively until the rats were running 60 minutes each day at 26.8 meters per minute (m/min) on a 10% grade, with a 30-second sprint at a speed of 40 m/min interposed every 10 minutes.

The caffeine was administered to 12 subjects by force feeding a caffeine solution. The amount of caffeine that was administered was related to each rat's body weight. Caffeine intake also included ad libitum

ingestion of diluted coffee administered in the drinking bottles.

At the end of 9 weeks all of the rats were sacrificed, and a homogenate of each whole animal was obtained for carcass analysis. Carcass composition, including carcass fat and carcass water, was determined on each rat.

Statement of the Problem

The purpose of this study was to determine the effect of caffeine when it was administered alone or in conjunction with daily training on carcass composition.

Hypothesis

The group of rats that received training and ingested caffeine will have the lowest body weight, lowest percentage of carcass fat, and highest percentage of carcass water among the four groups.

Assumptions

This investigation was conducted with the following assumptions:

1. A valid measure of carcass fat was obtained by the method of carcass analysis used in this study.

2. A valid measure of carcass water was measured by the method of carcass analysis used in this study.

3. A training effect was attained by all trained rats.

Definition of Terms

In this investigation, the technical terms used were defined as follows:

Caffeine is one of several xanthine derivatives which occur naturally in coffee beans, tea leaves, kola nuts, and cocoa beans (Graham, 1978). Caffeine stimulates the mobilization of FFA (Bellet et al., 1968), and has a stimulating effect on the central nervous system (Bugyi, 1980).

Carcass composition includes the percentage of fat, water, and dry, lean mass that comprise the body weight of each subject.

Homogenate is a slurry mixture of the whole carcass after it has been autoclaved, prepared in a grinder, and blended.

Training consisted of running 5 days per week for 9 weeks on a motor-driven treadmill. The duration of the training was increased progressively until the rats were running for 60 minutes a day at 26.8 m/min on a 10% grade, with a 30-second sprint at a speed of 40 m/min interposed every 10 minutes.

Delimitations

This investigation was conducted with the following delimitations:

1. The subjects consisted of 24 male Sprague-Dawley derived rats.

2. Carcass composition was determined by the analysis of samples for percent fat and percent water.

3. Training consisted of running on a motor-driven treadmill using a protocol such that the duration of exercise increased progressively until the rats were running for 60 minutes a day at 26.8 m/min on a 10% grade, with a 30-second sprint at a speed of 40 m/min interposed every 10 minutes.

Limitations

This investigation was conducted with the following limitations:

1. The results can be generalized only to male Sprague-Dawley derived rats used in this study.

2. Other carcass composition analysis procedures may yield different results.

3. Methods of training other than treadmill running may yield different results.

4. Other doses of caffeine may yield different results.

Chapter 2

REVIEW OF RELATED LITERATURE

This chapter has been organized according to the following topics: (a) the chemistry and properties of caffeine, (b) caffeine and lipid metabolism, (c) exercise and lipid metabolism, (d) lipid metabolism and body composition, and (e) summary.

The Chemistry and Properties of Caffeine

Caffeine, a widely consumed substance with active properties, has been shown to influence the central nervous system (Bugyi, 1980) and the mobilization of free fatty acids (Bellet, Kershbaum, & Finck, 1968). Recent concern regarding caffeine has included the effects on the body as well as its effects as an ergogenic aid (Costill, Dalsky, & Fink, 1978). Coffee and soft drinks have been reported to be some of the most common caffeine-containing beverages in the United States and in many other countries (Bellet et al., 1968). Caffeine has also been shown to be included in such popular products as tea and cocoa (Graham, 1978), milk chocolate, and many nonprescription drugs ("Caffeine: What it does," 1981).

An important factor in the popularity of caffeinated substances may be the stimulant factor. Graham (1978) reported that 200 mg, the usual pharmacologically active

dose of caffeine, initiated the refreshing feeling that people desire to attain. Ritchie (1975) reported that large doses of caffeine stimulated the entire central nervous system, and Ivy, Costill, Fink, and Lower (1979) reported that caffeine increased work production during prolonged strenuous exercise.

Caffeine has been classified as a member of the group of substances known as methylxanthines. Caffeine has alkaloid properties and has been identified as 1,3,7-trimethylxanthine. This has been closely related to theophyline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine), both of which have been reported to occur naturally in tea and cocoa, respectively (Graham, 1978), Acheson, Zahorska-Markiewicz, Anantharamon, and Jequier (1980) have reported that methylation in the position-1 of the purine ring was responsible for the enhancement of metabolic activities by caffeine and theophyline.

The metabolic activity of cells has been shown to be modified by a dual messenger system involving some hormone that initiates the intracellular production of cyclic adenosine monophosphate (cAMP). The hormone has been postulated to act on a membrane-bound enzyme which in turn activates the enzyme adenylate cyclase to bring

about the conversion of adenosine triphosphate (ATP) to cAMP. The cAMP then initiates a specific enzyme action which includes turning on glycogen and triglyceride lipase. Catecholamine secretion is related to glycogen and triglyceride lipase, such that the maintenance of high levels of epinephrine (a catecholamine) delays the formation of phosphodiesterase (Lehninger, 1975). Phosphodiesterase has been shown to act against cAMP (Bellet et al., 1968). Ritchie (1975) has reported that phosphodiesterase was inhibited by caffeine and that the inhibition was due to a nonpolar substitution at positions-1 and 3 of the 1,3,7-trimethylxanthine. The inhibition of phosphodiesterase that is caused by caffeine intake is related to cAMP, and it is cAMP that ultimately controls the metabolic activity of the cell.

Caffeine and Lipid Metabolism

There has been considerable research dealing with the effects of caffeine on lipid metabolism. For example, the ingestion of caffeine has been shown to elevate plasma free fatty acids (Bellet et al., 1968), increase muscle glycogen reserves (Costill, Coyle, Dalsky, Evans, Fink, & Hoopes, 1977), increase lipolysis, and increase the oxidation of fats (Costill et al., 1978; Ivy et al., 1979).

Caffeine ingestion has resulted in elevated cAMP levels, and cAMP has been shown to be responsible for the stimulation of lipid metabolism (Lehninger, 1975; Postnov, 1980). Postnov stated that caffeine caused an increase in the cAMP content in adipocytes of normotensive rats that were subjected to adrenalectomy. Cyclic AMP can be destroyed by the enzyme phosphodiesterase, which has been found in most animal tissues (Lehninger, 1975). Caffeine has been shown to inhibit phosphodiesterase, and that inhibition may result in elevated intracellular cAMP levels.

The activation of glycogen and triglyceride lipase was reported to be under the control of cAMP, which was related to catecholamine secretion (Lehninger, 1975). For example, when epinephrine was secreted into the blood by the adrenal medulla, the liver adenylate cyclase system remained activated and maintained cAMP at high levels (Lehninger, 1975). A high concentration of cAMP was shown to persist until epinephrine stopped, and the epinephrine bound on the liver cell membrane dissociated. When epinephrine levels fell, the formation of new cAMP ceased, and that which remained was destroyed by phosphodiesterase (Lehninger, 1975). Although the process by which caffeine increased catecholamine

secretion occurs has not been well established (Bellet, Roman, DeCastro, Kim, & Kershbaum, 1969), norepinephrine has been shown to be a potent inhibitor of insulin release (Porte, 1966), and there is an inverse relationship between increased lipolysis and decreased insulin secretion after caffeine ingestion (Wachman, Hattner, & George, 1970). Bellet et al. (1969) reported that ingestion of moderate doses of caffeine and coffee resulted in a significant increase in catecholamine release. The increased catecholamine secretion was due to caffeine's central nervous system stimulating effect. Also, catecholamine excretion during the 6 hours immediately following ingestion of 150 mg of a caffeinated beverage was significantly increased (Van Handel et al., 1977).

It was suggested by Acheson et al. (1980) that the ingestion of caffeine in reasonable quantities would result in an increased lipid metabolism and an increased supply of free fatty acids if needed. Wilcox (1982) reported that the administration of caffeine prior to exercise increased the rate of fat loss. While studies on the effect of caffeine on lipid metabolism have been numerous and the results in good agreement, there have been very few studies on the effect of caffeine on body

composition, and the results of those studies have not been clear. For instance, Acheson et al. (1980) reported no significant differences in food intake while showing differences in energy expenditure following caffeine ingestion. Parsons and Nadeau (1981) reported that moderate caffeine intake in the weanling rat appeared not to affect the growth pattern as reflected by food intake, weight gain, and the weight gain:food intake ratio. Wilcox (1982) reported that caffeine intake reduced food consumption in sedentary and exercised rats, as well as decreased body weight, fat pad weight, and fat cell size in caffeine-sedentary and caffeine-exercise groups.

Exercise and Lipid Metabolism

Extensive research on exercise and lipid metabolism has resulted from an increased interest in physical fitness and weight reduction in this country. It has been reported that regular exercise initiated distinct changes in the balance of energy, and that a redistributed blood flow, with a higher supply to the muscles, enhanced FFA release from the muscle cells (Simko, 1970). Exercise training has been shown to influence the metabolism of smooth muscle cells in particular by altering the supply and distribution of some metabolic products (Simko, 1970). Simko also

reported that training increased the oxygen content of arterial blood and its diffusion throughout the arterial wall.

Training influences fat as well as muscle tissue, and a study by Askew, Huston, Plopper, and Hecker (1975) reported that adipose tissue from trained rats possessed a greater ability to release FFA. The effect of training on fatty tissue has been related to catecholamine stimulation (Askew & Hecker, 1976). Several theories have been proposed to explain how chronic exercise training affects lipolysis. Askew and Hecker (1976) suggested that fat metabolism was stimulated by an increase in catecholamine secretion. They proposed that training may have caused an increase in catecholamine-stimulated lipolysis either by altering the adipocyte hormone receptor sites due to a change in cellular surface area or by preventing the attainment of some critical degree of cellular enlargement. Training has been reported to increase FFA levels not only during exercise, but in the post-exercise state as well (Craig, Hammons, Garthwaite, Jaret, & Holloszy, 1981). Larsen, Myhre, Vik-mo, and Mjos (1981) suggested that the FFA mobilized in the adipose tissue during exercise was trapped within the tissue as a result of inadequate blood

perfusion of the tissue and that the post-exercise rise in FFA, which has been reported in arterial blood flow, may have been due to a washout effect.

Lipid Metabolism and Body Composition

Body composition has been divided into two main components: a fat component and a fat-free component (lean body mass). Average body fat percentages for human males and females have ranged between 19-21% and 24-26%, respectively (Johnson & Nelson, 1979). The number and size of fat cells in the body have been related to body fat. Since fat cell numbers have been reported to increase until 16-18 years of age and then remain fairly constant throughout adulthood (Lamb, 1978), adults that have desired to decrease body fat probably have decreased the size of fat cells rather than the number of fat cells (Lau, Flaim, & Ritchie, 1979).

The amount of stored lipid has been related to the amount of lipid extracted from the blood, the amount of lipid that is synthesized by enzymes in the cells, and the amount of lipid that is released and subsequently oxidized (Florkin & Stotz, 1970). The way that caffeine influences the amount of stored lipid has not been clear, however, there has been agreement in studies that have investigated the effect of training on lipid. Training

may increase hormone-sensitive lipase (McGarr, Oscai, & Borensztajn, 1976) and lipoprotein lipase (Narayan, McMullen, Butler, Wakefield, & Calhoun, 1975). These enzymes have been shown to influence the rate of lipid turnover (Herbert, Kerkhoff, Bell, & Lopez, 1975) and lipid metabolism (Herbert et al., 1975; Vaughan, 1966). Therefore an increase in lipoprotein lipase and hormone-sensitive lipase may facilitate a decrease in fat storage due to increased mobilization of FFA and a fatty tissue clearing factor.

Summary

A major reason for the popularity of caffeinated beverages may be the stimulant property, which also enhances lipid metabolism. An increased lipid metabolism results in an increased energy expenditure, which, in turn, ultimately affects body composition. Training has also been shown to increase lipid metabolism, whereby energy expenditure is increased and body composition is changed. Catecholamines have been shown to influence lipid metabolism, and the amount of stored lipid has been related to the enzymes, lipoprotein lipase, and hormone-sensitive lipase, which are increased by training.

Chapter 3

METHODS AND PROCEDURES

This chapter includes the methods and procedures used in this investigation. The methods of animal care, caffeine treatment, training, homogenate preparation, carcass fat determination, carcass water determination, and analysis of data are outlined.

Animal Care

Twenty-four male Sprague-Dawley derived rats were used in this study. The rats were 4 weeks of age and weighed between 38.4 g and 65.3 g upon arrival at the laboratory. They underwent a preliminary stabilization period of 4 weeks to verify a pattern of normal weight gain. During the stabilization period all rats were exposed to daily runs on a motor driven treadmill. The duration of those runs was approximately 10 minutes on a flat treadmill at a speed of 26.8 m/min. After several practice sessions, 12 rats were selected as superior runners and were randomly assigned to either a caffeine- or no-caffeine-trained group. The remainder of the rats were randomly assigned to either a caffeine- or no-caffeine-sedentary group. The experiment was conducted in a 2 X 2 factorial design which included the variables of training (training or sedentary) and diet (caffeine or

no-caffeine).

All of the animals were housed in individual wire cages 23.75 cm long, 16.38 cm high, and 16.38 cm wide. They were maintained in an animal room with a thermostatically-controlled temperature of 23.5° C, a humidity of 88%, and an automatic 14-hour light, 10-hour dark cycle. A commercial ration (Charles River Rat and Hamster Chow, R-M-H 3000) was fed ad libitum throughout the experiment to all animals. The weight of the food was measured on a Sartorious balance (Brinkman Instruments). Food consumption was measured daily by subtracting the amount of uneaten chow, including spillage, from the original weight of the food given to the rats on the previous day. Over the weekends, extra food was provided, and a mean of the weekend consumption was recorded. All members of the caffeine groups received 60 ml of a diluted coffee solution in their drinking bottles each day, while the no-caffeine rats received 60 ml of tap water. Food and water were consumed ad libitum by all animals from the day of arrival up to, but not including, Day 1 of training and caffeine treatment. From Day 1 of the treatment and continuing throughout the study, the caffeine-trained and sedentary groups received a coffee solution in place of

water, while the no-caffeine-trained and sedentary groups continued to receive tap water ad libitum. Also, from Day 1 of the treatment and continuing throughout the study, body weights of all animals were taken weekly. All rats were weighed on the same day on an Ohaus Model 700 Triple Beam Balance (serial number 34438).

Caffeine Treatment

All members of the caffeine groups received 60 ml of a diluted coffee solution each day, while the no-caffeine rats received 60 ml of tap water each day. A stock solution was made by boiling 1 teaspoon of Maxwell House instant coffee, weighing approximately 1.5 g, in 80 ml of tap water. After cooling the solution and checking the volume, 3 ml of the coffee solution were drawn by a pipet into a drinking bottle, and tap water was added to provide a final volume of 60 ml. The animals in the caffeine groups received a fresh 60 ml supply of the diluted coffee solution each day except on weekends, when 200 ml were provided to last through 2 days. The ad libitum coffee dosage was calculated to provide an amount of caffeine at a dosage equivalent to that of the normal daily human consumption, based on previous reports that have shown that the average coffee consumption of humans was 3 cups per day, with each cup containing about 150 mg

of caffeine (Bellet, Kershbaum, & Fink, 1968). Assuming the average weight of a human male to be 70 kg, the average human consumption of caffeine would be approximately 6.5 mg/kg. The diluted coffee solution provided caffeine at a concentration of approximately 1.0 mg/ml, and since the rats consumed about 35 ml of liquid per day, the caffeine concentration in this resulted in a caffeine dose of approximately 7.5 mg/kg per day.

Liquid consumption was measured daily by pouring the unconsumed coffee solution or water into a graduated cylinder and subtracting that amount from the original 60 ml. On weekends, extra volumes of coffee solution and water were available to the animals, and the means of the liquid comsumption were calculated to express the amount over the weekends.

In addition to the voluntary ingestion of the coffee solution, the two caffeine groups received 10 mg/kg which was administered by force feeding. That caffeine dosage was chosen since Costill, Dalsky, and Fink (1978) have shown that it significantly increased lipid oxidation in humans. A fresh caffeine stock solution was made each week at a concentration of 10 mg/ml and stored in a refrigerator. The solution was made from anhydrous caffeine purchased from Sigma Chemical Company (number C-

0750, Lot number 25C-0144). The caffeine was administered from a 1-ml syringe equipped with a 4-cm long stainless steel intubation needle. The caffeine-trained group was force fed caffeine 0.5 hours prior to running in order to duplicate the conditions of Costill et al. (1978). Research has shown that once digested, caffeine diffuses rapidly within the body (Graham, 1979) and has influenced metabolism within 1 hour after ingestion (Costill et al., 1978; Ivy, Costill, Fink, & Lower, 1979). The force feeding altered the caffeine consumption relative to human consumption by increasing it by the equivalent of approximately 2 cups of coffee.

Training

The six rats in the caffeine-trained group and the six rats in the no-caffeine-trained group were trained 5 days each week for 9 weeks on a motor-driven treadmill according to the protocol of Holloszy (1967). The training began with runs of 15-minutes duration at 26.8 m/min on a 10% grade. Three minutes were added to the run each day, and when the duration reached 30 minutes, 30-second sprints at 40 m/min were interposed every 10 minutes. The running time increased until the rats were running for 60 minutes at 26.8 m/min on a 10% grade, with 30-second sprints at 40 m/min interposed every 10

minutes. The rats were stimulated by gentle prodding or an electric shock delivered from a grid at the back of the treadmill, but only if they avoided running. After exercise, Betadine solution was applied to the soles of the animals' feet to protect them from infection due to blisters or abrasions.

Homogenate Preparation

At the termination of the 9-week experiment, all rats were sacrificed by ether while restrained in a desiccator. Each animal was shaved with an animal clipper, and the remainder of the hair on the body was removed with a solution containing 200 g barium sulfide (Sigma Chemical Company), 30 g Tide detergent, and 500 ml 10% glycerol (Baker Analyzed Reagent, Lot number 517051).

The gastrointestinal tract was removed, cleansed of its contents, and replaced in the carcass. The carcass was then weighed on a triple beam balance, placed in an autoclave for 15 minutes at 120° C and 9-10 kg pressure, cooled, and ground in a Universal 72 food grinder. Care was taken to remove all obvious carcass tissue from the various parts of the grinder. Next, the tissue was blended in a Waring Commercial Blender, Model 5011 S. Small amounts of the ground tissue were blended to a smooth consistency prior to the addition of more tissue.

The entire carcass contents were homogenized in this manner and weighed on a triple-beam balance.

Carcass Fat Determination

The percent carcass fat was determined by the method of Clark and Tarttelin (1976). For each animal, triplicate homogenate aliquots ranging from 2 to 3 g were placed into three large screw-cap glass tubes which had been pre-weighed on a Mettler scale. The weight of the homogenate in the tube was recorded and the cap secured. About 10 boiling stones (Cargille Scientific, Catalog number 54010) were placed inside each tube, and the tubes were secured tightly. Next, a pipet was used to draw 14.25 ml of a solution of methanol water (1:.9) into each tube. Finally, 7.5 ml of chloroform was added, and the tubes were immediately recapped. The methanol and chloroform used were of reagent grade and were purchased from the Fisher Scientific Company. The samples were left overnight at room temperature, and on the following day the tubes were shaken horizontally for 20 minutes in a Shaker from Lab-Line Instruments (Model 3564) at 49 strokes per minute. The samples were again left overnight at room temperature, and on the 3rd day a 5-ml plastic syringe (Luer-Lab Tip B-D from Becton Dickinson and Company) was used to withdraw 5 ml of chloroform from

the lower phase. The chloroform samples were transferred to pre-weighed aluminum trays (Fisher Scientific Company, Catalog number 8-732, size 57) which were immediately placed in an oven (Single-Wall Transite from Blue M. Electric Company) set at 70° C. After 2 hours, the trays were removed and weighed on the Mettler balance. The weight of the contents of the trays represented the amount of lipid extracted from that sample. The lipid values were expressed as a percentage of wet carcass, and a mean was determined from the three samples. The coefficient of variation between samples from previous experiments was 5.5%.

Carcass Water Determination

The percentage of carcass water was determined by the method of Frisch, Hegsted, and Yoshinaga (1977). Triplicate aliquots of homogenate weighing between 7 and 11 g were moved from the blender and placed into pre-weighed aluminum trays. The empty trays and the aliquots were weighed on a Mettler type H-16 balance. The trays containing the samples of homogenate were immediately placed in a Thelco oven (Precision Scientific Model 17) set at 35° C. The samples were weighed daily until there was no further water loss, which was indicated by a constant weight measured over a period of 2 or more days.

The coefficient of variation for carcass water in previous experiments was 3.1%.

Analysis of Data

Data for body weights, carcass fat, and carcass water were analyzed by the Kruskal-Wallis one-way analysis of variance (ANOVA), and when a significance was indicated, the Mann-Whitney U test was used. The data for food consumption and liquid consumption were analyzed by ANOVA with repeated measures. If the ANOVA indicated significant interactions, then tests of simple main effects as described by Kirk (1968) were used. All statistical procedures were conducted at the .05 level of significance.

Summary

Twenty-four male Sprague-Dawley derived rats were used in this study. Twelve rats were selected as superior runners and were randomly assigned to either a caffeine- or a no-caffeine-trained group. The remainder of the rats were randomly assigned to either a caffeine- or a no-caffeine-sedentary group. Food and liquid consumption were consumed ad libitum by all animals throughout the study. Rats in the caffeine groups were force fed a caffeine solution that contained a caffeine dosage equivalent to that of the normal daily human

consumption plus a diluted coffee solution, which they ingested ad libitum. After 9 weeks of treatment, the rats were sacrificed by ether, and the carcass fat and carcass water were analyzed.

Chapter 4

ANALYSIS OF DATA

This study was conducted to investigate the influence of caffeine consumption in combination with training on the body weight and carcass composition of rats. In this chapter the results of the statistical analysis of the data for body weight, carcass fat, carcass water, food consumption, and liquid consumption obtained from this study are presented.

Body Weight

All rats were weighed once a week from the day of arrival at the laboratory until the last day of treatment. Mean body weights for each group were recorded. The raw scores for each animal's weekly body weight are reported in Appendix A. To analyze body weights, an $\underline{H}$ value was calculated for all body weights on each weekly measurement using the procedures in the Kruskal-Wallis one-way analysis of variance (ANOVA) by ranks test. The $\underline{H}$ values were compared to a critical value of chi-square in order to determine if there was a significant ($p < .05$) difference among the four groups in body weight for each week. The critical value for chi-square, $\chi^2(3) = 7.81$, was obtained from a chi-square table. The $\underline{H}$ values for Weeks 1 to 6 and Week 8 were

lower than the critical value of 7.81, indicating that there was no significant difference in body weight among the four groups. On Week 7 and Week 9 the groups were significantly different.

The Mann-Whitney U test was used to determine which groups differed significantly on Week 7 and Week 9. The critical value for U was determined to be 8 according to the tables in Mendenhall (1979), with values less than 8 indicating a significant difference. Table 1 presents the mean body weights of the four groups on Weeks 7 and 9. On Week 7 the caffeine-trained group weighed significantly less than the caffeine-sedentary group (12% less) and the no-caffeine-sedentary group (9.6% less). On Week 9 the caffeine-trained group weighed significantly less than the caffeine-sedentary group (11% less), the no-caffeine-trained group (7.0% less), and the no-caffeine-sedentary group (9.0% less).

Carcass Fat

At the end of treatment carcass fat composition was determined for each rat, and the Kruskal-Wallis test was used to compare the values of the four groups. Significant differences on the Kruskal-Wallis test were followed by the Mann-Whitney U test to determine which groups differed significantly. The group means and standard errors for carcass fat are found in Table 2. An H value

Table 1

Body Weights of the Four Groups

At Weeks 7 and 9 of Treatment

Group	Week 7	Week 9
Caffeine		
Trained	338.3 ± 10.38[a]	399.7 ± 9.39[b]
Sedentary	436.8 ± 16.05	449.4 ± 17.94
No-Caffeine		
Trained	406.0 ± 15.63	429.6 ± 12.90
Sedentary	424.0 ± 11.17	439.0 ± 10.00

Note. All values are means and standard errors of the mean, in grams.

[a] The caffeine-trained group weighed significantly less ($p < .05$) than the caffeine-sedentary and no-caffeine-sedentary groups.

[b] The caffeine-trained group weighed significantly less ($p < .05$) than the caffeine-sedentary, no-caffeine-sedentary, and no-caffeine-trained groups.

Table 2

Carcass Fat of the Four Groups of Rats at the End of Treatment

Group	No-Caffeine	Caffeine
Sedentary	31.9 ± 1.09	28.8 ± .55
Trained	20.1 ± .34[a]	26.3 ± 1.58

Note. All values are means and standard errors of the mean, expressed as mean percentages.

[a] The no-caffeine-trained group had significantly less ($p < .05$) fat than the caffeine-sedentary and no-caffeine-sedentary groups.

of 9.04 was determined by the procedures of the Kruskal-Wallis one-way ANOVA by ranks. The H value of 9.04 exceeded the critical chi-square value of 7.81, which indicated that there were significant differences among the four groups in carcass composition. The Mann-Whitney U test demonstrated two significant U values. The no-caffeine-trained group had a lower mean percentage of carcass fat than either the caffeine-sedentary (30% lower) or the no-caffeine-sedentary group (37% lower).

Carcass Water

At the end of treatment carcass water composition was determined for each rat, and the Kruskal-Wallis test was used to determine if the means for the four groups differed significantly. The group means and standard errors for carcass water are found in Table 3. An H value for the groups' percentage of carcass water was calculated to be 12.0, as compared to the critical value, $\chi^2(3) = 7.81$. The larger H value of 12.0 indicated that there was a significant difference in percentage of carcass water among the four groups. The Mann-Whitney U test was used in order to determine which groups differed significantly. The critical value of 8 was determined according to the tables in Mendenhall (1979). A significant U value of 2 was found, indicating that the

Table 3

Post Treatment Carcass Water of the Four Groups of Rats at the End of Treatment

Group	No-Caffeine	Caffeine
Sedentary	60.9 ± .71[a]	62.9 ± .66[b]
Trained	67.7 ± .43	66.5 ± .62

Note. All values are means and standard errors of the mean, expressed as mean percentages.

[a] The no-caffeine-sedentary group had significantly less ($p < .05$) water than the no-caffeine-trained and caffeine-trained groups.

[b] The caffeine-sedentary group had significantly less ($p < .05$) water than the no-caffeine-trained group.

caffeine-trained group had a larger percentage of carcass water than the no-caffeine-sedentary group. Other U values indicated that the no-caffeine-trained group had a larger percentage of carcass water than the caffeine-sedentary group (U = 3) and the no-caffeine-sedentary group (U = 0).

The combination of both trained groups contained 7.7% more carcass water than the combination of both sedentary groups. The caffeine-trained group had an 8.4% larger percentage of carcass water than the no-caffeine-sedentary group. Also, the no-caffeine-trained group had a 7.1% larger percentage of carcass water than the caffeine-sedentary group, and a 10% larger percentage of carcass water than the no-caffeine-sedentary group.

Food Consumption

Food consumption was recorded daily, and weekly average consumptions were calculated for each rat during the final 5 weeks of the study. The weekly means and standard errors for food consumption are found in Table 4. The raw scores for each animal's weekly food consumption are reported in Appendix B. A repeated measures ANOVA design was used to analyze the data collected for the weekly food consumption.

Table 4

Mean Food Consumption During the Last 5 Weeks of Treatment

	Week				
Group[a]	5	6	7	8	9
Caffeine					
Trained	25 ± .8	27 ± 1.3	27 ± 1.0	28 ± 1.5	26 ± 2.1
Sedentary	27 ± .8	31 ± .8	29 ± .8	28 ± 1.5	29 ± .6
No-Caffeine					
Trained	26 ± .7	29 ± 1.5	29 ± .8	28 ± .7	28 ± .9
Sedentary	24 ± 1.3	27 ± 1.5	25 ± 1.6	25 ± .7	24 ± .8

Note. All values represent the means and standard errors of the mean expressed in grams of food.

[a] $n = 6$ for each group.

Table 5

ANOVA Summary Table of Simple Main Effects For Food Consumption During the Last 5 Weeks of Treatment

Source	SS	df	MS	F
Caffeine				
Caffeine at Trained	66.13	1	66.13	2.51
Caffeine at Sedentary	153.00	1	153.00	5.80*
Trained				
Trained at Caffeine	30.56	1	30.56	1.16
Trained at No-Caffeine	224.20	1	224.20	8.51*
Error	526.70	20	26.34	

*$p \leq .05$.

The 3-way interactions did not indicate any significant differences, however, when the 2-way interactions were investigated, a significant interaction ($p < .05$) for caffeine by weeks was shown. Because of the interaction of caffeine by weeks, the main effects of caffeine and of weeks would be misleading. Therefore, simple main effects were examined. The ANOVA summary table is shown in Table 5.

Since training was not involved in a significant interaction, the main effects can be interpreted directly. They indicate that there was no effect of training on food consumption. A graph of the food consumption of the four groups during the last 5 weeks of treatment is found in Figure 1. The caffeine-trained group consumed 7.3% less food than the caffeine-sedentary group, and the no-caffeine-trained group consumed 2.2% less food than the caffeine-sedentary group.

Liquid Consumption

Average weekly liquid consumption for each rat was recorded. The weekly means for the final 5-week training period are found in Table 6. The raw scores for each animal's liquid consumption are reported in Appendix C. A repeated measures ANOVA design was used to analyze the data collected for the weekly liquid consumption. Then

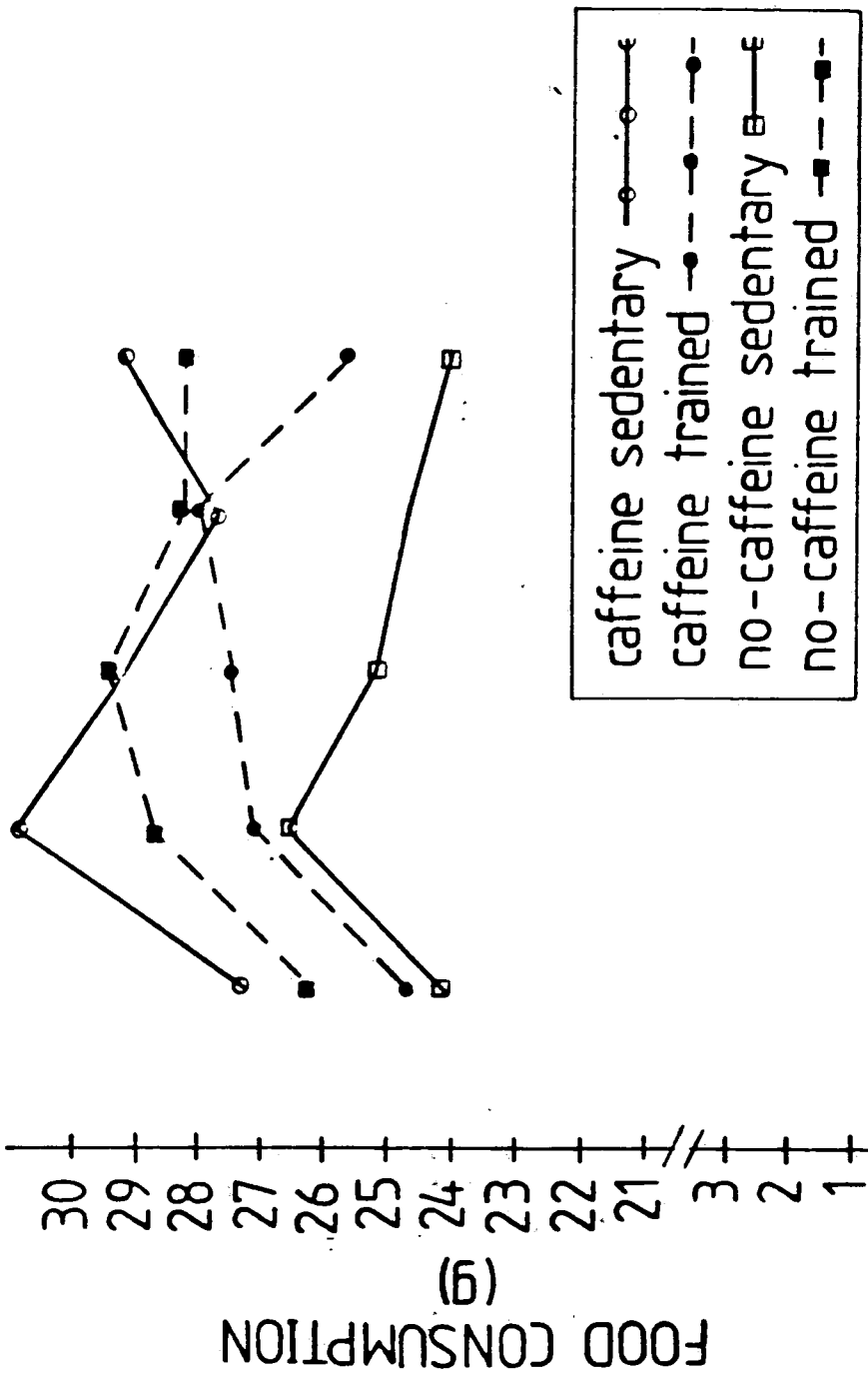

Figure 1. Mean food consumption during the last 5 weeks of treatment of the 4 groups of rats.

Table 6

Mean Liquid Consumption of Caffeine and No-Caffeine Groups During the Last 5 Weeks of Treatment

	Week				
Groups[a]	5	6	7*	8	9*
Caffeine	41 ± 1.9	38 ± 2.0	36 ± 1.8	36 ± 1.7	36 ± 3.4
No-Caffeine	39 ± 1.3	41 ± 1.8	44 ± 2.0	40 ± 1.6	40 ± 2.2

Note. All values are expressed in ml.

[a] $n = 6$ for each group.

* The no-caffeine groups consumed significantly ($p < .05$) more liquid than the caffeine groups.

Table 7

ANOVA Summary Table of Simple Main Effects for Liquid Consumption During the Last 5 Weeks of Treatment

Sources	SS	df	MS	F
Caffeine				
Caffeine at weeks	32.90	4	8.23	.89
No-Caffeine at weeks	108.00	4	27.00	2.93*
Trained vs. Sedentary				
At Week 5	.00	1	.00	.00
At Week 6	2.10	1	2.10	.23
At Week 7	27.70	1	27.70	3.00
At Week 8	2.14	1	2.14	.23
At Week 9	40.50	1	40.50	4.38*
Error	738.40	80	9.23	

*$p \leq .05$.

the Tukey test was used on the significant simple main effects of caffeine at weeks (Table 7). The results of the Tukey test showed that on Weeks 3 and 5 the trained groups (caffeine and no-caffeine) consumed significantly less liquid than the sedentary groups (caffeine and no-caffeine). On Week 3 the trained groups consumed 5.4% less liquid than the sedentary groups, and on Week 5 the trained groups consumed 6.4% less liquid than the sedentary groups. The caffeine-trained group consumed the least amount of liquid of all four groups on Weeks 3, 4, and 5, while the caffeine-sedentary group consumed the largest amount of liquid of all four groups during Weeks 3, 4, and 5.

Summary

Mean body weights, carcass fat composition, and carcass water composition for each group were analyzed according to the Kruskal-Wallis one-way ANOVA by ranks test, followed by the Mann-Whitney U test when appropriate. On Week 7 the caffeine-trained group weighed significantly ($p < .05$) less than the caffeine-sedentary and no-caffeine-sedentary groups. On Week 9 the caffeine-trained group weighed less than all of the other groups. The no-caffeine-trained group had a lower mean percentage of carcass fat than either the caffeine-

sedentary or no-caffeine-sedentary groups. The combination of both trained groups (caffeine and no-caffeine) contained larger percentages of carcass water than the combination of both sedentary groups (caffeine and no-caffeine).

Food consumption and liquid consumption data were analyzed by ANOVA with repeated measures, followed by a simple main effects test when a significance was found. The caffeine-trained group consumed significantly more food than the caffeine-sedentary group (7.3%), while the no-caffeine-trained group consumed less food than the caffeine-sedentary group (2.2%). It was found that both of the trained groups and the caffeine-sedentary group consumed significantly more food than the no-caffeine-sedentary group.

On Weeks 3 and 5 both trained groups (caffeine and no-caffeine) consumed less liquid than the sedentary groups (caffeine and no-caffeine). The caffeine-trained group consumed the smallest amount of liquid of all groups on Weeks 3, 4, and 5, and the caffeine-sedentary group consumed the largest amount of liquid of all groups during Weeks 3, 4, and 5.

Chapter 5

DISCUSSION OF RESULTS

The purpose of this study was to investigate the effect of caffeine ingestion in combination with training on body composition. The mean body weights of 24 rats were measured each week during the 9-week treatment. Food and liquid consumption were recorded daily during the final 5 weeks of the study, and weekly averages were calculated. Carcass fat was analyzed at the end of treatment according to the method of Clark and Tarttelin (1976). Carcass water was analyzed according to the method of Frisch, Hegsted, and Yoshinaga (1977). A discussion of the results from this investigation is presented in this chapter. The discussion has been divided into the following sections: (a) body weight, (b) carcass fat, (c) carcass water, (d) food consumption, (e) liquid consumption, and (f) summary.

Body Weight

The mean body weights of the trained rats (caffeine and no-caffeine) were significantly lower than the mean body weights of the sedentary rats (caffeine and no-caffeine). The mean body weights of both the trained and the sedentary groups were consistent with those of previous studies (Herbert, Kerkhoff, Bell, & Lopez, 1975;

Lau, Flaim, & Ritchie, 1979; McGarr, Oscai, Borensztajn, 1976). The rank order of the distribution of weekly mean body weights of the four groups were very similar to the rank order of the weekly mean body weights reported by Wilcox (1982), with the caffeine-trained group having the lowest mean body weight.

On Week 7 the caffeine-trained group weighed significantly less than the caffeine-sedentary group and the no-caffeine-sedentary group. On Week 9 the mean body weight of the caffeine-trained group was significantly lower than all other groups. These results suggested that the addition of caffeine to a training program may assist in reducing body weight.

As a diet aid, caffeine may reduce body weight either by enhancing caloric expenditure or by decreasing caloric intake. Lau et al. (1979) reported that regular training reduced the body weights of rats, and Wilcox (1982) reported that caffeine has also been associated with a decrease in body weight in rats. Since training alone or caffeine intake alone has been shown to decrease body weight, a potentiated effect was expected from the combined treatment administered in this study. In the present study, there was a significant decrease in body weight reported when caffeine intake was combined with

training. The caffeine-sedentary group and no-caffeine-trained group had the greatest weekly mean consumptions of all four groups. The increased food intake probably hindered body weight reduction, which may explain the discrepancy between the results of this investigation and other studies that have shown weight loss in caffeine-only and training-only groups.

The addition of stimulants to a diet program is not new, but in the past the side effects of such practices, such as increased blood pressure and irritability (Krupka & Vener, 1981; Reiman, 1967), have restrained the popularity of this practice. The results of this study indicated that the combination of training and caffeine may be conducive to a weight loss program.

Carcass Fat

Training, with or without caffeine, caused a decrease in the percentage of carcass fat (see Table 1). The findings of a reduced carcass fat was consistent with the of Herbert et al. (1975) and Wilcox (1982), in which training lowered the percentage of carcass fat by increasing lipid metabolism and changing the fat content of the body. In this study the significant differences in percentage of carcass fat between the no-caffeine-trained group and both sedentary groups (caffeine and no-

caffeine) supported the hypothesis that training reduced fat content.

Wilcox (1982) investigated the effect of caffeine and training on fat-pad weight and fat-cell size. He reported that the caffeine-trained group had the lowest fat-pad weight and the smallest fat-cell size when compared with the caffeine-sedentary, no-caffeine-trained, and no-caffeine-sedentary groups. In the present study, however, the no-caffeine-trained group had a lower percentage of carcass fat than any other group. The difference in the results between this study and that of Wilcox (1982) may be due to the different modes of training, since Wilcox used swimming instead of running. Swimming for 2 hours has heen shown to decrease food intake in rats (Oscai & Holloszy, 1969). Instead, in the present study, trained rats reduced food consumption but body weight continued to increase. The continued increase in body weight may have been due to factors not measured in this study, such as a decrease in spontaneous activity or basal metabolic rate.

The mean values for the percentage of carcass fat were 8% higher than the values reported by Frisch et al. (1977). Therefore, the difference in fat percentage

reported in previous studies and this study is very likely due to the different analysis methods employed.

Although the caffeine-trained group had the second lowest mean percentage of carcass fat, no significant difference was found between the caffeine-trained group and any of the other groups. The caffeine-trained group consumed more food than the no-caffeine-trained group, which may explain why the caffeine-trained group did not show a significant decrease in the percentage of carcass fat as was expected. Wilcox (1982) reported that caffeine intake with training reduced food intake. The discrepancy between the results of the present investigation and the study by Wilcox may be indicative of the variability of responses to chronic caffeine intake and training. It is not possible to make a direct comparison between the study of Wilcox (1982) and this study since the caffeine dosage varied between the two studies. It is possible that the increased caffeine dosage used in this study may have augmented food consumption.

Carcass Water

The results obtained from previous carcass analysis studies have shown that an inverse relationship existed between the percentage of carcass fat and the percentage

of carcass water (Lau et al., 1979; Herbert et al., 1979). Therefore, animals that had a low percentage of carcass fat usually had a high percentage of carcass water.

Training has been shown to produce a slight increase in the percentage of carcass water (Borer, Hallfrisch, Tsai, Hallfrisch, & Kuhns, 1979). The values obtained in this study are in agreement with the values of the percentage of carcass water obtained from a study by Frisch et al. (1977) and are approximately 11% higher than the values reported by Borer et al. (1979). The no-caffeine-trained group had the largest percentage of carcass water of all groups, a value that was significantly larger than the values for the caffeine-sedentary and the no-caffeine-sedentary groups. The caffeine-trained group had the second highest percentage of carcass water, a value significantly higher than the no-caffeine-sedentary group.

A high percentage of carcass water accompanied by a low percentage of carcass fat represents a desirable body composition. The large percentage of carcass water reported in both the caffeine-trained and the no-caffeine-trained groups was accompanied by a small percentage of carcass fat for both groups. The carcass

fat percentages of the caffeine-trained group and the no-caffeine-sedentary group were not significantly different in this study. The fact that the caffeine-trained group consumed significantly more food than the no-caffeine-sedentary group should be considered in an interpretation of this study. Perhaps the caffeine-trained group would have had an even greater increase in carcass water as well as a greater reduction in the percentage of carcass fat if the food consumption of that group had not been elevated.

Food Consumption

The effects of training or caffeine ingestion on food consumption have been pursued by many investigators. While some studies have reported that training depressed food intake (Askew & Hecker, 1976; Askew, Huston, Plopper, & Hecker, 1975), food consumption did not decrease in response to training in the present study, except when training was combined with caffeine intake. However, food consumption was increased in the caffeine-sedentary group. The reasons for the increase in food consumption in the caffeine-sedentary group and the very low food consumption by the no-caffeine-sedentary group are not clear. Parsons and Nadeau (1981) reported that caffeine intake increased food consumption only during

reported that caffeine-sedentary and caffeine-trained groups consumed less food than the no-caffeine-sedentary and no-caffeine-trained groups. The difference between the food consumption of the caffeine-sedentary group in this study and previous studies may be due to the different dosages of caffeine administered. The rats in the present study consumed coffee ad libitum and received a force-fed 10 mg-kg caffeine solution on 5 days per week. The increase caffeine administration in this study may have enhanced the drug's effect on the central nervous system.

Liquid Consumption

Previous studies have not reported the effects of training or caffeine intake on weekly liquid consumption. In the present study training decreased weekly liquid consumption. The greatest decrease in liquid consumption occurred in the caffeine-trained group. The caffeine-trained group consumed significantly less liquid than the other three groups on Weeks 3, 4, and 5, while both sedentary groups (caffeine and no-caffeine) increased their liquid consumption significantly. The caffeine-sedentary group consumed the largest amount of liquid during Weeks 3, 4, and 5. In this study, the addition of caffeine to a training group decreased liquid

consumption, while the addition of caffeine to a sedentary group increased liquid consumption.

The caffeine-sedentary group may have increased liquid consumption to compensate for the diuretic effect caused by caffeine intake (Graham, 1978). Filtration rate has been shown to decrease during exercise (Kachadorian & Johnson, 1970), therefore less water may have been excreted by the caffeine-trained group which may have resulted in a lower total body water loss. A reduced body water loss could explain the reduction in liquid consumption by the caffeine-trained group. Further research on the effects of caffeine intake and training on liquid consumption is needed.

Summary

The caffeine-trained group weighed significantly less than the caffeine-sedentary group and the no-caffeine-sedentary group on Week 7 of this study. On Week 9 the caffeine-trained group weighed significantly less than all other groups.

At the end of treatment, the no-caffeine-trained group had a significantly lower mean percentage of carcass fat than the caffeine-sedentary group and the no-caffeine-sedentary group. Also, at the end of treatment, the caffeine-trained group had a significantly larger

mean percentage of carcass water than the no-caffeine-sedentary group.

The fact that the caffeine groups (trained and sedentary) consumed significantly more food than the no-caffeine-sedentary group may have affected body composition. The caffeine-trained group consumed the smallest amount of liquid on Weeks 3, 4, and 5, while the caffeine-sedentary group consumed the largest amount of liquid on Weeks 3, 4, and 5. The addition of caffeine to a training group decreased liquid consumption, while the addition of caffeine to a sedentary group increased liquid consumption.

Chapter 6

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS FOR FURTHER STUDY

Summary

This investigation was conducted to study the effect of caffeine intake in combination with chronic training on body composition. The subjects were 24 male Sprague-Dawley derived rats that were assigned to four groups: caffeine-trained, no-caffeine-trained, caffeine-sedentary, and no-caffeine-sedentary. The two trained groups (caffeine and no-caffeine) trained 5 days a week for 9 weeks by running on a motor driven treadmill. The animals ran for 60 minutes at 26.8 m/min on a 10% grade, with 30-second sprints at 40 m/min interposed every 10 minutes. The animals in the caffeine groups (trained and sedentary) ingested a coffee solution ad libitum, which provided approximately 7.5 mg/kg per day. They were also force-fed a caffeine stock which provided 10 mg/kg per day. Body weights were averaged weekly. The percentage of carcass water for each rat was determined by the method of Frisch, Hegsted, and Yoshinaga (1977). The percentage of carcass fat for each rat was determined by the method of Clark and Tartellin (1976). During the last 5 weeks of this study, food and liquid consumption were

recorded daily, and weekly averages were calculated. The Kruskal-Wallis one-way analysis of variance was used to analyze body weight, carcass fat, and carcass water in the animals. A repeated measures ANOVA design was used to analyze food and liquid consumption.

The results showed that the caffeine-trained group weighed significantly less than the caffeine-sedentary and no-caffeine-sedentary groups on Week 7 of the treatment. The caffeine-trained group had the lowest body weight of all groups on Week 9. The no-caffeine-trained group had a lower mean percentage of carcass fat than either the caffeine-sedentary or no-caffeine-sedentary groups at the end of the treatment. The caffeine-trained group had a larger percentage of carcass water than the no-caffeine-sedentary group. The no-caffeine-trained group had a larger percentage of carcass water than the caffeine-sedentary and no-caffeine-sedentary groups.

Conclusions

The findings of this investigation support the following conclusions concerning the effects of caffeine intake in combination with training on body composition:

1. The combination of caffeine intake and training yielded the lowest mean body weight on Week 9 of treatment.

2. Training alone yielded the lowest mean percentage of carcass fat at the end of treatment.

3. The combination of caffeine and training yielded a larger percentage of carcass water than was seen in either the caffeine-only group or the training-only group.

4. The no-caffeine-trained group was shown to have a larger percentage of carcass water than the caffeine-sedentary group and the no-caffeine-sedentary group.

Recommendations for Further Study

The findings of this investigation suggested the following recommendations for further study on the influence of caffeine intake and training on body composition:

1. Use different caffeine doses and different time periods for forced caffeine feeding.

2. Use caffeine substances other than coffee for ad libitum caffeine consumption.

3. Use running protocols of varying durations and intensities.

4. Use forms of training other than running.

5. Involve a larger number of rats in each group to control for misinterpretations that may develop from responses to training and caffeine that may be unique to the individual.

Appendix A

RAW SCORES OF EACH ANIMAL'S

WEEKLY BODY WEIGHT

Groups	Rat	Weeks 1	2	3	4	5	6	7	8	9
Caffeine-Trained	# 1	346	351	358	376	393	391	393	405	371
	# 2	322	342	353	361	360	351	365	384	391
	# 3	344	357	365	390	410	406	415	434	421
	# 4	349	350	361	335	371	354	350	394	388
	# 5	300	318	345	361	386	381	396	412	426
	# 6	332	340	338	342	365	364	380	393	400
Caffeine-Sedentary	# 7	363	359	403	412	440	445	464	477	482
	# 8	379	378	414	425	460	460	472	482	488
	# 9	300	313	321	336	372	361	375	382	380
	# 10	355	363	412	425	454	447	456	468	470
	# 11	332	355	381	395	414	415	430	440	440
	# 12	306	316	356	362	404	411	423	428	435
No-Caffeine-Trained	# 13	305	344	367	376	393	407	416	429	437
	# 14	360	374	399	400	420	429	444	467	456
	# 15	312	330	366	364	382	389	403	427	427
	# 16	335	355	378	361	364	365	358	381	463
	# 17	375	417	444	429	431	438	441	427	405
	# 18	296	326	334	339	357	362	374	388	389
No-Caffeine-Sedentary	# 19	291	319	356	419	444	448	452	460	423
	# 20	372	400	425	434	448	455	451	458	457
	# 21	334	366	378	385	406	407	415	422	408
	# 22	364	385	407	424	397	406	409	426	466
	# 23	330	349	375	387	421	398	428	440	430
	# 24	264	312	336	320	361	370	388	404	450

Note. All values are expressed in grams.

Appendix B

RAW SCORES OF EACH ANIMAL'S

WEEKLY FOOD CONSUMPTION

Groups	Rat	Weeks 5	6	7	8	9
Caffeine-Trained	# 1	23.4	24.9	25.1	24.2	20.4
	# 2	23.4	23.1	25.7	24.2	26.1
	# 3	24.4	27.6	27.3	27.5	25.6
	# 4	24.9	31.9	31.8	32.6	33.4
	# 5	28.4	28.8	28.8	30.8	28.4
	# 6	23.4	26.3	25.7	24.8	25.9
Caffeine-Sedentary	# 7	25.6	32.5	32.4	31.0	31.3
	# 8	30.5	33.7	30.2	31.0	29.6
	# 9	25.7	28.6	28.2	26.1	27.4
	# 10	28.1	29.2	27.4	29.0	29.8
	# 11	25.8	29.5	28.5	21.7	28.4
	# 12	28.1	31.2	27.9	26.7	26.7
No-Caffeine-Trained	# 13	28.0	28.6	28.4	27.1	29.6
	# 14	27.5	29.4	30.8	29.6	29.7
	# 15	25.9	29.4	27.5	27.6	26.9
	# 16	24.2	22.6	28.7	27.9	26.7
	# 17	27.7	33.7	32.7	30.7	30.2
	# 18	24.1	28.3	27.5	26.0	25.1
No-Caffeine-Sedentary	# 19	27.7	28.9	27.6	26.4	26.5
	# 20	28.2	32.0	28.3	27.0	25.9
	# 21	21.4	26.1	25.8	23.8	22.3
	# 22	20.9	25.3	28.4	25.1	23.3
	# 23	22.6	22.2	19.4	22.1	22.0
	# 24	23.6	23.7	21.4	24.1	23.8

Note. All values are expressed in grams.

Appendix C

RAW SCORES OF EACH ANIMAL'S WEEKLY LIQUID CONSUMPTION

Groups	Rat	Weeks 5	6	7	8	9
Caffeine-Trained	# 1	38.4	37.2	31.3	36.9	34.9
	# 2	36.3	32.2	32.1	30.3	35.3
	# 3	37.1	32.8	32.5	32.3	33.3
	# 4	43.4	43.9	44.6	41.4	41.3
	# 5	48.1	38.4	34.8	39.0	39.8
	# 6	33.5	31.5	32.6	32.3	38.0
Caffeine-Sedentary	# 7	34.2	34.3	33.7	35.8	36.1
	# 8	54.1	54.9	49.1	50.1	54.0
	# 9	39.9	38.5	35.4	32.3	34.8
	# 10	33.6	34.1	32.9	33.6	32.3
	# 11	39.7	31.8	30.6	29.3	32.6
	# 12	49.1	42.5	43.3	42.1	45.0
No-Caffeine-Trained	# 13	35.7	40.0	40.4	37.7	41.3
	# 14	40.2	39.3	40.2	38.4	37.9
	# 15	40.7	41.8	43.2	40.4	40.0
	# 16	45.0	51.8	53.2	54.3	49.0
	# 17	44.9	45.6	46.8	42.8	56.0
	# 18	36.4	33.2	35.6	33.2	35.6
No-Caffeine-Sedentary	# 19	32.4	32.4	36.5	34.9	30.4
	# 20	33.6	32.3	37.9	41.9	37.0
	# 21	42.7	41.6	42.1	38.4	35.3
	# 22	41.9	39.8	41.8	39.2	36.4
	# 23	35.2	43.9	53.3	35.4	31.6
	# 24	43.4	48.6	55.9	38.6	46.7

Note. All values are expressed in ml.

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