INDIVIDUAL DIFFERENCES IN THE PROPENSITY OF FEMALE RHESUS
MONKEYS (*MACACA MULATTA*) TO GAIN AND LOSE BODY WEIGHT

by

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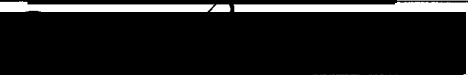Oregon Health & Science University

CERTIFICATE OF APPROVAL

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TABLE OF CONTENTS

LIST OF FIGURES

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ABSTRACT

Weight gain has escalated over the past two decades, such that currently 65% of American adults are overweight. Not surprisingly, weight-related illnesses have also increased. The overall goal of this dissertation was to examine individual differences in adult weight gain and weight loss to identify physiological mechanisms that predispose an individual toward adult weight gain, or conversely predispose an individual to maintain a healthy body weight throughout adulthood. This thesis addressed three forms of weight change that are common in adult women: menopausal weight gain, slow progressive weight gain that is unintentional, and weight change occurring in response to dietary change. In these studies food intake, body weight, activity and energy expenditure were directly measured in a population of 18 female rhesus monkeys over a series of experimental manipulations. To examine the role of menopause in causing weight gain, monkeys were ovariectomized and then received estrogen replacement therapy. Ovariectomy led to a rapid increase in body weight and food intake, suggesting that at least part of menopausal weight gain is due a decrease in ovarian hormones. Estrogen replacement therapy decreased body weight and food intake, and increased the level of physical activity, suggesting that estrogen replacement therapy may help postmenopausal women counteract midlife weight gain. These same monkeys were later studied during a period when their food intake and activity were stable, to identify physiological mechanisms that underlie slow weight gain in adulthood in many individuals. Neither food intake, nor basal metabolic rate were associated with weight gain, but the level of physical activity that an individual routinely undertook strongly predicted weight gain, such that the most active monkeys gained significantly less weight

than sedentary monkeys. Lastly, all monkeys were put on a uniform diet for a two-month period (a 30% calorie reduction for one month, and a 60% calorie reduction during a second month). Overall weight loss was only $6.4\pm1.7\%$, but some monkeys lost a considerable amount of weight (22%) while others showed no weight loss. The heaviest fattest monkeys lost the most weight on the diet, and weight loss was negatively correlated with initial energy balance (calories consumed — calories expended), such that monkeys with the lowest initial energy balance lost the most weight while dieting. All monkeys compensated for the reduction in calorie intake by substantially decreasing total energy expenditure, basal metabolic rate and physical activity. However, the degree of compensation did not predict individual differences in weight loss. We conclude that it is difficult to promote weight loss by dieting alone, as the body compensates for a reduction in calorie intake by markedly decreasing energy expenditure. Unfortunately, the individuals least likely to experience diet-induced weight loss are those who are overeating the most prior to dieting. In summary, the metabolic regulatory processes that predict individual differences in weight change depend on the circumstance under which weight change occurs.

Chapter 1

INTRODUCTION

1.1 Obesity Epidemic

The increasing prevalence of overweight and obese individuals is a global problem that affects over one billion adults and even 17.6 million children under the age of 5 (Flegal et al. 2002; Waxman 2004; Strychar 2006). Weight gain has escalated over the past two decades, such that currently only a minority (<34%) of adults in the United States have a healthy body mass index (BMI), and 65% of adults are either overweight or obese (>25.0 kg/m^2;(Flegal et al. 2002). The increasing prevalence of obesity has large implications for the health of the human population. Obesity is associated with overall increases in morbidity and mortality (Paffenbarger et al. 1993), resulting from an increased risk of diabetes mellitus (Colditz et al. 1990; Kujala et al. 1994; Folsom et al. 1996; WHO 2000), gall bladder disease (WHO 2000), coronary heart disease (Hamm et al. 1989; Manson et al. 1990; Kujala et al. 1994; Rimm et al. 1995; Willett et al. 1995; 2000; Klein et al. 2004), hypertension (Kujala et al. 1994; 2000), stroke (WHO 2000), dyslipidemia (WHO 2000), osteoarthritis (WHO 2000), gout (WHO 2000), pulmonary diseases (WHO 2000), colon cancer (Giovannucci et al. 1995), and breast cancer (Ziegler et al. 1996). If the rates of obesity continue to rise at the current pace, obesity will exceed smoking as the leading preventable cause of death in the United States within the next few years (Mokdad et al. 2004). Accordingly, the health care costs associated with obesity exceed $70 billion per year. Additionally, consumers spend $33 billion annually on weight loss products and services (Stern et al. 1995). Thus, it is becoming increasing important to better understand factors that prevent adult weight gain.

Moderate weight loss is associated with significant health benefits as it reduces the risk of a number of obesity-related diseases [heart disease (Krauss et al. 2000), stroke (Krauss et al. 2000), type 2 diabetes (Tuomilehto et al. 2001; Knowler et al. 2002), hypertension, hyperlipidemia, hypercholesterolemia, cardiovascular disease, osteoarthritis and depression (Goldstein 1992; Stern et al. 1995)]. Thus, it is not surprising that, at any time, two-thirds of obese adults are trying to lose weight (Serdula et al. 1999; Strychar 2006). Unfortunately, most of the individuals trying to loose weight are unable to do so. Success rates are very low and range from 2 to 20% of individuals who try to lose weight actually being successful (Wing and Hill 2001). To more effectively promote weight loss, a greater understanding is needed of factors that predispose certain individuals to be successful at losing weight.

In women, obesity is especially problematic. Women are more likely to be obese than men (Lovejoy 1998; Prevention 2004) and the women who are obese are more susceptible to obesity-related diseases, such as diabetes and coronary heart disease compared to obese men (Hu 2003). In addition to the health risks that are commonly associated with obesity, in women obesity is associated with increased health risks that are more specific to women. These included increased risk of breast (Schindler 1997; Hu 2003) and endometrial cancer (Schindler 1997), and increased incidence of conditions associated with infertility, such as polycystic ovarian syndrome and luteal phase defects (Lovejoy 1998). Moreover, weight loss has been shown to improve fertility (Clark et al. 1995). In women, the risk of becoming obese increases through adulthood (Flegal et al. 2002). In general, women gain weight and body fat during the fourth and fifth decades of life, which is during the time that menopause occurs (Williamson et al. 1990; Haffner et

al. 1991; Ley et al. 1992; Kotani et al. 1994; Aloia et al. 1995; Lovejoy 1998; Tchernof and Poehlman 1998; Chang et al. 2000; Blumel et al. 2001). The current life expectancy for women in the United States is 80 years of age (Prevention 2004) which implies that the average woman lives over a third of her life after menopause. Thus, it is becoming increasingly important to understand what causes individual differences in weight gain in postmenopausal women, as this is the population that is most at risk for obesity and obesity-related diseases. To begin to understand what causes individual differences in weight gain in postmenopausal women this thesis examines factors that predict individual differences in weight change in three circumstances that commonly cause weight change in women (menopause, slow progressive weight gain over adulthood and dieting) using a monkey model of postmenopausal women (i.e. ovariectomized female rhesus monkeys).

1.2 Causes of Obesity Epidemic

Obesity occurs when individuals consume more calories than they expend over a prolonged period of time. Studies show that both genetic and environmental factors, and the interactions between them, contribute to obesity. The increased prevalence of obesity in the last decade is unlikely to have resulted solely from genetic factors, as the genetic make up of the human population has not changed substantially during this brief time period. Most studies agree that environmental factors and lifestyle decisions, such as a decrease in physical activity and an increase in caloric intake play a large role in causing the increasing prevalence of obesity. To an extent, modernization over the past several hundred years has allowed a situation where individuals no longer need to be active to survive, and food supply is abundant and readily available in quantities that exceed

individual needs. This environment could easily lead to a situation where the average individual eats more calories than they expend.

Factors such as the availability of a wide array of highly palatable energy dense foods, large portion sizes, and increased snacking, could all contribute to increased energy intake. A number of studies report an association with the amount of high fat foods consumed and BMI (Lissner et al. 1987; Miller et al. 1990; Kendall et al. 1991; Klesges et al. 1992; Larson et al. 1995). Surprisingly, some studies indicate that the amount of food that individuals consume has remained relatively stable (Alexy et al. 2002) or declined (Cavadini et al. 2000) over the last few decades and that the amount of energy derived from fat has decreased (Willett and Leibel 2002) However, sales of most food items have increased over the last two decades (Speakman and Selman 2003). Thus, it appears likely that increased calorie intake is at least partially responsible for the increasing prevalence of obesity.

It is also likely that decreased energy expenditure plays a large role in causing the increased prevalence of obesity. There is no indication that basal metabolic rate has declined during the last two decades (Frankenfield et al. 1998; De Lorenzo et al. 2001), thus it seems likely that decreased physical activity has significantly contributed to the obesity epidemic. This claim is supported by a number of lines of evidence. Studies that measure activity in human subjects that are living in primitive rural societies find that they expend 40% more calories a day than the average modern man (Speakman and Selman 2003). The amount of energy expended to complete daily tasks has been reduced by technological advancements such as dishwashers, washing machines, vacuums and escalators. Recently, Levine and colleagues (Lanningham-Foster et al. 2003) found that

the amount of energy expended doing daily tasks such as washing clothes and dishes with modern technology accounted for significantly fewer calories per day than performing the same tasks without technological advancements. Another factor that has played a role in reducing physical activity is the shift from walking places to driving. The number of people that own automobiles has dramatically risen in developed countries over the last 50 years (Speakman and Selman 2003). These changes in mobility have had a large impact on many of our daily activities. Modern cities are built for convenience so restaurants and banks are set up so that you don't have to leave your car to get service (Levine 2004). Schools are often located out of walking distance for children, and sidewalks are less frequently available in a number of cities (Levine 2004). If people spent the amount of time saved with technological advancements participating in activities that require vigorous activity, such as sports or working out at the gym, this would not be a problem. Unfortunately, most people spend much of their time participating in sedentary activities such as working on computers and watching television (Speakman and Selman 2003). A number of studies have found a positive correlation between obesity and the number of hours spent watching television (Tucker and Bagwell 1991; Vioque et al. 2000; Dennison et al. 2002; Janz et al. 2002) In fact, currently 40% of women and 35% of men don't engage in any type of physical activity at all (Daniels 2006). In conclusion, there is ample evidence to suggest that decreased physical activity is an important factor contributing to the obesity epidemic.

Though genetic factors are unlikely to account for the increased prevalence of obesity, genetic factors appear to play an important role in determining who will become obese. Some groups of individuals such as Pima Indians and Pacific Islanders are

particularly prone to weight gain (Friedman 2003) and it has been postulated that these individuals possess a "thrifty gene" that was beneficial for survival during times of famine, but now predisposes them to morbid obesity (Bell et al. 2005). Additional evidence for a role of genetics in predisposing an individual to obesity comes from twin, adoption and family studies which have found that genetic factors account for up to 70% of the variability in obesity (Hebebrand et al. 2003; Bell et al. 2005; Farooqi and O'Rahilly 2005). Studies in mice have identified genes that are associated with obesity (Zhang et al. 1994) and have allowed identification of single gene mutations in humans that cause obesity (Montague et al. 1997; Comuzzie and Allison 1998; Perusse et al. 1999). However, single gene mutations only account for obesity in a very small percent of obese individuals (Montague et al. 1997; Comuzzie and Allison 1998; Perusse et al. 1999). Rather, linkage studies suggest that many distinct genetic loci contribute to the heritability of obesity. Recent studies are trying to identify the genes underlying energy intake (Perusse et al. 1988; Tarasuk and Beaton 1991) and energy expenditure (Ravussin and Bogardus 1989; Bouchard and Perusse 1993; Rho et al. 2004). Identifying genes associated with obesity will no doubt be important, as it will allow individuals who are at high risk of developing obesity to be identified before they become obese, allowing intervention to be initiated before the problem develops.

In women, there is limited evidence suggesting that ovarian hormones play an important role in body weight regulation. Epidemiologic studies show that women gain weight and body fat during the time when they undergo menopause (Williamson et al. 1990; Haffner et al. 1991; Ley et al. 1992; Kotani et al. 1994; Aloia et al. 1995; Lovejoy 1998; Tchernof and Poehlman 1998; Chang et al. 2000; Blumel et al. 2001). Moreover, a

number of studies report that hormone replacement therapy decreases the body weight of postmenopausal women (Espeland et al. 1997; Gambacciani et al. 1997; Khoo et al. 1998; Chmouliovsky et al. 1999; Sirola et al. 2003). In animals there is stronger evidence for a role of ovarian hormones in regulating body weight. Numerous studies have shown that surgical menopause (ovariectomy) causes an increase in food intake and body weight in rodents (Grunt 1964; Hervey and Hervey 1965; Kakolewski et al. 1968; Wade and Zucker 1970; Mook et al. 1972; Leshner and Collier 1973; Tarttelin and Gorski 1973; Landau and Zucker 1976; McElroy and Wade 1987; Chu et al. 1999; Ainslie et al. 2001; Chen and Heiman 2001; Shinoda et al. 2002) and domestic animals (Fettman et al. 1997; Harper et al. 2001; Martin et al. 2001). It is also well documented that treating ovariectomized rodents with estradiol lowers both food intake and body weight to levels found in ovary-intact controls (Grunt 1964; Hervey and Hervey 1965; Kakolewski et al. 1968; Wade and Zucker 1970; Mook et al. 1972; Leshner and Collier 1973; Tarttelin and Gorski 1973; Landau and Zucker 1976; McElroy and Wade 1987; Shinoda et al. 2002). Studies in monkeys find that ovariectomized monkeys have higher food intake than intact controls (Kemnitz et al. 1989), and treatment of ovariectomized monkeys with estradiol causes a significant decrease in food intake (Czaja and Goy 1975; Kemnitz et al. 1986). However, in monkeys as in women, there is little evidence that a decrease in ovarian hormone secretion (either with naturally-occurring menopause or surgical ovariectomy) leads to an increase in body weight. Thus, one goal of this thesis is to determine the role that ovarian hormones play in body weight regulation in primates, focusing on what causes individual differences in body weight change in response to changes in the levels of circulating ovarian hormones.

1.3 Physiologic Processes Regulating Body Weight

The balance between energy consumed and energy expended determines body weight. Body weight increases when energy intake exceeds energy expenditure and weight loss occurs when energy expenditure exceeds energy intake. Energy intake is energy consumed corrected for the amount of energy that is actually absorbed by the gastrointestinal tract (digestible energy). The main components of energy expenditure are basal metabolic rate, activity-associated energy expenditure and the thermic effect of food (Van Gaal et al. 1992; Levine 2004; Levine 2004). Basal metabolic rate (BMR) is the energy used by cellular metabolism. It is defined as the energy expended when an individual is lying down in the post-absorptive state (Levine 2004). BMR accounts for the majority (60%) of total energy expenditure (Van Gaal et al. 1992; Levine 2004) and is largely dependent on lean body mass (Ford 1984; Deriaz et al. 1992; Van Gaal et al. 1992; Levine 2004; Levine 2004), such that the greater the lean body mass the more energy is expended when the individual is at rest. The thermic effect of food is the energy expended as a result of increased cellular activity associated with digestion, absorption and storage of food and makes up approximately 10% of total energy expenditure (Hill et al. 1985; D'Alessio et al. 1988; Kinabo and Durnin 1990; Reed and Hill 1996; Levine 2004; Levine 2004). Energy expenditure associated with physical activity makes up the remaining 30% of energy expenditure and can be further subdivided into energy expenditure associated with volitional exercise and nonexercise associated thermogenesis (NEAT; (Levine 2004). Volitional exercise includes behaviors such as sport and fitness-related activities. NEAT is the thermogenesis that accompanies physical activities other than volitional exercise, such as the activities of daily living,

fidgeting, spontaneous muscle contraction, and maintaining posture when not recumbent (Levine et al. 1999; Levine 2004). NEAT ranges from 15% of total energy expenditure in sedentary individuals to 50% or more in active individuals (Livingstone et al. 1991; Jakicic et al. 1995; Blundell and King 1999; Levine 2004; Levine 2004). It is also important to keep in mind that a small amount of energy is lost in the feces, urine, combustible gases, and in waste products lost from the exterior surface of the animal such as hair, skin cells and oils (surface energy) (Kleiber 1961; Blaxter 1989).

1.4 Individual Differences in Adult Weight Gain and Loss

Though the mechanisms that regulate both energy intake and energy expenditure have been the subject of a number of studies, less attention has been paid to understanding what causes some individuals to be more susceptible to gaining weight than others and why some individuals can effectively lose weight while other can not. Large differences between individuals in weight gain or weight loss has been documented in animals and humans in response to the same intervention. For example, some individuals are able to resist weight gain in response to overconsumption of calories, whereas others readily gain weight (Sims et al. 1973; Bouchard et al. 1990; Diaz et al. 1992; Levine et al. 1999). Similarly, some individuals readily lose weight on a diet, whereas others do not (Keim et al. 1991; Van Gaal et al. 1992; Wadden 1993; Hainer et al. 2000; Fairfield et al. 2002). However, it remains unclear what causes these individual differences and whether the determinants of weight gain and loss vary depending on the mechanisms promoting weight change. Each of the components of energy balance influence weight change and

there is evidence for each of these factors being important in predicting individual differences in weight change.

It is well documented that increasing calorie intake increases body weight (Curb and Marcus 1991; Chalkley et al. 2002; Warwick et al. 2002; Estadella et al. 2004; Huang et al. 2004; MacLean et al. 2004) and decreasing calorie intake leads to weight loss (Adachi et al. 1996; Cox et al. 2003). However, a number of studies find that change in food intake is not correlated with change in body weight (Johnson et al. 1956; Stefanik et al. 1959; Maxfield and Konishi 1966; Ries 1973; Yearick 1978; Matter et al. 1980; Birkbeck 1981; Baecke et al. 1983; Bellisle et al. 1988; Guillaume et al. 1998; Lorenzo et al. 2003). There are only a few studies that show that individuals with high calorie intake (Larson et al. 1995; Tataranni et al. 2003) are more prone to weight gain and obesity than individuals with lower food intake. In contrast, other studies suggest that obese individuals have lower food intake than lean individuals (Keen et al. 1979), or that there are no differences in food intake between obese and lean individuals (Rolland-Cachera and Bellisle 1986). This apparent lack of correlation between food intake and body weight may be because many studies have relied on self report of food intake, and several studies have shown that self-report of food intake is inaccurate (Champagne et al. 1998; DeLany et al. 2002). Overall, the role that differences in food intake play in determining individual differences in weight change remains unclear.

A large body of epidemiologic data shows an association between low levels of physical activity and a higher rate of adult weight gain, and a greater increase in percent body fat, throughout adulthood (Rissanen et al. 1991; Klesges et al. 1992; Williamson et al. 1993; Weinsier et al. 1995; Kyle et al. 2001; Di Pietro et al. 2004; Sternfeld et al.

2004; Brown et al. 2005; Hunter and Byrne 2005; Littman et al. 2005). Several studies have shown that obese adults and children are less active than their lean counterparts (Fontvieille et al. 1993; Rising et al. 1994; Pratt et al. 1999; Livingstone 2000; Ekelund et al. 2002; Janz et al. 2002; Trost et al. 2003; Abbott and Davies 2004; Treuth et al. 2004; Sternfeld et al. 2005). However, other studies show no association between physical activity and body weight or show that weight gain is higher in individuals with high levels of physical activity (Rising et al. 1994; Weinsier et al. 1995; Kyle et al. 2001; Tataranni et al. 2003; Di Pietro et al. 2004; Kyle et al. 2004; Sternfeld et al. 2004; Ekelund et al. 2005; Hunter and Byrne 2005; Littman et al. 2005; Sternfeld et al. 2005). Studies in rodents find that obese rats generally have lower activity levels compared to lean individuals (Clark and Gay 1972; Levin 1991), and that initial activity level predicts future weight gain such that the most active individuals gain the least amount of weight (Dunnington et al. 1977; Brownlow et al. 1996). Further evidence that activity is important in determining differences in weight gain comes from a recent study by Levine et al. (1999) which found that NEAT (nonexercise activity thermogenesis) predicted individuals differences in weight gain. Thus, there appears to be a large amount of evidence that activity is important in predicting weight gain.

There is also evidence that suggests that low metabolic rate is an important factor in determining individual differences in weight gain. There are several studies that have found that in Pima Indians, a population that has a very high rate of obesity, a low resting metabolic rate adjusted for differences in lean tissue mass was associated with greater weight gain (Ravussin et al. 1988; Ravussin 1995; Tataranni et al. 2003). Several studies have found that post-obese individuals have a lower metabolic rate normalized for fat

free mass than individuals that have never been obese (Weigle et al. 1988; Buemann et al. 1992; Larson et al. 1995; Astrup et al. 1996; Astrup 1999). However, other studies find no difference in the metabolic rate of never obese and post-obese subjects (Bessard et al. 1983; Amatruda et al. 1993; Wyatt et al. 1999; Filozof et al. 2000). A recent study found that individuals with low resting metabolic rate normalized for lean tissue mass gained more weight over a 10 year period than those with a higher resting metabolic rate normalized for lean tissue mass (Buscemi et al. 2005), providing further support that low metabolic rate may play an important role in contributing to individual differences in weight gain. In contrast, other studies have found no association between low metabolic rate and weight gain (Seidell et al. 1992; Weinsier et al. 1995). In conclusion, the role that low metabolic rate plays in determining individual differences is unclear. It may depend on the population that is being studied or the circumstance under which the weight change occurs.

Individual differences in weight change may depend not only on the initial metabolic state that the body is in, but also on the changes that occur in metabolic regulatory systems over the period of weight change. There is a large amount of evidence that there are compensatory changes in energy expenditure in response to changes in energy intake. A number of studies show that energy expenditure is increased in response to increased calorie intake (Miller et al. 1967; Apfelbaum et al. 1971; Sims et al. 1973; Dauncey and Ingram 1979; Dauncey 1980; Hill et al. 1983; Ravussin et al. 1988; Diaz et al. 1992; Leibel et al. 1995; Levine et al. 1999). Also, it is well documented that energy expenditure decreases in response to a decrease in calorie intake in humans (Dauncey 1980; Bessard et al. 1983; de Boer et al. 1986; Henry et al. 1988; de

Groot et al. 1989; Heyman et al. 1992; Leibel et al. 1995; Heilbronn et al. 2006), nonhuman primates (Lane et al. 1996), and rodents (McCarter and McGee 1989; Duffy et al. 1990; Dulloo and Calokatisa 1991; Even and Nicolaidis 1993; Levin and Keesey 1998). Several studies find large variability in the magnitude of the changes in energy expenditure between individuals when they are increasing or decreasing food intake (Hill et al. 1983; Hainer et al. 2000).

Overall, our understanding at this time of which regulatory systems underlying energy balance (food intake, basal metabolic rate, physical activity, and the thermic effect of food) usually contribute to the large individual differences in body weight seen in many populations, or the individual differences in weight change in response to various environmental conditions (i.e., dieting, consuming a high fat diet, leading a sedentary lifestyle) is limited. It is likely that the mechanisms that predict individual differences in body weight and weight change differ depending on the circumstances, but little work has been done to elucidate the differential mechanisms underlying various weight gain and weight loss regimens. This dissertation will examine how food intake, activity, metabolic rate and the initial metabolic state of an individual predict weight change in three circumstances that generally leads to weight change in women over adulthood (menopause, slow weight gain over adulthood and going on a reduced calorie diet).

1.5 Physiological Systems Regulating Food Intake

As consumption of food and maintenance of adequate energy stores is critical for survival, it is not surprising that the physiological systems that control food intake are complex. The systems that control food intake involve both central neural circuits in

several brain regions, as well as peripheral systems that sense the metabolic status of the body and transmit information to the brain that can be used to guide activity in the circuits that regulate food intake. Having redundant systems ensures that if one system is not functioning properly other systems are available to take over and ensure that the body receives adequate nourishment. Both the short-term and long-term energy status of the body is monitored and regulated. Short-term energy availability is regulated by modulation of the initiation, size, and termination of a single meal. Regulation of long-term energy availability acts to maintain body weight and body composition so that the body's energy stores are maintained, and if necessary improved.

Central regulation of food intake is dependent on the brain receiving information from the periphery about the current nutritional status of the body. There are three mechanisms by which signals in the periphery that change in response to food consumption can transmit their signals to the brain. Information is sent to the brain through nerves, some small molecules enter the brain where the blood brain barrier is porous (e.g. the area postrema and the median eminence) and others use specific transport mechanisms to get into the brain. For example, when food enters the gastrointestinal (GI) tract, mechanoreceptors that detect distention of the stomach and chemoreceptors that are activated in response to nutrients in the GI tract or gut peptides, transmit signals to the brain via the vagus nerve and sympathetic spinal nerves to limit meal size (Konturek et al. 2004). Peptide hormones, such as insulin (Schwartz et al. 1991), leptin (Banks et al. 1996) and pancreatic peptide (PP) (Banks et al. 1995; Banks et al. 1995) are transported into the brain by a receptor-mediated mechanism that is saturated at high concentrations. Both insulin and leptin cross the blood brain barrier, most likely in areas

where the blood brain barrier is more porous, and act on receptors in the arcuate nucleus (ARC) to decrease food intake (Baskin et al. 1988; Banks et al. 1996). Other hormones, such as peptide tyrosine-tyrosine (PYY) (Nonaka et al. 2003), cross the blood brain barrier by passive diffusion and then act on receptors present on ARC neuropeptide Y (NPY) neurons to decrease food intake (Broberger et al. 1997).

The hypothalamus is a key area of the brain governing the regulation of food intake. A number of different subregions in the hypothalamus are involved both in stimulating food intake and in suppressing food intake. Early studies identified the ventromedial hypothalamus (VMH) as the area associated with inhibiting food intake and the lateral hypothalamus (LH) as the area associated with stimulating food intake (Stellar 1954). These conclusions were based on studies that showed that electrical stimulation of the VMH suppresses food intake and lesions of this area cause hyperphagia and obesity (Stellar 1954). In contrast, electrical stimulation of the LH increased food intake and lesions in this area suppressed food intake (Stellar 1954). Further studies identified a number of other hypothalamic areas involved in food intake regulation, including the ARC, the paraventricular nucleus (PVN), and the perifornical area (PFA) (Wynne et al. 2005). The ARC was identified as particularly important in this regulatory process, as lesioning produces hyperphagia and obesity (Olney 1969). The ARC receives signals about the peripheral nutritional state both directly, as it is in close proximity to the median eminence, which lacks a blood brain barrier (Stanley et al. 2005), and from neural projections from the hindbrain (Cone 2005). The PVN was identified as being important in food intake regulation because microinjection of a large number of peptides involved in food intake regulation such as NPY (Lambert et al. 1995), ghrelin (Lawrence et al.

2002), orexin-A (Edwards et al. 1999; Shirasaka et al. 2001), CCK (Hamamura et al. 1991), leptin (Van Dijk et al. 1996; Elmquist et al. 1997), GLP-1 (Van Dijk et al. 1996) and melanocortin agonists (Giraudo et al. 1998; Kim et al. 2000) modulate food intake when given in the PVN. The PVN is also important in integrating information that it receives about the nutritional state of the periphery from many brain regions including the ARC, brainstem, limbic cortex, and the amygdala (Sawchenko 1983). Similar types of studies continue to support a role of both the VMH and LH in the regulation of food intake. Some neurons in the VMH increase their firing rate in response to glucose, while others decrease their activity in response to glucose (King 2006). The neurons of the VMH that play a role in regulating food intake are located in the dorsomedial section of the nucleus and are connected to neurons in the dorsomedial hypothalamus (DMH). Lesions of the DMH lead to hyperphagia and obesity (Bernardis and Bellinger 1987) and microinjection of orexigenic peptides such as NPY, (Stanley et al. 1985), galanin (Kyrkouli et al. 1990), and gamma-aminobutyric acid (GABA) (Kelly et al. 1979) into the DMH increase food intake. The LH contains a number of glucose sensing neurons (Bernardis and Bellinger 1996). Application of local anesthetics into the LH decrease sensitivity to low blood glucose suggesting that the LH is involved in regulation of blood glucose concentrations (Bernardis and Bellinger 1996).

In addition to multiple hypothalamic regions being important in the regulation of food intake, many regions of the hypothalamus contain multiple neuronal circuits that regulate food intake. Some systems are orexigenic (i.e. stimulation increases food intake) and other systems are anorexigenic (i.e. stimulation decreases food intake). Orexigenic NPY is expressed in several hypothalamic nuclei, although expression is species specific

to some extent (Woods et al. 1998). NPY-expressing neurons that originate in the ARC (Broberger et al. 1998; Hahn et al. 1998) and project to the PVN are important in food intake regulation (O'Donohue et al. 1985). Central NPY administration increases food intake, particularly when it is administered into the PVN and PFA (Stanley et al. 1986), where NPY receptors (Y1 and Y5 receptors) are plentiful (Gerald et al. 1996). Repeated NPY injection has been found to produce obesity within a relatively short period of time in rodents (Gerald et al. 1996). This pathway is activated in response to a decline in energy availability. Fasting increases NPY gene expression in the ARC (Kalra et al. 1991) and increases NPY release into the PVN (Sahu et al. 1988). Both leptin and insulin, which provide information about the body's peripheral metabolic status, have been shown to decrease fasting-induced stimulation of NPY gene expression (Schwartz et al. 1996; Seeley et al. 1996). Orexin A and B are also orexigenic peptides. Orexins are produced by neurons in the perifornical nucleus, and the dorsal and lateral areas of the hypothalamus and orexin-containing neurons project to many brain sites involved in food intake regulation including the PVN, ARC, and the NTS (Williams et al. 2001). Orexin-containing neurons have reciprocal connections with ARC neurons expressing NPY/AGRP and POMC/CART (Elias et al. 1998; Horvath et al. 1999). Central administration of orexin A and B stimulate food intake (Qu et al. 1996; Sakurai et al. 1998). Also the expression of these peptides increases with fasting (Qu et al. 1996; Sakurai et al. 1998). Melanin-concentrating hormone (MCH) is another orexigenic peptide found in the LH and perifornical nucleus (Marsh et al. 2002). Like, the other hypothalamic orexigenic peptides, MCH expression is increased with fasting (Qu et al. 1996; Sakurai et al. 1998) and intracerebroventricular injection of MCH increase food

intake and adiposity (Qu et al. 1996; Marsh et al. 2002). In summary, there are a number of hypothalamic orexigenic peptides that regulate food intake including NPY, the orexins and MCH. Each of these orexigenic circuits shows an increase in firing rate during fasting, and these neuropeptides stimulate food intake when they are injected centrally.

Anorexigenic peptides in the hypothalamus also play an important role in regulating food intake. Perhaps the best know anorexigenic system is the melanocortin system. Melanocortins, such as alpha melanocyte stimulating hormone (alpha-MSH), are cleaved from the precursor peptide pro-opiomelanocortin (POMC) (Woods et al. 1998). POMC is expressed in ARC neurons and project to other hypothalamic areas that regulate food intake such as the PVN (Kiss et al. 1984), which expresses melanocortin receptors (MC3 and MC4) (Mountjoy et al. 1994; Fan et al. 1997). MSH agonists which bind to melanocortin receptors are anorexigenic and antagonists are orexigenic (Mountjoy et al. 1994; Fan et al. 1997). Fasting reduces POMC mRNA expression in the ARC (Schwartz et al. 1997; Thornton et al. 1997). Another important anorexigenic peptide is cocaine and amphetamine regulated transcript protein (CART). CART-expressing neurons are abundant within the hypothalamus and are located in the ARC, LH, and PVN (Couceyro et al. 1997). Fasting reduces ARC expression of CART (Kristensen et al. 1998). Microinjection of CART into the third ventricle inhibits food intake (Kristensen et al. 1998; Lambert et al. 1998). Surprisingly however, injection of CART into hypothalamic nuclei such as the ARC and VMN increase food intake (Abbott et al. 2001). Thus, it appears that there may be several populations of CART-expressing neurons with different roles in the regulation of food intake. Another anorexigenic peptide that is involved in hypothalamic regulation of food intake is corticotrophin-releasing hormone (CRH). CRH

is widely distributed in the brain (Hillebrand et al. 2002), and has other functions in many brain areas outside the hypothalamus, specifically in the hypothalamus where it provides the central neural stimulation to the pituitary-adrenal axis. NPY projections from the ARC modulate CRH expression and release (Sarkar and Lechan 2003). Central administration of CRH decreases food intake and weight (Rothwell 1990; Spina et al. 1996). CRH expression in the hypothalamus is increased by leptin administration and decreased by glucocorticoids (Schwartz et al. 1996; Seeley et al. 1997). In conclusion, anorexigenic peptides such as alpha-MSH, CART and CRH act in the hypothalamus to inhibit food intake.

The brainstem receives information about the nutritional state of the periphery and sends this information to areas of the hypothalamus, predominantly the ARC (Ricardo and Koh 1978; Ter Horst et al. 1989). One of the key regions in the brainstem involved in the regulation of food intake is the nucleus tractus solitarius (NTS) (Broberger 2005). The NTS is close to the area postrema, which has an incomplete blood brain barrier, and so is able to directly receive peripheral signals about metabolic state of the individual but also receives vagal afferents from the GI tract and afferents from the glossopharyngeal nerves (Kalia and Sullivan 1982; Sawchenko 1983). The neurons of the NTS can be modulated by circulating factors such as leptin, which they have receptors for (Luckman and Lawrence 2003). Neurons of the NTS and ventromedulla of the brainstem respond to changes in blood glucose (Broberger 2005). The NTS also receives information from peripheral organs, such as the GI tract and liver, via the vagus nerve (Woods and Seeley 2002). For example, mechanoreceptors and chemoreceptors that are activated when food enters that GI tract transmit signals to the NTS via the vagus

nerve to limit meal size. The NTS contains NPY, melanocortin and glucagon-like-peptide-1 (GLP-1) neuronal circuits (Stanley et al. 2005). GLP-1 is synthesized by NTS neurons that also express leptin receptors (Stanley et al. 2005). These GLP-1 neurons project to the PVN and the dorsal motor nucleus of the vagus, with fewer projections to the ARC (Luckman and Lawrence 2003). GLP-1 receptors are located in areas of the hypothalamus such as the PVN and DMH, and in brain stem areas including the subfornical organ, area postrema, and organum vasculosim laminae terminalis (Stanley et al. 2005). Microinjection of GLP-1 into the third or fourth ventricle inhibits feeding in fasting rats and injection of a GLP-1 antagonist has been shown to block the inhibitory effect on food intake (Turton et al. 1996). The NTS also synthesizes prolactin-releasing peptide (PrRP) (Roland et al. 1999; Taylor and Samson 2001). PrRP expressing neurons transmit information to the hypothalamus and are distinct from GLP-1 expressing neurons (Luckman and Lawrence 2003). Central injections of PrRP decrease food intake (Luckman and Lawrence 2003).

The periphery sends information to the brain about both short-term (i.e. the amount of food consumed in a meal) and long-term energy status of the body (i.e., the availability of energy stores) by distinct mechanisms (Schwartz et al. 2000). Nutrients and gastrointestinal hormones that act as satiety factors and limit meal size regulate short-term food intake. Short-term food intake is regulated in part by nutrients (glucose, amino acids and fat), and metabolites (lactate, pyruvate, and ketones) that are released by digestion of a meal and act as satiety signals (Havel 2001). Nutrients activate chemo-receptors in the GI tract and mechano-receptors are activated in response to an increase in the volume of the GI tract (Konturek et al. 2004) Both mechano- and chemo-receptors

transmit signals to the NTS via the vagus nerve to limit meal size (Konturek et al. 2004). The GI tract also produces a number of peptides and hormones that regulate food intake including: PYY, ghrelin, GLP-1, oxyntomodulin, cholecystokinin (CCK), gastrin-releasing pepetide (GRP)/bombesin, and gastric inhibitory peptide (GIP) (Woods and Seeley 2002). All of these, with the exception of ghrelin, inhibit food intake (Bray 1995). Other peptides from the periphery are important in regulating food intake including glucagon and pancreatic polypeptide that are secreted by the pancreas in response to food consumption (Broberger 2005) Also, administration of peripheral peptides such as enterostatin, somatostatin, calcitonin gene related peptide and gastric inhibitory peptide have been shown to inhibit food intake (Havel et al. 2000), thus it is likely that these peptides also play a role in regulating food intake.

Long-term regulators of food intake provide information about energy stores and the amount of energy consumed over the long term (Havel 2001). An example of a long term regulator of food intake is leptin, which is primarily produced by adipocytes (Havel et al. 2000; Schwartz et al. 2000). Circulating leptin is proportional to fat mass and is influenced by food intake as calorie reduction decreases leptin concentration and increased food intake increases leptin levels (Havel et al. 2000; Schwartz et al. 2000). Mice without the gene encoding leptin get extremely fat, providing more evidence for leptin involvement in food intake regulation (Zhang et al. 1994). Also, humans with genetic mutations that cause leptin deficiency are characterized as having marked hyperphagia and being extremely obese (Montague et al. 1997; Strobel et al. 1998). Leptin receptors are located in brain regions involved in food intake regulation and central administration inhibits food intake, providing further evidence that leptin plays a

role in regulating food intake (Schwartz et al. 2000; Broberger 2005). For example, leptin receptors are located on NPY and POMC neurons and act to inhibit transcription of NPY and increase POMC RNA (Schwartz et al. 1991; Ahima et al. 1996; Schwartz et al. 1997; Thornton et al. 1997).

In addition to the brainstem and hypothalamus, the cortex is also involved in food intake regulation. The hypothalamus sends a large number of projections to the cortex and the cortex also sends input to the hypothalamus (Saper 1985; Risold et al. 1997). The insular cortex plays a role in an individual's determination of which food is being eaten by the taste, appearance, texture and smell of the food (Cechetto and Saper 1987). The orbitofrontal cortex, which receives direct input from the insular cortex, acts as a higher order taste cortex and determines how pleasant a particular food is (Broberger 2005). Studies in nonhuman primates that use fMRI imaging and electrophysiological neuronal recordings indicate that activation of orbitofrontal neurons decrease when a particular food is eaten to satiety (Kringelbach et al. 2003). This area is important in the pleasure of eating and sends information directly to the LH (Broberger 2005). Imaging studies find that hunger activates brain regions associated with the regulation of emotions, such as the limbic and paralimbic cortex, and satiety causes activation of the prefrontal cortex, which is hypothesized to play a role in stopping inappropriate behaviors (Tataranni et al. 1999). The insular cortex responds to the pleasure of food consumption as shown by the increase in signaling from the insular cortex when a sweet drink is consumed after a fast (Delparigi et al. 2005). Interestingly, changes in firing rate in the insular cortex in this paradigm have been show to be of greater magnitude in obese subjects than in normal weight control subjects (Delparigi et al. 2005), and a recent study revealed that post-

obese subjects also had a greater magnitude of change in brain areas in response to a palatable sweet drink than never obese controls (DelParigi et al. 2004). These findings suggest that there may be differences in the amount of pleasure perceived by obese and lean individual following food consumption and would be interesting to follow up with further study.

It is important to note that that the majority of studies examing the pathways that control body weight have been performed using rodent species. Studies have shown that many of the neuronal systems that modulate food intake are similar in rodents and primates including αMSH, AGRP (Koegler et al. 2001), modulators of the melanocortin system, leptin (Tang-Christensen et al. 1999), NPY (Larsen et al. 1999) and CCK (Moran and McHugh 1982). However, several studies provide evidence that differences exist in the neuronal circuitry that controls body weight between rodents and primates. For example in rodents physiologic doses of PYY have been shown to potently inhibit food intake however, in primates only supraphysiologic levels of PYY are able to cause a sustained decrease in food intake (Koegler et al. 2005). Monkeys show greater sensitivity to NPY-induced feeding than rodents (Koegler et al. 2001) and less sensitivity to AGRP-induced feeding (Larsen et al. 1999). The neuronal circuitry conrolling food intake appears to be more complex in primates than in rodents. For example primates have NPY mRNA in the ARC, SON and PVH where as in rodents NPY is only expressed in the ARC (Grove et al. 2003). Thus, it is important to confirm findings from rodent studies in nonhuman primates in order to understand the pathways that control food intake in primates.

In order to elucidate the many neuronal circuits and peptides involved in feeding a number of strategies have been used. A number of neuronal populations have been identified as being involved in food intake regulation as they change their activity in response to a change in metabolic state, for example NPY and POMC-expressing neurons in the ARC. These changes have been detected using electrophyiological recording from the neurons that show changes in the firing pattern in response to changes in metabolic state. Also, immediate early genes, such as c-Fos, have been used to look for changes in gene expression in response to change in the metabolic state. Secondly, studies have administered peptides that may be involved in food intake regulation either peripherally or centrally via microinjection to determine if the peptides change food intake. It is important to note that a peptide could decrease food intake by acting as a satiety signal or could decrease food intake by making the animal nauseous. Rats don't vomit, so in order to determine if a peptide is making a rat sick it is general practice to pair the compound with a distinctive flavor and look for the development of a taste aversion from that flavor. Monkeys, are more like humans and display nausea by drooling, yawning and eventually vomiting (Schreihofer et al. 1993). Lastly, once a neuron is identified that appears to play a role in food intake regulation, anteriorgrade tracing can be used to determine where the neuron projects to and posteriorgrade tracing can be used to determine which neurons project to it. In summary, there are a number of strategies that are widely used to identify neurons and peptides that play a role in food intake regulation, and using these strategies a great deal has been learned about brain regions and specific neural systems that play important roles in regulating food intake.

1.6 Physiological Systems Regulating Energy Expenditure

The regulation of energy expenditure is less well understood, but appears to also be regulated by a number of systems. These systems include thyroid hormones (de Lange et al. 2001), the sympathetic nervous system and noradrenaline (Ricquier et al. 2000). Also, recent studies have found that a number of peptides that regulate food intake also regulate energy expenditure, such as leptin (Ricquier et al. 2000), melanocortins (Pierroz et al. 2002), NPY (Billington et al. 1991) and CART (Havel 2001). For example, leptin has been shown to increase energy expenditure by increasing the activity of the sympathetic nervous system (Seals and Bell 2004). In contrast, NPY injection into the PVN reduces the activity of the sympathetic nervous system and decreases energy expenditure (Billington et al. 1991). The brain areas involved in metabolic rate regulation include the PVN and VMH, which communicate with the sympathetic nervous system (Szekely and Szelenyi 2005).

The mechanisms that regulate physical activity are also not well understood. However, studies have shown that a number of the peptides and neurotransmitters that regulate food intake, such as leptin (Pelleymounter et al. 1995; Nagy et al. 1997; Salbe et al. 1997; Rowland 1998), ghrelin (Castaneda et al. 2005; Matsuda et al. 2005), pancreatic polypeptide (Lassmann et al. 1980; Uhe et al. 1992; Nakajima et al. 1994), and cholecystokinin (Sei et al. 1999), and the neurotransmitters serotonin (Borer et al. 1988; Heisler et al. 1999; Nguyen et al. 1999; Nonogaki et al. 2003), glutamate (Donzanti and Uretsky 1983; Swanson and Kalivas 2000), dopamine (Fallon and Moore 1978; Zhou and Palmiter 1995; Swanson et al. 1997; Rowland 1998), norepinephrine (Rowland 1998), nitric oxide synthase (Dzoljic et al. 1997; Khedara et al. 1999) and *B*-endorphin (Hill et

al. 2002), and the hormone estrogen (Wade 1972; Thorburn and Proietto 2000), all play

potential roles in the regulation of physical activity. Additionally, several brain regions

involved in arousal have been implicated in the regulation of activity, predominantly the

reticular activating formation (Rowland 1998). Lesion studies have provided evidence

that areas of the basal forebrain, VMH, hypothalamus, PVN, amygdala, and thalamus also

are important in activity regulation (Kennedy and Mitra 1963; Rowland 1998).

Chapter 2

OVARIECTOMY LEADS TO RAPID CHANGES IN FOOD INTAKE,

BODY WEIGHT AND METABOLIC REGULATION

IN FEMALE RHESUS MONKEYS *(MACACA MULATTA)*

2.1 INTRODUCTION

In women, an increase in body weight, BMI, and percent body fat generally

occurs over the fourth and fifth decades of life, as women undergo the menopausal

transition (Williamson et al. 1990; Haffner et al. 1991; Ley et al. 1992; Kotani et al.

1994; Aloia et al. 1995; Lovejoy 1998; Tchernof and Poehlman 1998; Chang et al. 2000;

Blumel et al. 2001). It is unclear, however, whether weight gain results from the decline

in circulating levels of ovarian steroid hormones, or reflects age-related changes in

metabolism, or results from lifestyle changes such as a decrease in physical activity or an

increase in consumption of high calorie foods. Reports of hormone replacement therapy

decreasing the body weight of postmenopausal women (Espeland et al. 1997;

Gambacciani et al. 1997; Khoo et al. 1998; Chmouliovsky et al. 1999; Sirola et al. 2003)

support the hypothesis that menopausal weight gain is linked to changes in ovarian

steroid hormone levels. However, several studies tracking body weight in

postmenopausal women with and without hormone replacement therapy have shown no

difference in weight gain between the two groups (Jensen et al. 1986; Reubinoff et al.

1995; Blumel et al. 2001).

Studies using animal models (mice, rats, cats, monkeys) provide stronger evidence that decreases in circulating levels of ovarian steroid hormones cause an increase in body weight and percent body fat. Numerous studies have shown that rats increase food intake and body weight relative to intact controls after ovariectomy, and that treatment of ovariectomized rats with estradiol lowers both food intake and body weight to levels found in ovary-intact controls (Grunt 1964; Hervey and Hervey 1965; Kakolewski et al. 1968; Wade and Zucker 1970; Mook et al. 1972; Leshner and Collier 1973; Tarttelin and Gorski 1973; Landau and Zucker 1976; McElroy and Wade 1987; Shinoda et al. 2002). The post-ovariectomy increase in body weight in the rat occurs rapidly, within the first several weeks after surgery (Chu et al. 1999; Ainslie et al. 2001). Ovariectomy also increases food intake in the first several weeks post-ovariectomy in the rat (Chen and Heiman 2001). Weight gain is associated with ovariectomy in cats [i.e., spaying; (Harper et al. 2001; Martin et al. 2001)], although most studies compare weight before and one year after spaying. However, Fettman et al. (1997) reported a significant gain in body weight at one month post-spaying compared to nonspayed controls. Ovariectomized monkeys have higher food intake than intact controls (Kemnitz et al. 1989) and treatment of ovariectomized monkeys with estradiol causes a significant decrease in food intake (Czaja and Goy 1975; Bielert and Busse 1983; Kemnitz et al. 1986; Kemnitz et al. 1989). However, body weight and food intake have not been tracked longitudinally in monkeys post-ovariectomy, so it is unclear whether these changes in food intake are accompanied by changes in body weight, and how rapidly changes occur in monkeys post-ovariectomy, is unknown.

To examine in further detail whether the robust relationship between decreased ovarian hormone status and rapid increases in body weight and food intake that has been documented in small animals has cross-species applicability, particularly to primates, the present study was conducted examining the effects of ovariectomy on food intake, body weight, percent body fat, and blood levels of gonadal hormones and several metabolic hormones before and after ovariectomy in a group of 16 adult female rhesus monkeys.

2.2 METHODS

Animals

Sixteen adult female rhesus monkeys (*Macaca mulatta*), 7-11 years of age, were used in this study to track the effects of ovariectomy on food intake, body weight and metabolism. Animals were used as their own controls, thus body weight and food intake measurements were made both pre- and post-ovariectomy. In addition, because the effects of ovariectomy on body weight have not been well studied in primate species, we added a control group that had ovarian surgery, but were not ovariectomized, to control for the effect of ovarian surgery on weight gain. This control group consisted of 28 adult female rhesus monkeys, 5-12 years of age that underwent a laparoscopic surgery in which ovaries were manipulated but not removed (aspiration of one or more follicles to collect oocytes).

All monkeys were housed in individual stainless steel cages in a temperature-controlled room (24±2 C), with lights on for 12 h a day (0700-1900 h). Monkeys were provided with two meals a day at 0930 h and 1300 h. At each meal, they received 12 high protein monkey chow biscuits (no. 5047, jumbo biscuits, Ralston Purina Co., St.

Louis, MO; approximately 16.5 g each, 3.11 metabolizable Cal/g, 616 Cal/meal). In addition, half of an apple was provided at 1900 hr. Water was available *ad libitum*. Monkeys had been adapted to these conditions for at least 18 months prior to the initiation of this study. All studies were reviewed and approved by the ONPRC Animal Care and Use Committee.

Surgery

Experimental animals were ovariectomized bilaterally using sterile laparoscopic techniques. Control monkeys also underwent a laparoscopic surgery however, instead of ovariectomy, control monkeys underwent ovarian follicular aspiration to retrieve oocytes, follicular fluid and granulosa cells from the ovaries. In preparation for this surgery, control animals received a regimen of Antide (Ares-Serono Inc., Randolph, MA), followed by recombinant human FSH to stimulate follicular development (Ouhibi et al. 2001).

Measurement of body weight, caloric intake and body fat

Measurements of body weight, calorie intake, body fat and several plasma hormone levels were made throughout the study in experimental monkeys. Body weight was tracked in control monkeys before and after ovarian surgery.

Body weight and length assessments: Experimental monkeys were weighed on the day of ovariectomy, and at weekly intervals for six weeks after ovariectomy. Control monkeys were weighed before and at 1, 2, 4 and 8 weeks after ovarian surgery. Weight measurements were made before consumption of the morning meal, at approximately 0800 h. In experimental monkeys 'crown rump length' was measured while animals were sedated with Ketamine Hydrochloride (10 mg/kg, IM.), and were laying flat on their

backs with their head against a fixed head rest. Body Mass Index (BMI) was calculated as body weight (kg) divided by the square of crown rump length (m^2).

Caloric intake assessments: Total food consumption at each meal was recorded throughout the study for experimental monkeys, by counting the amount of food remaining prior to the next meal presentation.

Hormone measurements: Blood samples (10 ml/sample) were obtained from each experimental monkey after an overnight fast once before ovariectomy and again 8 weeks after ovariectomy. On the evening prior to blood sample collection all food was removed from each animal's cage at 1700 h. For blood sample collection, monkeys were sedated with Ketamine Hydrochloride (10 mg/kg, IM) and blood was collected by femoral venipuncture into sterile heparinized syringes, transferred into glass tubes, and centrifuged at 1125 g at 4 C for 10 min. Plasma was removed and stored in plastic vials (with 40 µl of a 50:50 mixture of 1000U sodium heparin and 38% sodium citrate heparin per 1 ml of plasma, to prevent clotting of plasma proteins), at –20 C until assays were performed.

DEXA-measurements: Body fat was determined using dual energy X-ray absorptiometry (DEXA) scanning at 7 weeks post-ovariectomy in the experimental monkeys. DEXA was utilized as a way to measure body composition as the technology was readily available at the ONPRC. DEXA has been shown to have good precisision (Kohrt 1995; Lohman 1996) and has been validated against a number of other methods of determining body composition (Van Loan and Mayclin 1992; Prior et al. 1997; Kohrt 1998; Bolanowski and Nilsson 2001). The perecent variation between scans was 3%. Animals were sedated with Telazol (3 mg/kg; IM., Fort Dodge Animal Health, Fort

Dodge, Iowa), supplemented with Ketamine Hydrochloride (10 mg/kg, IM) as needed, and were positioned supine on the bed of a Lunar DPX scanner (Lunar Corporation, Madison, WI, software version 3.4). Total body scans were done in the Pediatric Medium scan mode with a voltage of 76 kv. Lunar software version 3.4 calculated the total percent body fat of each scan. Two to three scans were performed for each monkey and the average percent body fat was calculated.

Assays

Plasma leptin, insulin, T_3, estrogen, progesterone and testosterone concentrations were measured by the Endocrine Services Core Facility at the ONPRC. Leptin was measured using a commercially available double antibody RIA kit for Primate Leptin (PLR-1101) from Linco Research, Inc. (St. Charles, MO)(Downs and Urbanski 2006). The limit of sensitivity of this assay was 0.5 ng/ml. All samples were measured in the same assay, and intra-assay variability was 2.8%. Plasma insulin, T_3, progesterone, estrogen and testosterone were measured using a Roche Elecsys 2010 clinical instrument and assay reagents for human insulin and T_3 from Roche Diagnostics (Indianapolis, IN) as previously described (Xu and Stouffer 2005; Downs and Urbanski 2006). Inter-assay variabilities were 4.1%, 7.2%, 3.6%, 7.5% and 4.6% for insulin, T_3, progesterone, estrogen and testosterone, respectively. The limits of assay sensitivity were 2μU/ml, 0.19 ng/ml, 0.05 ng/ml, 5 pg/ml and 0.05 ng/ml for insulin, T_3, progesterone, estrogen and testosterone, respectively. Glucose, total cholesterol, HDL cholesterol, triglyceride, nonesterified free fatty acids (NEFA), glycerol, and ß-hydroxybuturate (ß-HBA) were assayed by the Metabolic Disease Core Assay Facility of GlaxoSmithKline, Research Triangle Park, North Carolina using an automated chemistry analyzer (Technicon Axon,

Tarrytown, NY). The with-in run variability is 0.7%, 0.91%, 0.85%, 1.4% for glucose, total cholesterol, HDL cholesterol, and triglyceride, respectively. The limits of assay sensitivity are 0.6 mmol/l, 0.5 mmol/l, 0.05 mmol/l. 0.10 mmol/l for glucose, total cholesterol, HDL cholesterol, triglyceride, respectively.

Statistical Analyses

To determine if ovariectomy had an effect on body weight, food intake and metabolic substrate and hormone levels statistical analyses was performed comparing measurements before and after ovariectomy. Changes in weight and calorie intake are expressed as percent of pre-ovariectomy levels. The assumptions of normality and homoscedacity were tested for all analyses. The body weight measurements of the control monkeys were not normally distributed and could not be normalized by transformation, thus a Friedman Rank Sum Test was used to look for significant differences between multiple measurements and the Wilcoxon signed ranks test was used to look for differences between pairs of measurements. Additionally, changes in insulin and leptin concentrations were also made using the nonparametric Wilcoxon Signed Rank Test. All other measurements were normally distributed or were normally distributed after a square root or log transformation. Measurements made multiple times throughout the study were analyzed using a one-way repeated measures analysis of variance. The assumption of sphericity (that the variance of the difference scores in a within-subjects design are equal across all the groups) was examined with Mauchly's Test. The Greenhouse-Geiser correction factor was used in cases where the assumption of sphericity was violated. If a significant difference was found, planned contrasts were

utilized to compare measurements to baseline, using the least significant difference test. Ovarian hormones and metabolic parameters measured before and after ovariectomy were analyzed using a paired Student's t-test. Correlations between parameters were determined using a Pearson product moment correlation. For all tests, alpha values were considered significant with $p \leq 0.05$. Data are presented as mean ± SEM. All statistical analyses were conducted using the SPSS software package (SPSS Inc., Chicago, Illinois).

2.3 RESULTS

Ovariectomy led to a significant decrease in circulating levels of estradiol ($t=9.28$, $df=15$, $p<0.001$), with no significant changes in mean plasma progesterone or testosterone concentrations (Table 2.1).

Upon initiation of the study, individual differences in daily calorie intake (153 to 1,128 calories/day) and body weight (4.6 to 9.1 kg) were substantial. However, there was no correlation between caloric intake and body weight ($r=-0.31$, $p=0.25$; Figure 2.1).

Body weight significantly changed over the experimental period ($F_{2.9, 44.7}=14.48$, $p<0.0001$). By six weeks post-ovariectomy, there was a significant elevation in body weight (4.81±0.31%; $p=0.005$), compared to pre-ovariectomy levels (Table 2.2, Figure 2.2A). Similarly, there was a small but significant increase in body mass index ($t=-2.36$, $df=15$, $p=0.03$), from 25.35±1.32 kg/m^2 to 25.91±1.17 kg/m^2. In contrast, there was no significant change in body weight in the control group that underwent laparoscopic surgery without ovariectomy ($X^2=4.32$, $df=3$, $p=0.23$; Figure 2.2B).

Table 2.1 Ovarian hormones before and after ovariectomy.

Variable	Before OVX	After OVX	P value
Estradiol (pg/ml)	50.94±4.56	11.63±0.91	<0.001
Progesterone (ng/ml)	0.61±0.26	0.15± 0.02	0.10
Testosterone (ng/ml)	0.04±0.01	0.05± 0.01	0.44
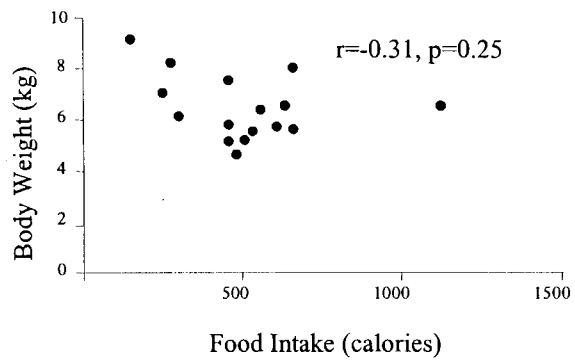
Figure 2.1 Correlation between initial food intake and initial body weight before ovariectomy.

The percent change in body weight was negatively correlated with initial body weight (r=-0.68, p=0.004), with the lightest monkeys gaining the most weight. Also, the percent change in body weight was negatively correlated with initial BMI (r=-0.70, p=0.003), such that the leanest monkeys gained the most weight.

Food intake changed significantly over the experimental period ($F_{3.6,50.0}$=3.1, p=0.03). Food intake was stable during the three weeks prior to ovariectomy and then increased significantly by two weeks post-ovariectomy and remained elevated through six weeks post-ovariectomy (16.1±6.5, p=0.02, Table 2.2, Figure 2.3). Although both food intake and body weight increased with ovariectomy, there was no correlation between the increase in food intake and the increase in body weight (r=-0.20, p=0.46; Figure 2.4).

Ovariectomy caused an increase in circulating leptin concentration (p=0.04). Plasma leptin concentration correlated with body mass index both before (r=0.77; p=0.001) and after (r=0.72; p=0.002) ovariectomy and with percent body fat, seven weeks post-ovariectomy (r=0.79; p<0.0001; Figure 2.5). However, there was no correlation between the change in plasma leptin concentration and change in body mass index post-ovariectomy (r=0.19, p=0.47). There was no change in the circulating level of insulin, T_3, glucose, glycerol or NEFA post-ovariectomy. Circulating triglyceride concentrations decreased (t=4.29, df=15, p=0.001), whereas plasma levels of ß-hyrdroxybutyrate increased (t=-3.109, df=15, p=0.007) after ovariectomy. Changes in both were correlated with the change in body weight (triglycerides: r=-0.52, p=0.04; ß-hydroxybutyrate: r=-0.54, p=0.03).

Table 2.2 Metabolic changes with ovariectomy.

Variable	Before OVX	After OVX	P value
Weight (kg)	6.42±0.31	6.60±0.28	<0.001
Food Intake (calories/day)	511.3±33.4	593.4±38.7	0.02
Leptin (ng/ml)	2.06±0.68	2.62±0.95	0.04
T3 (ng/ml)	1.55±0.09	1.62±0.10	0.35
Insulin (uU/ml)	46.76±13.08	32.69±5.28	0.44
Glucose (mg/dL)	84.81±4.72	87.89±4.07	0.52
Cholesterol (mg/dL)	165.12±5.78	171.00±6.72	0.29
LDL-Cholesterol (mg/dL)	91.56±4.57	88.75±5.72	0.45
HDL-Cholesterol (mg/dL)	73.56±5.26	82.25±4.53	0.01
Triglycerides (mg/dL)	72.37±9.97	46.26±4.36	0.01
NEFA (mEq/L)	1.03±0.11	1.13±0.09	0.14
Glycerol (mg/dL)	17.94±1.63	18.19±2.15	0.88
ß-HBA (mg/dL)	0.96±0.19	1.80±0.39	0.01
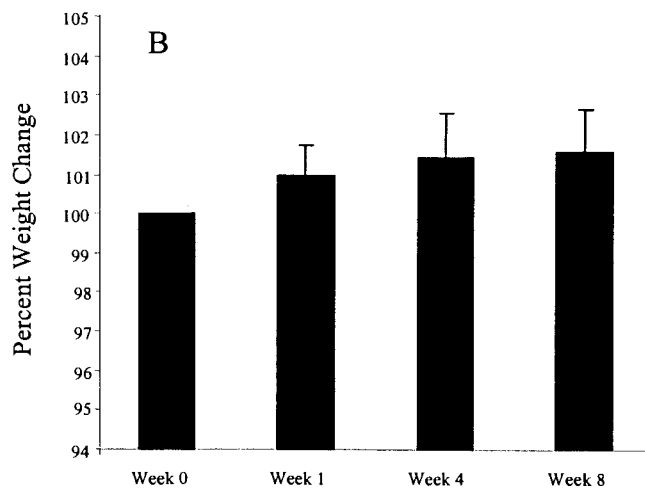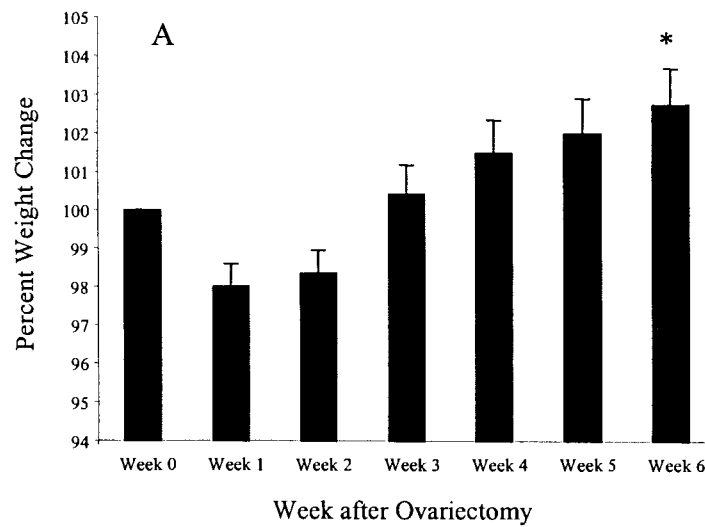
Figure 2.2 (A) Percent change in body weight in the experimental group from pre-ovariectomy (week 0) to six weeks after ovariectomy ($F_{2.9,44.7}=14.48$, $p<0.0001$). (B) Perent change in body weight in the control group from pre-ovarian surgery (week 0) to eight weeks post-surgery ($X^2=4.32$, $df=3$, $p=0.23$). Asterisk indicates a significant difference from week 0, $p<0.05$.
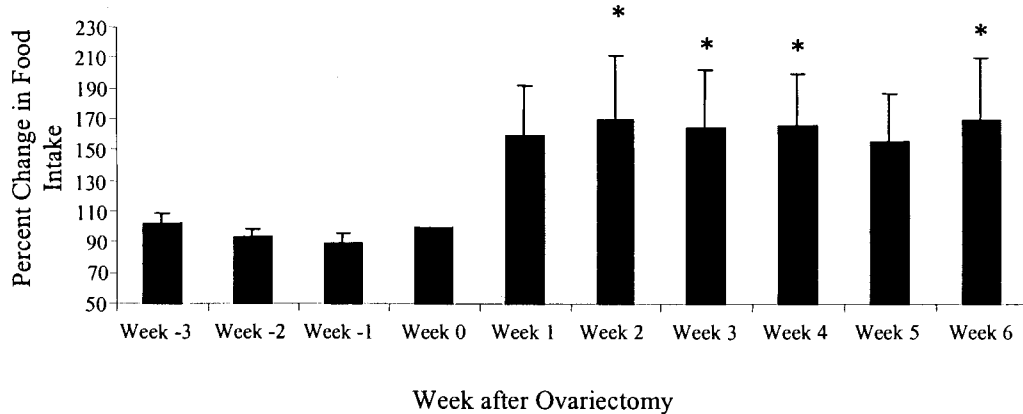
Figure 2.3 Percent change in food intake from three weeks pre-ovariectomy to six weeks after ovariectomy ($F_{3.6,50.0}=3.1$, p=0.03). Asterisks indicate a significant difference from week 0, p<0.05.
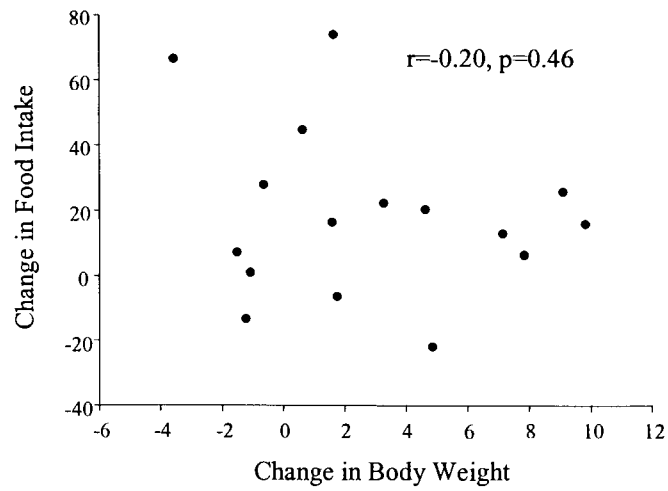
Figure 2.4 Correlation between percent change in body weight and food intake during

the 6 weeks post-ovariectomy (r=-0.20, p=0.46).
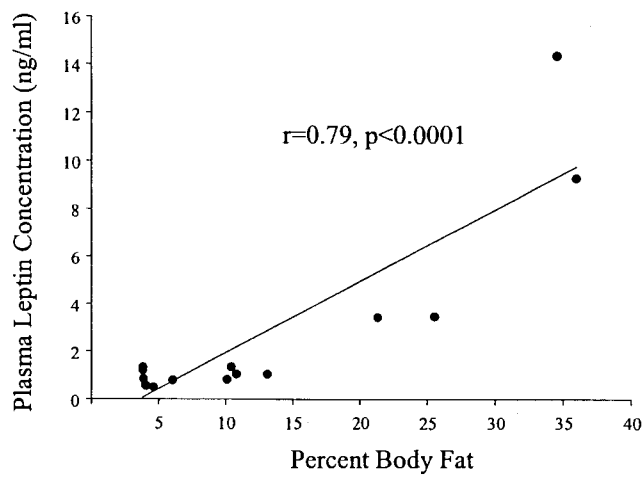
Figure 2.5 Correlation between plasma leptin concentration and percent body fat after ovariectomy (r=0.79, p<0.0001).

HDL-cholesterol was also significantly greater (t=-3.22, df=15, p=0.006) in the post-ovariectomy sample, but there was no change in total cholesterol or LDL-cholesterol.

2.4 DISCUSSION

The results of this study provide strong evidence that ovariectomy is associated with a rapid increase in caloric intake and a small, but significant, gain in body weight (about 5%) within the first two months post-ovariectomy that was not observed in the control group of monkeys that had ovarian surgery but no ovariectomy. The finding that ovariectomy rapidly increases weight in rhesus monkeys extends numerous previous experiments in small animals (mice, rats, cats; (Fettman et al. 1997; Chu et al. 1999; Ainslie et al. 2001; Geary et al. 2001), that have consistently shown that ovariectomy leads to a 14–21% increase in weight within several weeks of ovariectomy. Weight gain, an increase in body mass index (BMI) and increased adiposity during the menopausal transition in women is also well documented (Williamson et al. 1990; Haffner et al. 1991; Ley et al. 1992; Kotani et al. 1994; Lovejoy 1998; Tchernof and Poehlman 1998; Chang et al. 2000). However, whether menopausal weight gain results from decreased circulating ovarian hormones, age-related slowing of metabolic rate, or changes in lifestyle (such as a decreased exercise or increased consumption of high calorie foods) has been debated. The data we present here strongly supports the notion that weight gain at menopause, at least in part, results from a decrease in circulating levels of ovarian hormones.

The rise in body weight in the first two months post-ovariectomy was accompanied by a similarly rapid increase in food intake, confirming and extending

previous reports of an increase in food intake in longer-term ovariectomized monkeys (Czaja and Goy 1975; Bielert and Busse 1983; Kemnitz et al. 1986; Kemnitz et al. 1989). These results provide further evidence in a primate species that the decrease in ovarian hormones occurring over the menopause may play an important role in contributing to the weight gain, increased BMI and adiposity that have been well documented in women over the menopausal transition (Williamson et al. 1990; Haffner et al. 1991; Ley et al. 1992; Kotani et al. 1994; Aloia et al. 1995; Lovejoy 1998; Tchernof and Poehlman 1998; Chang et al. 2000). Moreover, these results suggest that changes in food intake occur rapidly in response to decreased ovarian function, suggesting that ovarian hormones acutely regulate physiological systems that control food intake.

Interestingly, body weight and BMI before ovariectomy correlated with weight gain post-ovariectomy, with the lightest leanest monkeys gaining the most weight after ovariectomy. This finding is supported by epidemiologic studies that have shown leaner adults gain more weight over adulthood than fatter adults (Sonne-Holm et al. 1990; Meltzer and Everhart 1995; Lahmann et al. 2000; Ball et al. 2003). Also, individuals with higher plasma leptin concentrations (a hormone produced by fat) gain less weight than individuals with lower levels of circulating leptin (Lindroos et al. 1998; Ravussin and Gautier 1999). Together, these findings suggest that lean women are more prone to postmenopausal weight gain than obese women. Further studies will be necessary to begin to understand the mechanism(s) underlying this difference in sensitivity to weight gain.

Although this study focused on early changes in body weight and food intake post-ovariectomy, some metabolic parameters were measured, and changes during the

first 6 weeks post-ovariectomy were detected, including an increase in fasting concentrations of leptin and ß-hydroxybutyrate. Considering the increase in body weight and body mass index that occurred in the first two months post-ovariectomy, the increase in plasma leptin levels was not surprising. In many species, including humans, even small changes in body weight, and more specifically body fat, are accompanied by changes in plasma leptin levels (Hassink et al. 1996; Havel et al. 1996; Havel et al. 1996; Kolaczynski et al. 1996; Ellis and Nicolson 1997). In this experiment, ovariectomy was accompanied by a 27% increase in leptin levels and a 5% increase in body weight, which is within the range of what has been reported for increases in plasma leptin levels in rodents with simple diet-induced weight gain (Ahren 1999). However, we found no correlation between change in body mass index and change in plasma leptin concentrations in this experiment. This may be due to the short period of study post-ovariectomy (i.e., 6 weeks), or because changes in body mass index are only an estimate for changes in body fat.

Fasting levels of plasma ß-hydroxybutyrate, a ketone body produced by liver metabolism of free fatty acids in states of negative energy balance (Palou et al. 1981; Gallen et al. 1990; Stannard et al. 2002), was also increased post-ovariectomy. The increase in this metabolite may suggest that post-ovariectomy monkeys metabolize energy stores at a faster rate than pre-ovariectomy. Faster energy metabolism may be the signal driving the increase in food intake. Alternatively, an increase in metabolic rate may be the consequence of increased food intake, representing a homeostatic mechanism to return the body to pre-ovariectomy weight. These possibilities deserve further investigation, with more detailed assessment of changes in metabolic rate with

ovariectomy and how they relate to changes in food intake (i.e., whether a change in metabolic rate occurs post-ovariectomy and whether it precedes or follows changes in food intake).

Resting metabolic rate decreases with age (Klausen et al. 1997; Van Pelt et al. 1997). However, a number of studies suggest that there is a reduction of resting metabolic rate at menopause that is independent of age-related changes in metabolic rate (Van Pelt et al. 1997; Poehlman and Tchernof 1998; Lynch et al. 2002). There is limited work examining the effects of estrogen on fasting ß-hyrdroxybutyrate levels. Estrogen given to ovary intact rats (Morrow et al. 1981) and women (Morrow et al. 1981) produced an increase in ß-hydroxybutyrate levels during brief periods of fasting. However, these results may reflect the effects of pharmacological levels of estrogen, above levels found in ovary intact individuals.

Lastly, we found changes in triglyceride and HDL-cholesterol concentrations comparing pre- to post-ovariectomy fasting samples. Triglyceride levels decreased and HDL-cholesterol levels increased, consistent with a typical inverse relationship between these two variables (Patsch et al. 1983). Numerous studies have found that post-menopausal hormone replacement therapy (HRT) is accompanied by an increase in plasma triglyceride levels (Walsh et al. 1991; Folsom et al. 1996; Barrett-Connor et al. 1997; Ritsch et al. 2002), suggesting that the post-ovariectomy decrease in triglyceride levels found in the present study may result from a decrease in plasma estradiol levels. However, there have been reports of increased triglyceride levels in post-menopausal women not on HRT (Torng et al. 2000), compared to pre-menopausal women. And, in this study the decrease in triglyceride levels was correlated with the post-ovariectomy

change in body weight. Thus, further studies, examining whether HRT can reverse the post-ovariectomy decrease in plasma triglyceride levels, are needed to definitively establish whether the post-ovariectomy changes in triglycerides are caused by decreased ovarian steroid hormones, weight gain, or a combination of these changes. Post-menopausal hormone replacement therapy has also been shown to increase fasting levels of plasma HDL- cholesterol (Walsh et al. 1991; Knopp et al. 1994; 1995; Folsom et al. 1996; Hulley et al. 1998; Ritsch et al. 2002). This may reflect an estrogen-mediated enhancement of cholesteryl ester transfer from high density lipoproteins to apolipoprotein B-containing lipoproteins (Ritsch et al. 2002). However, short-term (5 days) estrogen treatment has been found to decrease plasma levels of HDL-cholesterol in mice (Srivastava et al. 2001). Other studies have shown that estrogen treatment of ovariectomized cynomologus monkeys (Wagner et al. 1992; Manning et al. 1996) and estrogen plus progesterone treatment of postmenopausal women (Haarbo et al. 1991) has no effect on plasma concentrations of triglycerides or HDL cholesterol. It is also possible that these metabolic changes we measured eight weeks post-ovariectomy may not be due to decreased ovarian hormones, but may be a consequence of surgical stress, as surgery and trauma have been reported to alter plasma levels of trigylcerides and HDL-cholesterol for up to two months post-surgery (Vaughn 1999).

In summary, this is the first study documenting early changes in both food intake and body weight consequent to changes in ovarian steroid hormone availability in a primate species. Old world primates offer excellent models for studying the relationship between ovarian function and the body's metabolic regulatory systems, as they are the only animal model that demonstrates monthly menstrual cycles, like women. The

findings reported here suggest that further studies examining the mechanisms underlying changes in food intake and weight gain consequent to changes in ovarian steroid hormones are warranted. Such relationships could play a role in modulating of the body's metabolic and weight regulatory systems across the lifespan, including puberty, pregnancy and post-pregnancy, across the menstrual cycle and with the menopausal transition.

Chapter 3

ESTROGEN REPLACEMENT THERAPY DECREASES BODY WEIGHT IN OVARIECTOMIZED FEMALE RHESUS MONKEYS (*MACACA MULATTA*) BY DECREASING FOOD INTAKE AND INCREASING ACTIVITY

3.1 INTRODUCTION

Women gain weight in adulthood over the menopausal transition (Williamson et al. 1990; Haffner et al. 1991; Ley et al. 1992; Kotani et al. 1994; Aloia et al. 1995; Lovejoy 1998; Chang et al. 2000; Blumel et al. 2001). However, it is unclear if weight gain is due to age-related changes in metabolism, lifestyle changes with aging (such as eating more or exercising less), or the decrease in ovarian hormones that occurs with menopause. The effects of hormone replacement therapy (HRT) on body weight inpostmenopausal women are controversial. Many women believe that HRT increases body weight and 18% of hormone replacement users report weight gain as the main reason for discontinuing HRT (Nachtigall 1990; Ryan et al. 1992). However, the idea that HRT increases body weight is supported by the findings of only a few studies (Aloia et al. 1995; Hartmann et al. 1996). In contrast, the majority of studies examining this issue in postmenopausal women have reported that HRT users have lower body weight than nonusers (Sites et al. 2001; Sirola et al. 2003) and that HRT decreases (1995; Espeland et al. 1997; Gambacciani et al. 1997; Lahmann et al. 2000; Gambacciani et al. 2001; Gambacciani et al. 2001), or has no effect (Jensen et al. 1986; Reubinoff et al. 1995; Kritz-Silverstein and Barrett-Connor 1996; Khoo et al. 1998; Norman et al. 2000; Salbach et al. 2000; Blumel et al. 2001; Sites et al. 2001; Sorensen et al. 2001; Sumino et

al. 2003) on postmenopausal weight gain. A few studies have even shown that HRT causes weight loss (Ongphiphadhanakul et al. 1998; Chmouliovsky et al. 1999). The controversy around the effects of HRT on body weight in postmenopausal women may be related, in part, to differing effects of the various HRT regimens that are in common use. Differences in the timing of initiation of HRT, the variety of synthetic hormones used in HRT, as well as differing doses and routes of administration (i.e. oral vs. transdermal) also complicate comparisons between studies. For example, O'Sullivan et al. (1998) found differences in the body composition changes in postmenopausal women taking oral versus transdermal estrogen replacement therapy. Additionally, other variables that influence body weight, such as caloric intake, dietary composition, and level of physical activity have often not been controlled, or even measured, in the studies of HRT on body weight. Furthermore, because weight gain tends to occur in both women and men progressively over adulthood, it is possible that the cases in which weight gain has been noted in women during HRT is not caused by HRT but rather by a continuation of adult weight gain associated with a progressive decline in metabolic rate, eating more calories than are utilized, or a sedentary lifestyle.

In animals, the observed effects of estrogen on body weight and food intake are more consistent. Ovariectomy of rodents (Hervey and Hervey 1965; Landau and Zucker 1976; McElroy and Wade 1987; Chu et al. 1999; Ainslie et al. 2001; Chen and Heiman 2001; Shinoda et al. 2002) and domestic animals (Fettman et al. 1997; Harper et al. 2001; Martin et al. 2001) causes a rapid increase in food intake and body weight. Moreover, numerous studies show that treatment of ovariectomized rodents with estradiol decreases both food intake and body weight to levels of ovary-intact controls (Nyda et al. 1948;

Hervey and Hervey 1965; Wade and Zucker 1970; Shinoda et al. 2002). We have shown that ovariectomy rapidly increases body weight in non-human primates (Sullivan et al. 2005; Chapter 2). Also, previous studies have shown that treatment of ovariectomized monkeys with estradiol causes a significant decrease in food intake (Czaja and Goy 1975; Bielert and Busse 1983; Kemnitz et al. 1986; Kemnitz et al. 1989). Thus, data from studies in animals, including nonhuman primates, strongly suggest that estrogen modulates both body weight and food intake. To more thoroughly study the effects of estrogen replacement therapy on the mechanisms regulating body weight in primates, 18 ovariectomized female rhesus monkeys received an orally available synthetic estrogen for 3-months. Parameters measured included body weight, food intake, activity, total energy expenditure, basal metabolic rate and the thermic effect of food. The results show that estrogen replacement therapy significantly decreased body weight and food intake and increased physical activity.

3.2 MATERIALS AND METHODS

Animals

Eighteen adult female rhesus monkeys (*Macaca mulatta*), 7-11 years of age, living in individual stainless steel cages in a temperature-controlled room (24 ± 2°C), with lights on between 0700 and 1900 h, were studied. Approximately one year prior to the initiation of the study, these monkeys were ovariectomized and placed on a high fat diet (35% of total calories from fat) to approximate the conditions experienced by many postmenopausal women in the Western world (Williams et al. 2003). The high fat diet was formulated at our facility following a modification of the recipe developed by Clarkson and colleagues which was designed to study diet-induced atherosclerosis in

monkeys (Shadoan et al. 2003; Williams et al. 2003). The high fat diet that we used was modified to prevent loose stool by lowering the percent fat from 43 to 35% and increasing the amount of carbohydrate from 39 to 46% and reducing the amount of calcium and phosphorus. The diet had a wheat flour base and derived 35% of calories from fat, 19% from protein, and 46% from carbohydrate, lard, butter, beef tallow, cholesterol, and safflower oil provided the majority of the fat, while protein was derived from casein and lactalbumin as well as the wheat flour. Monkeys were fed *ab libitum* with meals provided at 0915 and 1515 h. All aspects of the study were reviewed and approved by the ONPRC Animal Care and Use Committee.

Compound Information

The synthetic estrogen used in this study was a novel cycloalkylidene compound, which binds to estrogen alpha and beta receptors with equal affinity (GSK232802A; GlaxoSmithKline, unpublished data).

Experimental Design

The goal of this study was to test whether estrogen replacement therapy would reverse the increase in body weight caused by ovariectomy, and if so, to determine the mechanisms by which estrogen replacement therapy decreases body weight. The testing phase was separated into three experimental periods. First, there was a one-month control period during which monkeys received vehicle daily, followed by a 3-month experimental period during which monkeys received GSK232802A (5 mg/kg, PO) daily, and lastly a two-month washout period during which they again received vehicle daily.

The vehicle (a small piece of palatable fruit, usually melon) or vehicle plus drug were provided to monkeys 15 minutes prior to the morning meal (0900 h). Measurements of body weight, food intake, and activity were made throughout the entire experiment. Body composition and metabolic rate were determined during the control period, at the end of the treatment period, and at the end of the washout period. Blood samples to measure lutenizing hormone (LH), follicle stimulating hormone (FSH), leptin, triiodothyronine (T_3), and alkaline phosphatase were collected during the control period, after 1 and 3-months of GSK232802A treatment, and at the end of the washout period.

Experimental Measures

Body weight: Body weight measurements were made weekly prior to consumption of the AM meal, at approximately 0800 h.

DEXA-measurements: Percent body fat, central fat mass, peripheral fat mass, and bone mineral density were determined using dual energy X-ray absorptiometry (DEXA) as previously described (Sullivan et al. 2005; Chapter 2). To delineate central fat mass from peripheral fat mass, fat in the trunk (including both the subcutaneous and visceral compartments), and fat in the extremities was calculated using a standard methodology (Clark et al. 2005; Tanko and Christiansen 2005).

Food Intake: Each monkey was fed more food than it routinely consumed at each meal to ensure *ab libitum* food intake. Total food consumption at each meal was estimated by quantifying the amount of food remaining prior to each subsequent next meal throughout the study.

Activity: Activity was measured continuously throughout the experiment using triaxial Actical accelerometers (MiniMitter, Bend, OR). The Actical monitors contain an omnidirectional sensor capable of detecting acceleration in all directions. The sensor integrates the speed and distance of acceleration and produces an electrical current that varies in magnitude depending on the change in acceleration. An increased speed or distance of the acceleration, or a change in direction, produces an increase in electrical current. The activity monitors store this information as activity counts.

Each monkey was fitted with a loose-fitting metal collar (Primate Products, Inc.; Immokalee, FL) with an attached activity monitor, housed in a snug protective stainless steel box. The monitor was programmed to store the total number of activity counts per minute. During the study period, monkeys were sedated with Ketamine HCl (10-20 mg/kg, IM; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and the data from each activity monitor was downloaded at least every 45 days (the maximum number of days that the monitor is capable of storing data). After activity data was downloaded and saved, the monitors were reprogrammed and replaced on the collar.

Metabolic Rate: Metabolic rate of each monkey was measured by placing the monkey in a sealed Lexan and stainless steel metabolic chamber (Columbus Instruments, Columbus, OH) and measuring the amount of carbon dioxide produced and oxygen consumed using a computer-controlled indirect open circuit calorimeter (Oxymax System, Columbus Instruments, Columbus, OH). The metabolic chamber was approximately the same size as the monkey's home cage (inside dimensions are 30" x 24" x 24"). To prevent social isolation during metabolic testing, two monkeys familiar with the test monkey were placed in cages across from and in clear view of the animal in

the metabolic testing chamber at all times. The familiar monkeys were animals that were housed in the same room as the test monkey prior to, and after, metabolic chamber test periods. Prior to each recording session, the oxygen and carbon dioxide sensors were calibrated with a standard mixture of gases (20.5 % oxygen, 0.5% carbon dioxide and a nitrogen balance). Fresh air was pumped into the chamber (12 – 40 L/min) using an external fresh air pump controlled by a flow meter (Columbus Instrument, Columbus, OH) and circulated within the chamber with a 4" fan. The flow rate into the chamber was adjusted for each monkey so that the difference in oxygen concentration between the chamber and the room air was above 0.2% and the carbon dioxide level in the chamber was below 0.6%. The chamber air was sampled at a rate of 0.5 L/min and was circulated over a water absorbent (Drierite) column prior to passing through the oxygen and carbon dioxide sensors. The oxygen and carbon dioxide concentrations of the ambient air and chamber air were recorded every 4 minutes. Oxygen consumption, carbon dioxide production, and total energy expenditure (kcal) were calculated using Oxymax Software version 2.3 (Columbus Instruments, Columbus OH). The Oxymax system calculated oxygen consumption (VO2) by taking the difference between input oxygen flow and output oxygen flow. Similarly, carbon dioxide production (VCO2) was calculated by taking the difference between output and input carbon dioxide flows. In order to calculate energy expenditure, the respiratory exchange ratio (RER), the ratio of CO_2 production (VCO_2) to O_2 consumption (VO_2), and the energy expenditure (EE) was calculated using the following equations: $EE = (3.82 + 1.23 \times RER) \times VO_2 \times 0.001$.

Twenty-four hour metabolic rate was assessed during the control period, at the end of GSK232802A treatment and at the end of the washout period. To determine total

daily energy expenditure, monkeys were placed in the metabolic chamber at 1000 h and remained in the chamber until 0900 h the next morning. Before placement in the chamber, monkeys were fed a standard meal at 0915 h and were then fed a banana (114 g ± 10g, 108 calories) at 1515 h while in the chamber. Water was available *ad libitum* through out metabolic testing. Basal metabolic rate was calculated as the average number of kilocalories expended per hour from 2300 to 0300 h. This time period was selected because this is when monkeys typically sleep and when heart rate is typically slowest (J Cameron, unpublished observations). In addition, this was the time when monkeys exhibited the lowest number of activity counts in this study. The thermic effect of an isocaloric meal (the banana fed at 1515h) was calculated by subtracting basal metabolic rate and activity-associated energy expenditure from total energy expenditure for the four hours after the banana was consumed. This practice was based on studies showing that the majority of energy expended due to meal digestion and processing is within the first four hours after a meal is eaten (Reed and Hill 1996; St-Pierre et al. 2004; Brehm et al. 2005).

Blood Sample Collection

All blood samples were collected by femoral venipuncture after sedation with Ketamine HCl (10-20 mg/kg, IM; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA). Blood samples (12 mLs) were collected during the control period, at the end of the first and third months of GSK232802A treatment, and at the end of the washout period to measure LH, FSH, leptin, T_3, and alkaline phosphatase. These blood samples were obtained between 0800 and 1000 h after an overnight fast. Blood was collected into

sterile heparin-rinsed syringes, transferred into glass tubes, and centrifuged at 1125 g at 4

°C for 10 min. Plasma was removed and stored in plastic vials (with 40 μl of a 50:50

mixture of 1000 U sodium heparin and 38% sodium citrate heparin per mL of plasma, to

prevent clotting of plasma proteins), at –20 °C until assays were performed.

Tissue Collection

A sample of the vaginal wall was collected from one monkey during the baseline

period and after 8 weeks of GSK232802A treatment. To collect samples of the vaginal

wall, the monkey was sedated with Ketamine HCL (10-20 mg/kg, IM; Ketaset, Fort

Dodge Animal Health, Fort Dodge, IA) placed in the prone position, and the perineum,

anus and labia were cleansed and prepared for sterile surgery. Biopsy forceps were

inserted retrograde to a depth of about 3 cm and a 1 cm by 1 cm sample of the lateral

vaginal wall was collected. Analgesia was provided by hydromorphone hydrochloride

(0.5-1.0 mg, i.v.; Elkins-Sinn, Inc., Cherry Hill, New Jersey) and Buprenorphine

hydrochloride (0.03 mg/kg, i.m., SID; Buprenex, Reckitt & Colman Pharmaceuticals,

Inc., Richmond, Virginia). Immediately after sample collection, tissue was placed in

Hank's Balanced Salt Solution. Samples were then fixed in 4% paraformaldehyde and

embedded in paraffin. Parraffin sections (5 um) were placed on SuperFrost Plus Slides

and deparaffinized by standard methods. Tissue sections were then stained with

Haematoxylin and Eosin followed by dehydration and mounting in Permount.

Micrographs were photographed through Zeiss planapochromatic lenses with the

Optronics DEI-750TD CCD camera (Optronics Engineering, Goleta, CA). The sharpness

and contrast of digital images were adjusted with Adobe Photoshop (Adobe Systems, Seattle, WA).

Assays

The Endocrine Services Core Facility at the ONPRC measured plasma leptin and T_3 concentrations. Leptin was measured using a commercially available double-antibody RIA kit for primate leptin (PLR-1101) from Linco Research, Inc. (St. Charles, MO) (Downs and Urbanski 2006). The limit of sensitivity of this assay was 0.5 ng/ml. All samples were measured in a single assay and the intra-assay variability was 3.5%. Plasma T_3 was measured using a Roche Elecsys 2010 clinical instrument and assay reagents for human T_3 from Roche Diagnostics (Indianapolis, IN). The assay variability was 6% and the limit of sensitivity was 0.19 ng/ml. LH and FSH were measured by RIA at the RIA Core Laboratory of the Center for Research in Reproductive Physiology, University of Pittsburgh (Williams et al. 2001). For FSH the limit of sensitivity was 0.62 ng/ml, the intra-assay variability was 2% and the inter-assay variability was 7%. For LH the limit of sensitivity was 0.70 ng/ml and the intra- and inter-assay variability were 4% and 11%, respectively. The Metabolic Disease Core Assay Facility of GlaxoSmithKline, Research Triangle Park, North Carolina assayed alkaline phosphatase using an automated chemistry analyzer (Technicon Axon, Tarrytown, NY) and reagents for human alkaline phosphatase (Olympus Life and Material Science, O'Callaghan's Mills, Ireland). For alkaline phosphatase the within-run variability was 2%, the between day variance was 4%, and the limit of sensitivity was 5 U/L.

Statistical Analyses

Body weight, activity and food intake measures were averaged during the control period to obtain mean control values for each monkey. Changes in food intake, body weight and activity were expressed as percent of control values for each monkey. Statistical comparisons were also made between the average body weight, food intake, and activity during each month of the experiment.

For all analyses, normality and homogeneity of variance were initially tested. If these criteria were met, a repeated measures ANOVA was utilized to examine differences in variables over time. The assumption of sphericity was examined with Mauchly's Test. The Greenhouse-Geiser correction factor was used in cases where the assumption of sphericity was violated. Least Significant Difference post-hoc tests were used to determine time periods that were significantly different from each other. Correlations were determined using a Pearson product moment correlation. An independent t-test was used to detect differences in the change in activity between the most sedentary and most active monkeys. If data was not normally distributed and could not be transformed (using a square root or log transformation) then nonparametric tests were utilized. Differences in non-normally distributed data over time were assessed using the Friedman test followed by the Wilcoxon Signed Ranks test. Spearman's rho correlation was used to analyze correlations between measures that were not normally distributed. Data are presented as mean $\pm$ SEM. Alpha values were considered significant with $p \leq 0.05$. All statistical analyses were conducted using the SPSS software package, version 13.0 (SPSS Inc., Chicago, Illinois).

3.3 RESULTS

Plasma LH concentrations changed significantly over the experiment ($F_{2,34}$=22.5, p<0.0001), with mean LH levels decreasing significantly from 12.9±1.0 ng/mL during the control period to 9.86±0.94 ng/mL during GSK232802A treatment (18.1 ± 4.3% decrease, p<0.0001), and returning to control levels by the end of the washout period (p=0.23; Figure 3.1A). Similarly, FSH levels changed significantly over the experiment ($F_{2,24}$=20.83, p<0.0001), with mean plasma FSH concentrations of 13.18±0.92 ng/mL during the control period decreasing significantly to 10.89±1.01 ng/mL during GSK232802A treatment (23.7±3.8% decrease, p=0.001), but increasing to levels significantly higher than during the control period (14.95±1.24 ng/mL; p=0.02) by the end of the washout period (Figure 3.1B). After two months of GSK232802A treatment there was a marked thickening of the vaginal epithelium with prominent spines (traiangular outgrowth of epithelium) visible (Figure 3.2A), as compared to the tissue sample collected before treatment (Figure 3.2B).

There was a significant change in body weight during the experiment (X^2=41.28, df=5, p<0.0001; Figure 3.3). Body weight was significantly reduced after the first month of treatment (p=0.01), and continued to progressively decrease throughout the 3-months of GSK232802A treatment. By the end of treatment, an average of 4.6±1.03% of initial body weight (p=0.002) was lost. At the completion of the washout period, body weight had returned to control levels (p=0.09). There was also a significant change in percent body fat across the three experimental periods (X^2=20.7, df=2, p<0.0001, Figure 3.4A). After 3-months of GSK232802A treatment, monkeys lost an average of 4.7±1.1% percent body fat (p=0.001). By the end of the washout period percent body fat returned to control
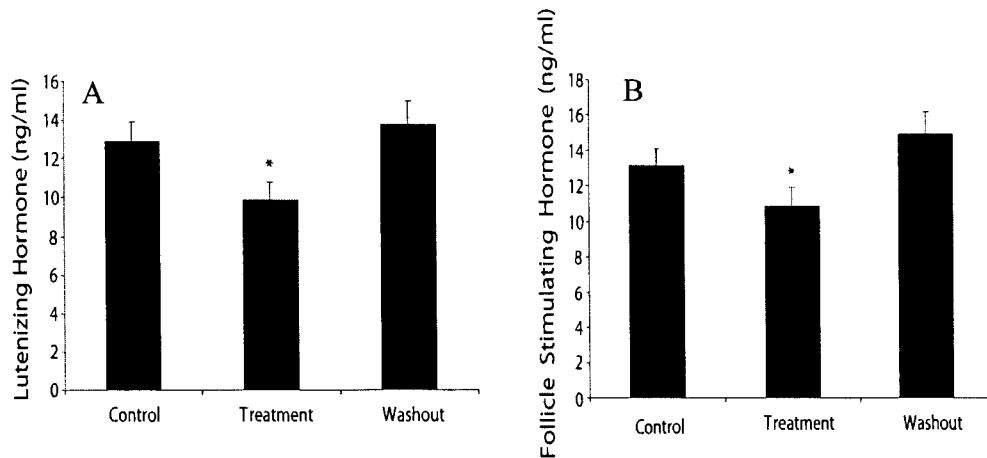
Figure 3.1 (A) Plasma LH concentrations changed significantly over the experiment ($F_{2,34}$=22.5, p<0.0001), with mean LH levels decreasing significantly from 12.9±1.0 ng/mL during the control period to 9.86±0.94 ng/mL during GSK232802A treatment (18.1 ± 4.3% decrease, p<0.0001), and returning to control levels by the end of the washout period (p=0.23). (B) FSH levels changed significantly over the experiment ($F_{2,24}$=20.83, p<0.0001), with mean plasma FSH concentrations of 13.18±0.92 ng/mL during the control period decreasing significantly to 10.89±1.01 ng/mL during GSK232802A treatment (23.7±3.8% decrease, p=0.001), but increasing to levels significantly higher than during the control period (14.95±1.24 ng/mL; p=0.02) by the end of the washout period.
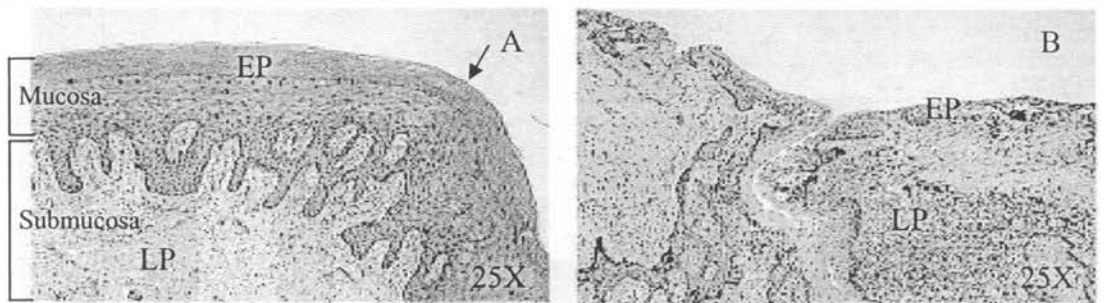
Figure 3.2 Photomicrographs of Haematoxylin and Eosin stained paraffin section of the lateral vaginal wall after two months of GSK232802A (A) and prior to treatment (B). EP, epithelium, LP, laminia propia, Arrow indicates spine.
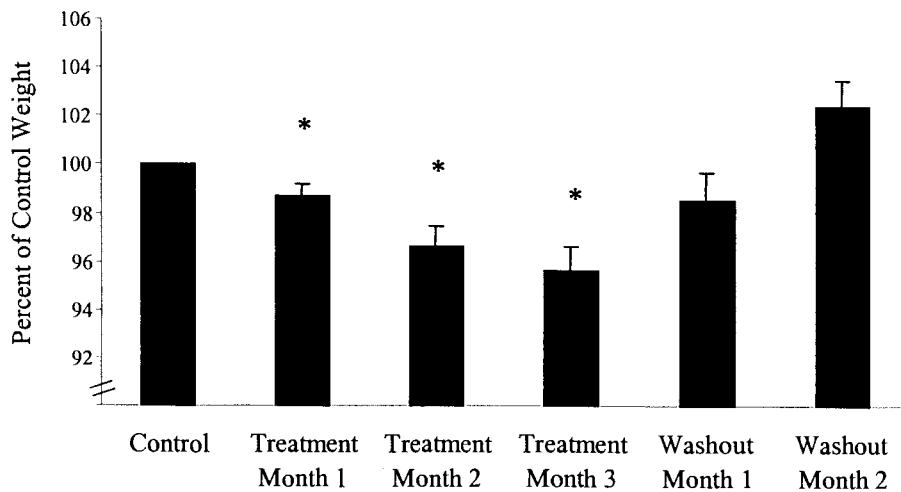
Figure 3.3 Body weight significantly decreased with estrogen replacement therapy (X^2=41.3, df=5, p<0.0001). Body weight was significantly lower than controls levels during the first (p=0.01), second (p=0.005) and third (p=0.002) months of treatment and was not different from control levels during the first (p=0.20) and second (p=0.09) months of the washout period. Asterisks indicate a significant difference from the control period, p<0.05.
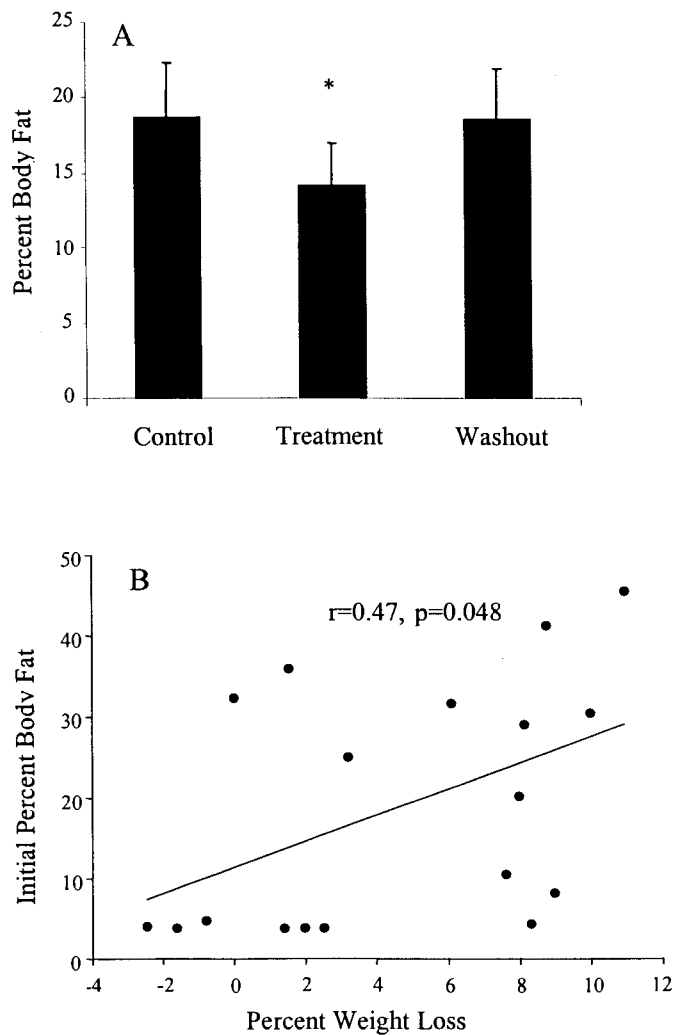
Figure 3.4 (A) Percent body fat was significantly lower than control levels after 3-months of estrogen replacement therapy (p=0.001) and returned to control levels by the end of the washout period (p=0.74). (B) There was a significant correlation between initial percent body fat and weight loss (r=0.47, p=0.05).

levels (p=0.74). The loss in percent body fat correlated with the loss in body weight, such that the monkeys that lost the most fat also lost the most weight (r=0.63, p=0.005). Initial percent body fat also correlated with weight loss, such that the fattest monkeys lost the most weight (r=0.47, p=0.05; Figure 3.4B). There was a significant change in central fat mass over the experiment (X^2=20.9, df=2, p<0.0001), with central fat mass decreasing from 21.0±4.0% during the control period to 15.6±3.3% upon completion of GSK232802A treatment (p=0.001), and returning to control values at the end of the washout period (p=0.88). There was also a significant change in peripheral fat mass (X^2=10.78, df=2, p=0.005) with peripheral fat mass significantly decreasing from 12.33±2.03% to 9.97±1.65% during GSK232802A treatment (p=0.006), and returning to control values at the end of the washout period (p=0.33). No difference was observed in bone mineral density between the three experimental periods ($F_{2,34}$=1.489, p=0.24). Leptin levels decreased after 3-months of GSK232802A, however, this was not statistically significant ($F_{1.3,23}$=0.423, p=0.58; Table 3.1). As expected, initial leptin levels correlated with initial percent body fat (r=0.96, p<0.001) and central fat mass (r=0.95, p<0.0001), such that the fattest monkeys had the highest leptin levels. Initial plasma leptin, like percent body fat, correlated with weight loss, such that monkeys with the highest leptin levels lost the most weight (r=0.57, p=0.01).

Treatment with GSK232802A decreased total daily food intake ($F_{2.6,45.0}$=8.6, p<0.0001). Total daily food intake was significantly lower than control levels during the first (p=0.04), second (p=0.04) and third (p=0.045) months of treatment (p=0.02; Figure 3.5A). Food intake was also significantly higher than control levels

Table 3.1 Metabolic changes occurring during hormone replacement therapy.

Asterisks indicate a significant difference from levels during the control period and a hatchmark indicates a significant difference from levels during the treatment period.

	Control	Treatment	Washout
Leptin (ng/mL)	5.48 ± 1.18	4.21 ± 0.61	5.12 ± 1.03
T3 (ng/mL)	2.27 ± 0.15	$2.85 \pm 0.23*$	$2.10 \pm 0.09*^{,\#}$
ALP (U/L)	133.5 ± 10.5	$77.4 \pm 5.0*$	$162.8 \pm 9.5*^{,\#}$
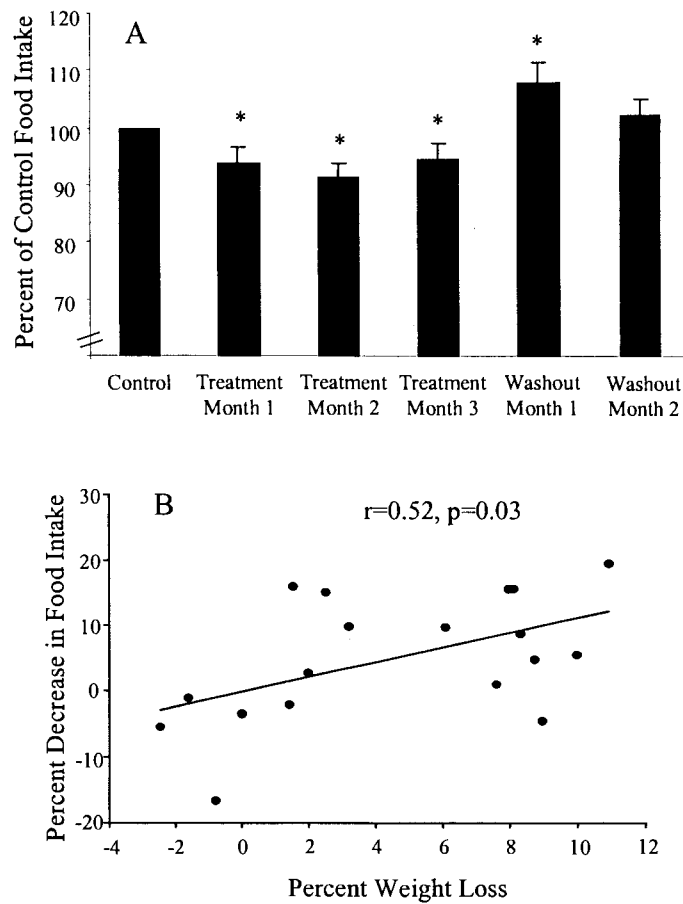
Figure 3.5 (A) There was a significant decrease in food intake over the course of the study ($F_{2.6,45.0}=8.6$, $p<0.0001$). Food intake was lower than control levels during the first ($p=0.04$), second ($p=0.04$) and third ($p=0.045$) months of treatment, was higher than control levels during the first month of the washout period ($p=0.03$), and then was not different from control levels during the second month of washout ($p=0.53$). (B) There was a significant correlation between the percent decrease in food intake during treatment and the percent weight loss over the treatment period ($r=0.52$, $p=0.03$).

during the first month of the washout period (p=0.03), but returned to control levels by the end of the second month of washout (p=0.53). The decrease in total daily food intake correlated with weight loss, such that monkeys that decreased their food intake the most were the ones that lost the most weight (r=0.52, p=0.03; Figure 3.5B).

No significant difference in the metabolic rate was observed at any time of day between the control, treatment and washout periods ($F_{2,34}$=0.551, p=0.58; Figure 3.6A). No significant differences in basal metabolic rate ($F_{2,34}$=1.0, p=0.38), or the thermic effect of an isocaloric meal ($F_{2,26}$=1.96, p=0.16) were observed over the course of the experiment (data not shown). However, initial metabolic rate (r=0.55, p=0.02), and initial basal metabolic rate (r=0.60, p=0.01; Figure 3.6B) correlated with weight loss, such that monkeys with the highest metabolic rate lost the most weight.

Physical activity level among the three experimental periods was significantly different ($F_{2.15,36.5}$=16.63, p<0.0001; data not shown). In the group, as a whole, the level of physical activity did not change from control levels during the first (p=0.40) and second (p=0.29) months of treatment. However, by the third month of treatment, activity was significantly higher than control levels (p=0.04). Activity remained elevated during the first (p<0.0001) and second (p<0.0001) months of the washout period. The percent change in activity correlated with initial activity, such that the most inactive monkeys increased their activity the most (r=-0.56, p=0.02; Figure 3.7A). The quartile of monkeys that were most sedentary increased their activity significantly during the first (p=0.02), second (p=0.01) and third (p=0.006) months of treatment (Figure 3.7B). Change in activity also correlated with initial percent body fat (r=0.72, p=0.001) and initial plasma leptin levels (r=0.62, p=0.008), such that the fattest monkeys increased their activity the
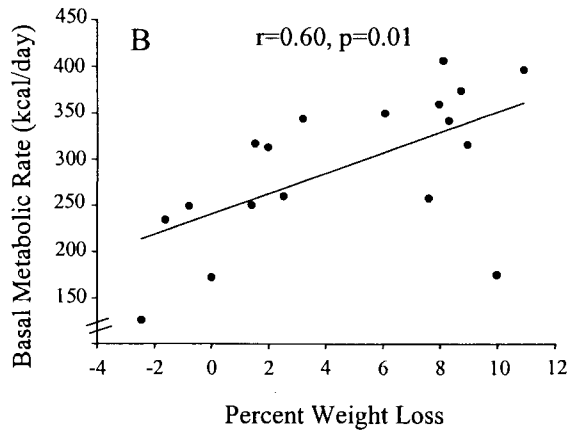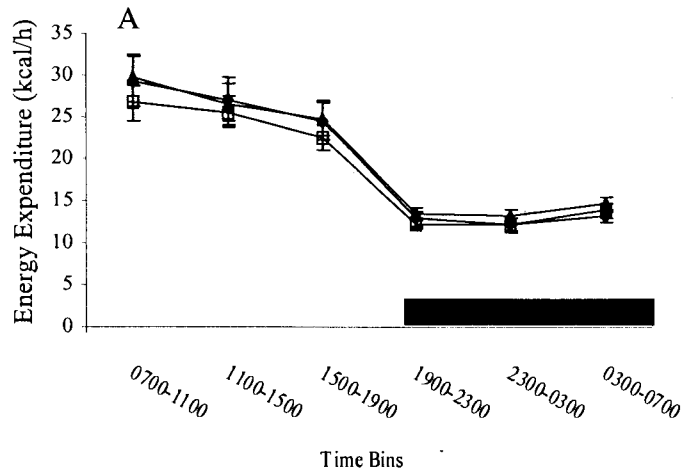
Figure 3.6 (A) There was no significant difference in the metabolic rate at any time of

day between the control (denoted with black diamonds), treatment (denoted with an open

box) and washout periods (denoted by a black circle) ($F_{2,34}=0.551$, $p=0.58$). (B) There

was a significant correlation between basal metabolic rate and weight loss ($r=0.60$,

$p=0.01$).
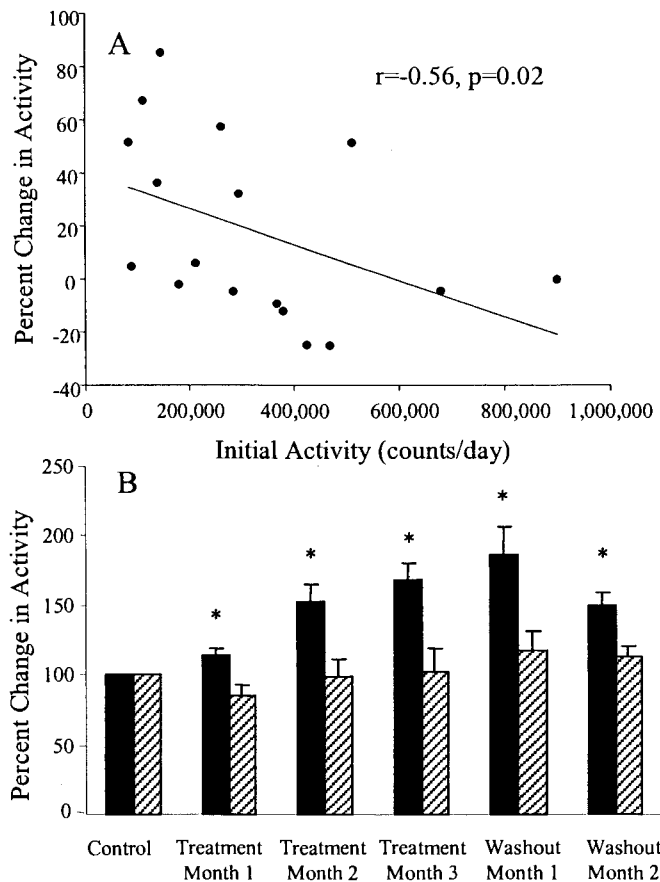
Figure 3.7 (A) There was a significant correlation between percent change in activity and activity at study initiation (r=-0.56, p=0.02). (B) The most sedentary quartile of monkeys (n=5) significantly increased their activity over the course of the study ($F_{1.8,7.34}$=11.6, p=0.0006). Activity was significantly higher than control levels during the first (p=0.02), second (p=0.01) and third (p=0.006) months of treatment and during the first (p=0.01) and second (p=0.005) months of the washout period. The black bars denote the activity of the most active quartile of monkeys and the hatched bars represent the activity of the most sedentary quartile of monkeys. An asterick denotes a significant difference from control levels for each group (active versus sedentary) of monkeys.

most (data not shown).

However, neither the change in activity (r=0.15, p=0.58), nor initial activity level (r=-0.16, p=0.55) correlated with weight loss (data not shown). However, neither the change in activity (r=0.15, p=0.58), nor initial activity level (r=-0.16, p=0.55) correlated with weight loss (data not shown). T_3 levels changed significantly over the experiment (F $_{1.2,21}$= 16.2, p<0.0001). T_3 levels increased during GSK232802A treatment ($26.5 \pm 5.7\%$ increase, p<0.001; Table 3.1). By the end of the washout period T_3 levels decreased to 2.10 ± 0.09 ng/mL, a level that was significantly lower than levels during the control (p=0.04) and treatment periods (p=0.001; data not shown). T_3 levels during treatment correlated with percent change in activity (r=0.69, p=0.002), such that monkeys who increased their activity the most also had the highest T_3 levels. T_3 levels during treatment also correlated with weight loss such

that monkeys with the highest levels of T_3 lost the most weight (r=0.51, p=0.03; data not shown).

Alkaline phosphatase levels changed significantly over the course of the study ($F_{2,34}$=59.1, p<0.0001, Table 3.1), decreasing during treatment (p<0.0001) and increasing during the washout period to levels higher than the control (p=0.002) and treatment (p<0.0001) periods.

3.4 DISCUSSION

Estrogen replacement therapy significantly decreased body weight (a 4.6% decrease in 3-months) and percent body fat (a 4.7% decrease in 3-months) in ovariectomized female monkeys. Of particular interest, the 4.6% weight loss over 3-

months with estrogen replacement was similar in magnitude to the weight gain that we observed in these monkeys after ovariectomy (4.8% increase; Chapter 2; Sullivan et al. 2005). Weight loss appears to have resulted, in part, from a decrease in food intake (5% decrease over the 3-month period of weight loss). The decrease in food intake was positively correlated with weight loss, such that the monkeys that decreased their food intake the most, lost the most weight. Interestingly, estrogen replacement therapy was most effective in causing weight loss in the heaviest individuals, those with the greatest fat mass. This is likely because the fattest individuals are also the most sedentary individuals, and in these animals estrogen replacement therapy robustly increased level of physical activity. We conclude that, as in non-primate species, estrogen replacement therapy causes weight loss in primates. This finding supports epidemiological studies showing that HRT decreases postmenopausal weight gain (1995; Espeland et al. 1997; Gambacciani et al. 1997; Lahmann et al. 2000; Gambacciani et al. 2001; Gambacciani et al. 2001) and clinical studies finding that HRT causes weight loss in obese postmenopausal women (Ongphiphadhanakul et al. 1998; Chmouliovsky et al. 1999).

The SERM (synthetic estrogen receptor modulator) that we utilized in this study, GSK232802A, has been shown to bind with equal affinity to both the alpha and beta estrogen receptors (GlaxoSmithKline, unpublished data). It is well documented that estrogen and other SERMs decrease plasma LH and FSH in women and in rhesus monkeys (Weick et al. 1983; Osborn et al. 1989; Weick et al. 1989; Asch et al. 1991; O'Sullivan et al. 1998; Kulkarni et al. 2001; Cheng et al. 2004; Gass et al. 2004). Thus, our finding that plasma LH and FSH were reduced during GSK232802A treatment is consistent with the proposed estrogenic action of this compound. Also, it is well

documented that estrogen and estrogen agonists induce cell proliferation in the vaginal epithelium and a thickening of this layer of tissue in women and rhesus monkeys (Otto et al. 2002; Ellmen et al. 2003; Slayden and Brenner 2004; Slayden et al. 2004). In addition, in rhesus monkeys estrogen treatment leads to the development of spines in the epidermal layer of the vagina (Slayden et al. 2004), similar to those that we found (see Figure 1A). Our finding that GSK232802A treatment caused a thickening of the vaginal epithelium and the development of spines, further confirms the *in vivo* estrogenic potency of this compound in monkeys.

The finding that estrogen replacement therapy with GSK232802A decreases food intake supports other studies in ovariectomized non-human primates showing that treatment with estradiol itself, decreases food intake (Czaja and Goy 1975; Bielert and Busse 1983; Kemnitz et al. 1986; Kemnitz et al. 1989). Likewise, treating ovariectomized rats with estradiol decreases food intake to the level of ovary-intact controls (Wade and Zucker 1970; Blaustein and Wade 1976; Asarian and Geary 2002; Shinoda et al. 2002; Roesch 2006). In the current study, estrogen replacement therapy decreased food intake during the first month of treatment. Similarly, other studies in ovariectomized monkeys have found a significant decrease in food intake within a few weeks of starting treatment (1-4 weeks) (Czaja and Goy 1975; Bielert and Busse 1983; Kemnitz et al. 1986; Kemnitz et al. 1989). Moreover, studies in rodents report that estrogen significantly decreases both food intake and body weight within a day to 2 weeks after initiation of treatment (Wade and Zucker 1970; Czaja and Goy 1975; Chu et al. 1999; Wallen et al. 2001). The ability of estrogen to decrease food intake rapidly is also consistent with studies in women (Dalvit 1981; Pliner and Fleming 1983; Gong et al.

73

1989; Lyons et al. 1989), non-human primates (Czaja and Goy 1975; Rosenblatt et al. 1980; Kemnitz et al. 1986; Kemnitz et al. 1989) and rodents (Slonaker 1924-1925; Brobeck 1947; Jennings 1969; Czaja and Goy 1975; Blaustein and Wade 1976) that have reported that food intake is lowest just prior to ovulation, the period during the menstrual cycle of peak estrogen secretion.

Physical activity was elevated during estrogen replacement therapy and this effect was greatest in the most sedentary monkeys. This finding is consistent with results of studies in rodents showing that activity is decreased after ovariectomy (Wang 1923; Slonaker 1924-1925; Wade 1972; Gerall et al. 1973; Takahashi and Menaker 1980; Adams et al. 1985; Shimomura et al. 1990) and that estrogen treatment increases activity to pre-ovariectomy levels (Young and Fish 1945; Colvin and Sawyer 1969; Gerall et al. 1973; Takahashi and Menaker 1980; Ravussin and Gautier 1999). Additional evidence that estrogen increases activity comes from studies showing that wheel-running activity in rodents increases near the time of estrus after the proestrus rise in estrogen; (Wang 1923; Brobeck 1947; Jennings 1969; Wollnik and Turek 1988). Interestingly, female rats have been reported to be more active than male rats, and transplanting ovaries into male rats has been shown to raise their activity level to that typical of a female rat (Wang et al. 1925). Further support for a role of estrogen in influencing activity comes from studies showing that estrogen knockout mice have lower levels of physical activity than wild type mice (Jones et al. 2000; Misso et al. 2003). The findings of this study suggest that estrogen increases activity in primates as it does in rodents.

In this study, the physical activity of the most sedentary individuals was elevated within the first month of estrogen replacement therapy, and their activity remained

elevated during the subsequent two months of treatment and throughout the washout period. In rodents, ovariectomy decreases activity within 1-9 days and estrogen treatment increases activity to post-ovariectomy levels within 1-12 days of treatment initiation (Colvin and Sawyer 1969; Gerall et al. 1973; Ahdieh and Wade 1982; Ogawa et al. 2003). The similar time course of estrogen action suggests that a similar mechanism underlies the increase in activity in primates and rodents. In this study, plasma T_3 levels increased during GSK232802A treatment. T_3 treatment has been reported to increase activity in rats (Levine et al. 2003). Additionally, men with high T_3 levels have been reported to be more active than men with lower T_3 levels (Ravaglia et al. 2001). Also, people with hyperthyroidism who produce excess T_3 have been reported to be hyperactive (Weetman 2000). However, in this study and in the studies by Weetman (2000) and Ravaglia et al. (2001) it is unclear if the increases in T_3 are causing the changes in activity or if the need for greater cellular energy caused by increased physical activity leads to the increase in T_3. Further studies are needed to define the mechanism whereby estrogen increases activity in primates.

The fact that activity increased during a period of weight loss suggests a direct effect of estrogen treatment on activity. Usually a period of weight loss is accompanied by a compensatory decrease in both activity (Keys et al. 1950; de Groot et al. 1989; Kemnitz et al. 1993; Leibel et al. 1995) and metabolic rate (Dauncey 1980; Bessard et al. 1983, Henry et al. 1988; Heyman et al. 1992; Leibel et al. 1995; Lane et al. 1996; see Chapter 5 for further discussion). In addition weight loss was not accompanied by a decrease in basal metabolic rate or total daily metabolic rate as you expect with a decrease in metabolically active tissue, suggesting estrogen treatment counteracts the

normal decrease in metabolic rate that would occur with weight loss. Further studies to better understand the actions of estrogen on activity and metabolic rate could compare changes in activity and metabolic rate occurring during estrogen-induced weight loss to changes in these parameters during diet-induced weight loss. It was surprising, that the increase in activity did not correlate with weight loss as both the increase in activity and weight loss correlated with initial percent body fat. Further analysis revealed that the change in activity and weight loss were not correlated because 3 monkeys that were initially lean and very active did not increase their activity over the course of the study, but they did loose weight. Also, one monkey greatly increased her activity but did not lose weight as she also increased her food intake. When these four monkeys were removed from the analysis the increase in activity was correlated with weight loss (r=0.63, p=0.02). In conclusion, there was a general trend for the monkeys that increased their activity the most to lose the most weight, this however was not true for all individuals. Monkeys who were very active at the beginning of the study did not become more active, and it appears that increased food intake could override the effect of increased activity on weight loss.

The fattest monkeys lost the most weight during estrogen replacement therapy. This finding is supported by a clinical study that found that HRT caused significant weight loss in the fattest individuals (Ongphiphadhanakul et al. 1998). Also, Chmouliovsky et al. 1999 found that HRT caused weight loss in obese postmenopausal women (Ongphiphadhanakul et al. 1998). This may not be surprising, in that the majority of studies of diet-induced weight loss show that obese individuals lose more weight than lean individuals (Stein et al. 1981; Wadden et al. 1992; Hoie and Bruusgaard

1995; Packianathan et al. 2005; Teixeira et al. 2005). This study is the first to report that estrogen replacement therapy preferentially increases the activity of the most sedentary individuals, probably because previous studies with HRT treatment of women and monkeys have not measured activity. And the fattest monkeys were the most sedentary, thus the effect of estrogen replacement therapy on activity may be the mechanism underlying the preferential effect of HRT to cause weight loss in the fattest monkeys.

Alkaline phosphatase is an enzyme that is produced primarily in the liver and bone (Vaughn 1999). There are multiple forms of alkaline phosphatase (isoenzymes) that have different chemical structures and are found in different tissues (Vaughn 1999). Alkaline phosphatase is considered a marker of bone remodeling (Woitge et al. 1996; Weaver et al. 1997; Minisola et al. 1998) and a high correlation exists between bone-specific and total plasma alkaline phosphatase (Woitge et al. 1996; Takahashi et al. 1997). In this study, alkaline phosphatase decreased with estrogen treatment suggesting a positive effect of estrogen replacement therapy on bone mineral density, as previous studies have shown that a decrease in bone specific alkaline phosphatase is associated with an increase in bone mineral density (Greenspan et al. 2005). Similar findings have been reported in both monkeys and postmenopausal women treated with estrogen or various SERMs (Rymer et al. 1994; 1996; Gallagher et al. 2001; Cakmak et al. 2005; Lees et al. 2005; Love et al. 2005). It is well documented that treating postmenopausal women or monkeys with HRT increases bone mineral density (1996; O'Sullivan et al. 1998; Gallagher et al. 2001; Gambacciani et al. 2001; Jirapinyo et al. 2003; Jolly et al. 2003; Lees et al. 2005; Mizunuma et al. 2006). Although an increase in bone mineral density has been detected in women after a year of HRT, several studies, which have

looked at bone mineral density after 6 months of HRT, have not been able to detect a significant change in this shorter time period. This may explain why we did not see a significant increase in overall bone mineral density measured by DEXA-scan (data not shown) in our study, as our treatment period was only 3 months in duration.

In conclusion, this study shows that estrogen replacement therapy decreased body weight and percent body fat in ovariectomized primates, similar to its effect in rodents. Additionally, estrogen replacement therapy was associated with both a decrease in food intake and an increase in physical activity level which are likely to have both contributed to the weight loss. The increase in activity was particularly marked in the fattest, most sedentary monkeys. These findings suggest that HRT may be most effective in promoting weight loss in the population of individuals that could most benefit from weight loss, obese sedentary individuals.

Chapter 4

INDIVIDUAL DIFFERENCES IN PHYSICAL ACTIVITY ARE STRONGLY PREDICTIVE OF SLOW ADULT WEIGHT GAIN IN FEMALE RHESUS MONKEYS (*MACACA MULATTA*)

4.1 INTRODUCTION

Epidemiological studies indicate that body weight and body fat increase through early and middle adulthood, such that by late middle age there is an increased percentage of overweight and obese individuals compared to the early adult period (Folsom et al. 1996; Lewis et al. 2000; Vardi and Pinhas-Hamiel 2000; Lara-Castro et al. 2002; Wilson and Kannel 2002). Weight gain over the adult years has escalated over the past two decades, such that 65% of adults in the United States have a BMI above the healthy range (>25.0 kg/m^2) (Flegal et al. 2002). Weight gain and obesity in adulthood have been associated with overall increases in morbidity and mortality (Paffenbarger et al. 1993), and in the risk of diabetes mellitus (Colditz et al. 1990; Kujala et al. 1994; Folsom et al. 1996; WHO 2000), gall bladder disease (WHO 2000), coronary heart disease (Hamm et al. 1989; Manson et al. 1990; Kujala et al. 1994; Rimm et al. 1995; Willett et al. 1995; 2000; Klein et al. 2004), hypertension (Kujala et al. 1994; WHO 2000), stroke (WHO 2000), dyslipidemia (WHO 2000), osteoarthritis (WHO 2000), gout (WHO 2000), pulmonary diseases (WHO 2000), colon cancer (Giovannucci et al. 1995), and breast cancer (Ziegler et al. 1996).

A large body of epidemiologic data shows an association between low levels of physical activity and a higher rate of adult weight gain, and a greater increase in percent body fat, throughout adulthood (Rissanen et al. 1991; Klesges et al. 1992; Williamson et

al. 1993; Kyle et al. 2001; Di Pietro et al. 2004; Sternfeld et al. 2004; Brown et al. 2005; Hunter and Byrne 2005; Littman et al. 2005). However, most of these studies rely on self-report of physical activity and several studies have shown that self-reporting of activity can be inaccurate and problematic (Klesges et al. 1990; Matthews and Freedson 1995; Melanson and Freedson 1995; Treuth et al. 2004). Accelerometry, an objective way to monitor physical activity, has recently been used to show that obese children and adults have lower activity levels than their lean counterparts (Ekelund et al. 2002; Janz et al. 2002; Trost et al. 2003; Abbott and Davies 2004; Treuth et al. 2004; Sternfeld et al. 2005). Similarly, studies in rodents that objectively measure activity also show that obese individuals have lower activity levels compared to lean individuals (Clark and Gay 1972; Levin 1991). However, it is unclear from these studies whether low activity is a cause or consequence of obesity. Accelerometry would be ideal for measuring the contribution of individual differences in activity to adult weight gain, but would require wearing activity monitors during the prolonged periods over which adult weight gain takes place. In fact, two studies in mice that objectively measured activity found that weight gain in adulthood was negatively correlated with activity level (Dunnington et al. 1977; Brownlow et al. 1996), supporting the notion that low activity is a cause of obesity.

In the current study, we maintained adult monkeys wearing accelerometers over a prolonged period (i.e., 9 months), allowing us to determine whether individual differences in adult weight gain differed in monkeys exhibiting low activity versus high physical activity. Food intake and metabolic rate were measured to allow assessment of the relative contribution of physical activity level to adult weight gain. Results of this study show that of all of the parameters that we measured, an individual's level of

physical activity is the strongest predictor of weight gain in ovariectomized female monkeys.

4.2 MATERIALS AND METHODS

<u>Animals</u>

Eighteen adult female rhesus monkeys (*Macaca mulatta*), 9 - 13 years of age, weighing between 4.7 and 11.1 kilograms, living in individual stainless steel cages (32″ x 24″ x 27″ or 32″ x 34″ x 27″) in a temperature-controlled room (24 ± 2 C), with lights on for 12 h a day (0700 - 1900 h), were studied. Approximately one year prior to the initiation of this study, the monkeys had been ovariectomized and maintained on a high fat diet (35% fat) to approximate the conditions experienced by many post-menopausal women in the Western world (Williams et al. 2003). The high fat diet was formulated according to the recipe developed by Clarkson and colleagues to study diet-induced atherosclerosis (Shadoan et al. 2003; Williams et al. 2003). Monkeys were fed *ab libitum* with meals provided at approximately 0915 and 1515 h. All aspects of the study were reviewed and approved by the ONPRC Animal Care and Use Committee and performed according to federal guidelines.

<u>Experimental Design</u>

The goal of this experiment was to determine if the activity level of an individual is predictive of weight gain over a period of time in adulthood during which food intake is stable and there is slow progressive weight gain. Additionally, other parameters known to influence weight gain, such as food intake and metabolic rate, were measured.

The experimental period was 9 months in duration, during which time activity level of each monkey was measured continuously by a 3-way accelerometer. During the first 3 months of the study, the weight of each monkey was measured weekly, food intake was quantified at each meal, and percent body fat was determined at the beginning and end of this period. Metabolic rate was measured over a 4-hour period at the beginning of the study and was measured for 24 hours at the end of the first three months. Morning metabolic rate during fasting was compared between the two time points. During the last 6 months, activity was continuously monitored to allow assessment of the stability of this physiological measure.

Experimental Measures

Body weight: Body weight was measured weekly at approximately 0800 h, prior to the AM meal.

DEXA-scans: Percent body fat was determined using dual energy X-ray absorptiometry (DEXA) as previously described (Sullivan et al. 2005).

Calorie Intake: Each monkey was fed more food than she routinely consumed at each meal to ensure *ab libitum* food intake. Total food consumption at each meal was recorded daily throughout the study, by quantifying the amount of food remaining prior to the next meal. On one day during the study the total amount of stool excreted in a 24 hour period was collected from each monkey by placing a metal pan covered with wire mesh under each monkey's cage for 24 hours. The amount of stool was weighed and a representative sample was collected at 9 AM the next morning and frozen at $-20\,^{\circ}C$. The caloric content of a sample of stool from the two monkeys that consumed the most

calories and the two monkeys that consumed the least number of calories was determined

by bomb calorimetry (Kinetica Inc., Franklin, Ohio), to quantify differences in calories

excreted versus calories absorbed.

Metabolic Rate: Metabolic rate of each monkey was measured by placing the

monkey in a sealed Lexan and stainless steel metabolic chamber (Columbus Instruments,

Columbus, OH) and measuring the amount of carbon dioxide produced and oxygen

consumed using a computer-controlled indirect open circuit calorimeter (Oxymax

System, Columbus Instruments, Columbus, OH) as previously described (Chapter 3).

Upon study initiation, monkeys were individually placed in the metabolic chamber at

0900 h and remained in the chamber until 1300 h. The day prior to testing each monkey

was fed its standard meal and at 1700 h all food was removed from the monkey's cage

and the monkey was fasted until completion of metabolic testing. After 3 months, 24-

hour metabolic rate of each monkey was assessed. Monkeys (n=16) were placed in the

metabolic chamber at 1000 h and remained in the chamber until 0900 h the next morning.

Prior to placement in the chamber, monkeys were fed a standard meal at 0915 h and were

fed a 114 ± 1 g banana at 1515 h while in the chamber. Basal metabolic rate was

calculated as the average number of kilocalories expended per hour from 2300 to 0300 h,

as previously described (Chapter 3). The thermic effect of an isocaloric meal (the banana

fed at 1515h) was calculated by subtracting basal metabolic rate and activity-associated

energy expenditure from total energy expenditure for the four hours after the banana was

consumed, as previously described (Chapter 3).

Activity: The naturally occurring activity level of each monkey was assessed

using triaxial Actical accelerometers (MiniMitter, Bend, OR) and previously described

methods (Chapter 3). Activity counts recorded from 0700 to 1900 h (when lights were on) were considered daytime activity and activity counts recorded from 1900 h to 0700 h (when lights were off) were considered nighttime activity. Activity-associated energy expenditure was calculated by determining the energy expended (in kcal) per activity count. This was calculated by measuring total energy expenditure at times of day in which there would be little thermic effect of food contributing to the metabolic rate (from 1400-1500 h and 1800-1900 h), subtracting basal metabolic rate, and dividing the remaining energy expenditure by the number of activity counts occurring during this time period. The number of calories expended per activity count was multiplied by total daily activity counts to determine daily activity-associated energy expenditure.

The duration of time that the monkeys were sedentary was assessed during a representative day from the week of initial activity measurement by determining how many minutes each monkey had no activity counts.

Data Analysis

Activity during a representative week at the beginning of the study, after 3 months, and again after 9 months was analyzed. Body weight, average weekly food intake and fasting morning metabolic rate were compared upon study initiation and after 3 months. The associations between all measurements (food intake, total energy expenditure, basal metabolic rate, thermic effect of food, and activity) and weight gain were determined. Regression analysis demonstrated that lean body mass was the best predictor of total energy expenditure (R^2=0.52) and basal metabolic rate (R^2=0.58), thus

after analyzing the raw values the adjusted residuals were also analyzed for their association with weight gain.

For all analyses, normality and homoscedacity were initially tested. Initially, multivariate regression analysis was used to determine which variable (basal metabolic rate, activity and food intake) was best able to predict weight gain. If data was normally distributed, paired t-tests were used to evaluate differences between measures made at two different time points and a repeated measures ANOVA was used to evaluate differences in activity measurements made upon initiation of the study, after 3 months, and after 9 months. The assumption of sphericity was examined with Mauchly's Test. The Greenhouse-Geiser correction factor was used in cases where the assumption of sphericity was violated. Independent t-tests were utilized to assess differences in amount of weight gained by monkeys divided into groups of the highest and lowest quartiles based on food intake, activity, and metabolic rate. Correlations between measurements were determined using a Pearson product moment correlation. If data was not normally distributed, and could not be transformed (using a square root or log transformation) then nonparametric tests were utilized. The Wilcoxon Signed Ranks test was utilized to assess differences in non-normally distributed data over time. Spearman's rho correlation was used to analyze correlations between parameters that were not normally distributed. Linear regression analysis was performed to develop an equation for predicting the energy expenditure in kilocalories per activity count. Data are presented as mean $\pm$ standard error of the mean (SEM). Alpha values were considered significant if p ≤ 0.05. Statistical analyses were performed with SPSS software, version 13.0 (SPSS Inc., Chicago, Illinois).

4.3 RESULTS

During the first three months of the study, the group showed a small but significant gain in body weight (5.5 ± 0.88%, t=-6.3, df =17, p<0.0001; Table 4.1). There were large differences in weight gain between individual monkeys, with several monkeys gaining no weight during this experimental period, while others gained up to 13% of their initial weight in 3 months. Body fat also increased significantly in the group from 15.9 ± 3.0% to 18.87 ± 3.4% of total body mass during this time period (z=-3.17, p=0.002; Table 4.1). Initial body fat was not correlated with weight (r=-0.19, p=0.45) or fat gain (r=-0.17, p=0.51), and there was no difference in weight gain or fat gain between the monkeys who were in the leanest versus fattest quartile at the beginning of the study (t=0.33, df=8, p=0.75; t=-1.3, df=8, p=0.24 for weight and fat gain, respectively). Additionally, the age of the monkeys was not correlated with weight gain (r=-0.25, p=0.33) or fat gain (r=0.05, p=0.86), and there were no differences in the amount of weight or fat gained between the youngest and oldest monkeys (t=0.04, df=8, p=0.97; t=-0.11, df=8, p=0.92 for weight and fat gain, respectively).

Initially, a multivariate regression analysis determined that when food intake, basal metabolic rate and activity were used as independent variables, that body weight gain could not be significantly predicted (R^2=0.27, $F_{3,12}$=1.50, p=0.27). However, activity was a better predictor of weight gain (p=0.07) than food intake (p=0.73) and basal metabolic rate (p=0.87).

Food intake was not significantly changed during the three-month period (t=1.04, df=17, p=0.31; Table 4.1). Although there were considerable individual differences in the amount of calories consumed by individual animals (a 5-fold difference, ranging from

**Table 4.1 Metabolic Parameters Across Three Months of Weight Gain (mean ±
SEM).** Asterisks indicate a significantly different from initial measures using a paired t-
test.

	Initial	Final
Body Weight (kg)	7.54 ± 0.48	7.94 ± 0.49*
Percent Body Fat (%)	15.9 ± 3.0	18.8 ± 3.5*
Food Intake (kcal)	1188 ± 129	1113 ± 105
Energy Expenditure (kcal/h)	21.3 ± 2.3	28.7 ± 2.9*
Activity (counts/day)	291,296 ± 35,805	233,651 ± 37,983

411 –2210 kcal/day), the food intake of individual monkeys was consistent over this three

month time period such that the initial food intake of each individual was highly

correlated with food intake after three months (r=0.95, p<0.0001; Figure 4.1A).

However, food intake did not correlate with the amount of weight gained by each

monkey during this time period (r=0.15, p=0.56). In addition, there was no difference in

the weight gain of the quartile of monkeys that ate the most, compared to the quartile of

monkeys that ate the least (t=-0.20, df=8, p=0.85; Figure 4.1B). To begin to look at

whether food intake accurately predicts calorie absorption, we measured the caloric

content of the stool in the two monkeys eating the most calories and the two eating the

least. Mean caloric content of the four stool samples was 1.70 ± 0.28 kcal/g, and the

caloric content of the stool samples was correlated with food intake, such that the

monkeys that ate the most excreted more calories per gram of stool (r=0.95, p=0.046, 2.1-

fold difference between monkeys with the highest and lowest food intake).

Daily energy expenditure significantly increased from 21.3 ± 2.3 kcal/h to 28.7 ±

2.9 kcal/h (t=-3.46, df=17, p=0.003; Table 4.1) over the first three months of the

experimental period. Initial daily energy expenditure was correlated with final daily

energy expenditure (r=0.68, p=0.002, Figure 4.2A). Moreover, the change in daily

energy expenditure correlated with weight gain (r=0.47, p=0.05); such that the monkeys

that gained the most weight increased their daily energy expenditure the most. There was

a 6-fold difference in daily energy expenditure between individual monkeys. However,

initial daily energy expenditure did not correlate with weight gain (r=-0.35, p=0.16) and

although percent weight gain was somewhat higher in the quartile of monkeys with the

lowest daily energy expenditure (7.4% weight gain) compared to the quartile of monkeys
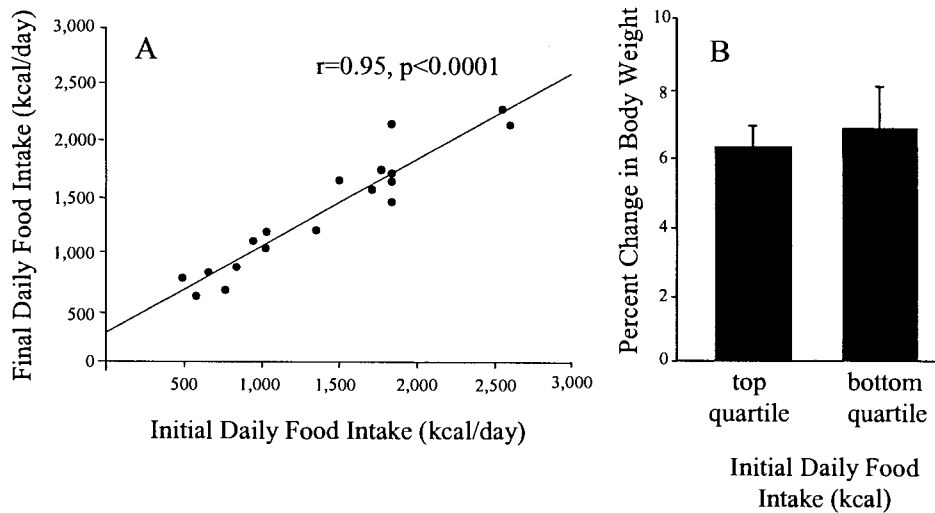
Figure 4.1. (A) Food intake for individual monkeys remained stable. There was a correlation between food intake at study initiation and after 3 months (r=0.95, P<0.0001). (B) However, the quartile of monkeys eating the most food showed a similar percent change in body weight as the quartile that ate the least amount of food (t=-0.20, df=8, p=0.85).
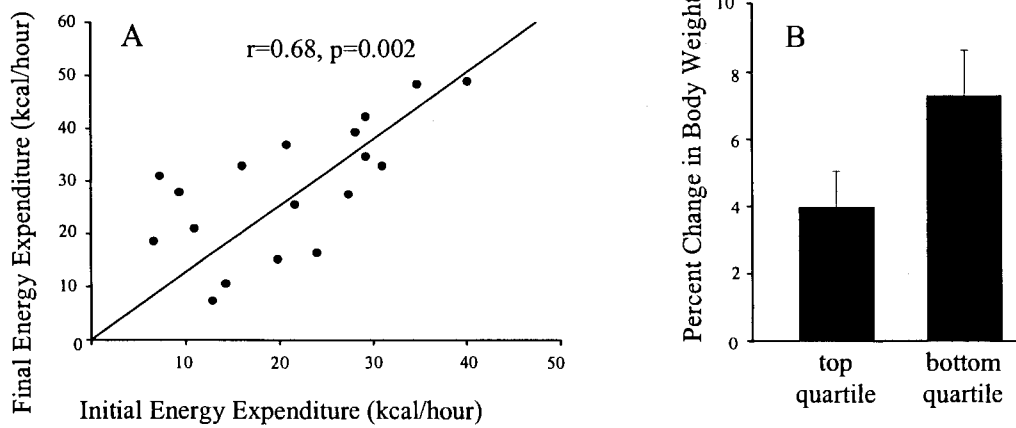
Figure 4.2. (A) Correlation between daily energy expenditure at study initiation and after

3 months (r=0.68, p=0.002). (B) The change in body weight over three months between

the monkeys in the top and bottom quartiles of energy expenditure was not significantly

different (t=2.08, df=8, p=0.07).

with the highest daily energy expenditure (4.0% weight gain), this was not a significant difference (t=2.08, df=8, p=0.07, Figure 4.2B). Once total energy expenditure was adjusted for lean body mass by regression analysis there was only a 2.6-fold difference in energy expenditure between individual monkeys. However, adjusted daily energy expenditure did not correlate with weight gain (r=-0.04, p=0.89) and there was no difference in weight gain between monkeys in the quartile with the highest adjusted daily energy expenditure compared to the quartile of monkeys with the lowest adjusted daily energy expenditure (t=0.04, df=6.1, p=0.97).

The average basal metabolic rate was 291±19 kcal/day and ranged from 172 to 406 kcal/day. On average, basal metabolic rate accounted for 61% of total daily energy expenditure, ranging from 47–83% of total energy expenditure in individual monkeys. Basal metabolic rate did not correlate with weight gain (r=0.08, p=0.75) and weight gain was not different between the quartile of monkeys with the highest basal metabolic rate and the quartile of monkeys with the lowest basal metabolic rate (t=0.40, df=8, p=0.70). Additionally, basal metabolic rate adjusted for lean body mass using regression analysis was not correlated with weight gain (r=-0.04, p=0.89) and there was no difference in weight gain between the quartile that had the highest adjusted basal metabolic rate and the quartile with the lowest adjusted basal metabolic rate (t=0.04, df=6.1, p=0.97).

The mean thermic effect of a 108 calorie meal was 19.9 ± 3.2 kcal and ranged from 8.5–59.3 kcal (a 7-fold difference) between individual monkeys. There was no significant difference in the weight gain in the monkeys with the highest thermic effect of the meal and the monkeys with the lowest thermic effect of the meal (t=-1.81, df=8, p=0.11).

There was an 8-fold difference in activity between the most active and most sedentary monkey (Figure 4.3), with the most sedentary monkey displaying a mean of 92,110 ± 7,873 activity counts per day and the most active monkey displaying 770,446±110,476 activity counts per day. The number of activity counts per day did not change significantly during the three-month period (t=1.15, df=15, p=0.27; Table 1) and each monkey's daily activity level (counts/day) was consistent over time, such that the number of activity counts per day initially recorded for each monkey was highly correlated with the number of activity counts per day recorded after three months (r=0.79, p<0.0001, Figure 4.4A). There was a significant correlation between the number of daily activity counts and weight gain such that the most active monkeys gained less weight than the least active monkeys (r=-0.52, p=0.04). The quartile of monkeys that were most active gained significantly less weight during the three month period than the quartile of monkeys that were least active (t=-2.7, df=8, p=0.03, Figure 4.4B). To follow up this initial finding we measured activity over an additional 6 months and found that the number of activity counts per day remained stable ($F_{1,16}$=1.13, p=0.30), and that the number of activity counts initially recorded for each monkey was highly correlated with the number of activity counts recorded for that monkey after nine months (r=0.85, p<0.0001). There was a 10-fold difference in the number of activity counts recorded during the day between individual monkeys and 96% of total daily activity occurred during daylight hours. Interestingly, although nighttime activity accounted for only 4%
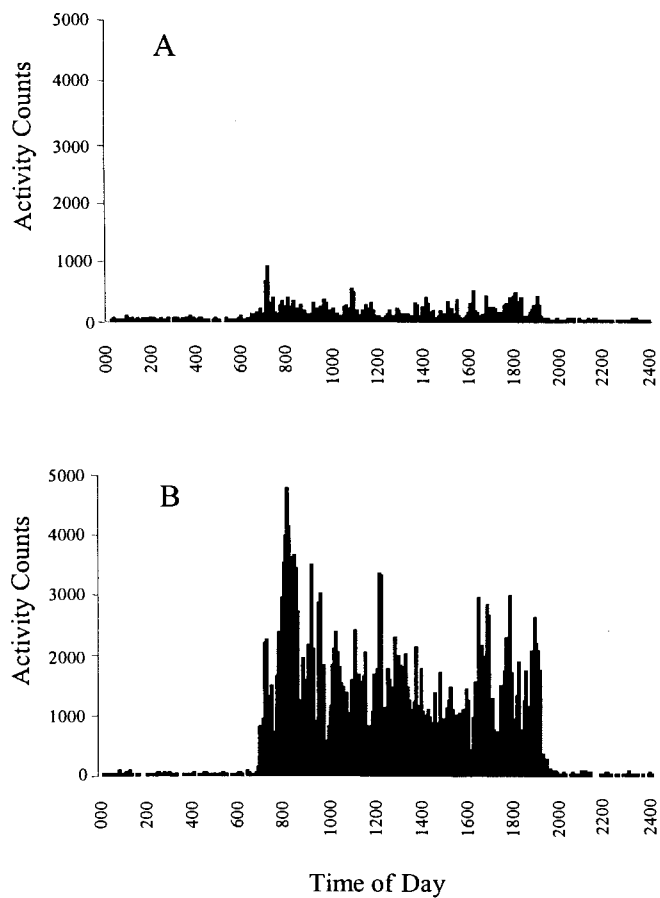
Figure 4.3 The most sedentary monkey (A) was 8 times less active than the most active

monkey (B).
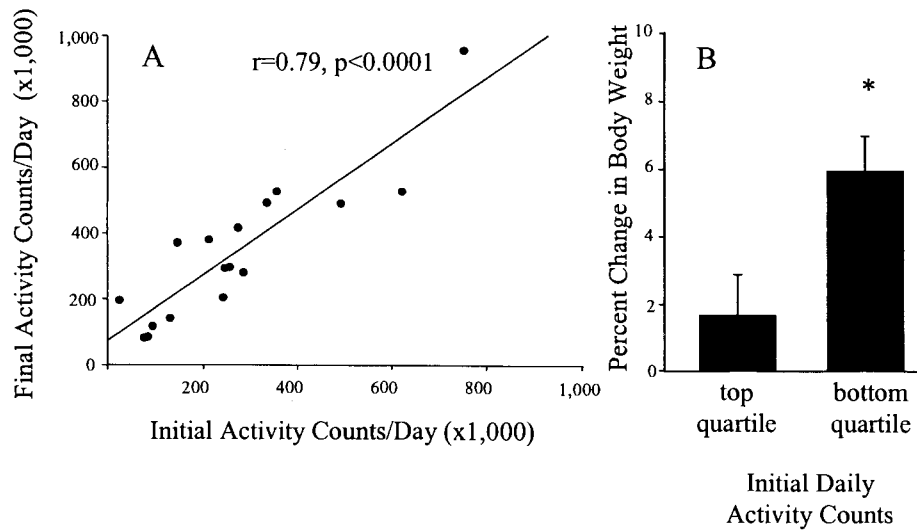
Figure 4.4 (A) There was a significant correlation between physical activity at study initiation and after 3 months (r=0.79, p<0.0001). (B) The quartile of monkeys that had the lowest physical activity had significantly greater weight gain than the quartile of monkeys that had the highest physical activity (t=-2.7, df=8, p=0.03). The asterisk indicates a significant difference in percent change in body weight between groups.

of total daily activity there was also a 10-fold difference in nighttime activity. Nighttime activity was positively correlated with daytime activity such that the monkeys that were the most active during the day were also the most active at night (r=0.57, p=0.02; Figure 4.5).

Activity counts correlated strongly with activity-associated energy expenditure (AEE) (adjusted for body mass) during both the time periods from 1400-1500 h (r=0.80, p<0.0001; Figure 4.6) and 1800-1900 h (r=0.74, p=0.001; data not shown). The regression equation for calculation of activity-associated energy expenditure (AEE) was similar at both times of day [2-3 PM: AEE = (number of activity counts x 0.000025) + 0.71; 6-7 PM AEE = (number of activity counts x 0.000021) + 0.54]. On average, 0.045 ± 0.006 kcal were expended per kilogram of body weight per 1,000 activity counts. Average AEE was 109 ± 14 kcal/day and ranged from 24 to 206 kcal/day. On average, 18 ± 3% of total energy was expended by physical activity, with physical activity accounting for 8 – 43% of total daily energy expenditure in individual monkeys. The quartile of monkeys that expended the most calories due to activity gained significantly less weight than the quartile of monkeys that expended the least amount of energy due to activity (t=-2.85, df=4.6, p=0.04).

Further analysis of the activity data revealed that there was an inverse correlation between the number of daily activity counts and the number of minutes that the monkeys were completely inactive such that the least active monkeys were inactive more than the most active monkeys (r=-0.51, p=0.046). However, there was no correlation between the number of minutes that the monkeys were inactive and weight gain (r=0.39, p=0.13).
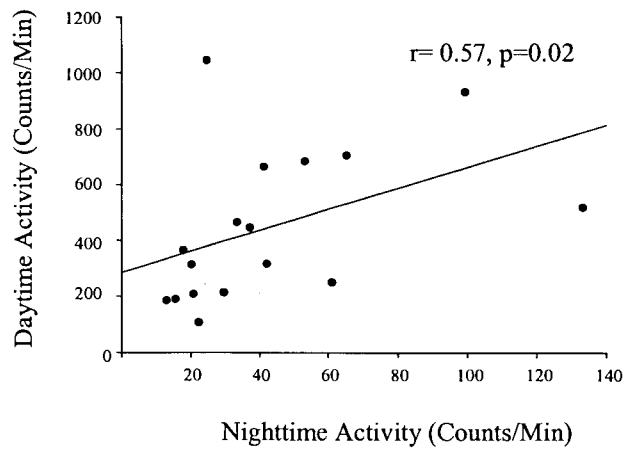
Figure 4.5 Nighttime activity was significantly correlated with daytime activity ($r=0.57$, $p=0.02$).
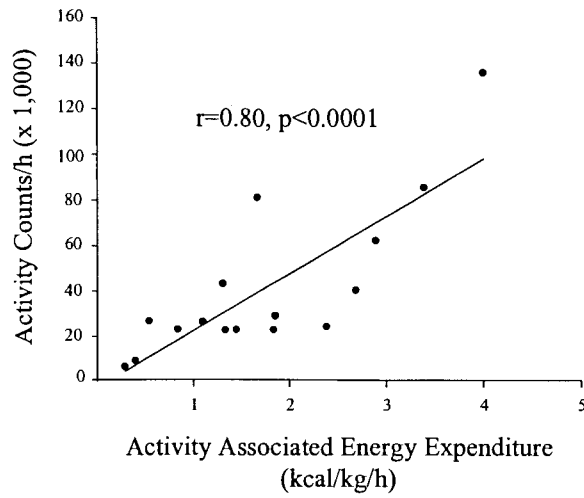
Figure 4.6 Correlation between activity counts and activity-associated energy

expenditure measured simultaneously from 1400-1500 h (r=0.80, p <0.0001).

4.4 Discussion

In this study we objectively measured individual monkey's levels of physical activity as well as other components of energy balance (caloric intake, metabolic rate) over a period of weight gain in adulthood when monkeys were eating a stable diet. There was a slow but significant increase in body weight (5.5%), during the experimental period. However, calorie intake and physical activity level remained stable during this period. The amount of weight gained was predicted by physical activity level, such that the most active monkeys gained significantly less weight than the least active monkeys. This finding shows a very strong relationship between an individual's physical activity level and its tendency to gain weight, and suggests that physical activity is an important determinant of body weight gain in adulthood. Activity level differed 8-fold between monkeys but the activity level of an individual was remarkably consistent throughout the 9-month experimental period, suggesting that activity level is an intrinsic property of an individual.

In this study, the most active individuals were less likely to gain weight than the most sedentary individuals during a period of stable dietary intake in adulthood. This finding supports the epidemiologic data showing that low levels of physical activity predict greater increases in body weight and body fat, and high levels of physical activity prevent or limit weight and fat gain (Rissanen et al. 1991; Williamson et al. 1993; Kyle et al. 2001; Di Pietro et al. 2004; Sternfeld et al. 2004; Brown et al. 2005; Hunter and Byrne 2005; Littman et al. 2005). Most of these reports relied on self-report of physical activity. However, Levine et al. (Levine et al. 2005) directly assessed posture allocation in 10 obese and 10 lean individuals and found that obese individuals were seated

significantly more than lean individuals and that lean individuals were standing more than obese individuals. Our findings also support findings in mice reporting that activity level is negatively correlated with weight gain (Dunnington et al. 1977; Brownlow et al. 1996). Our study represents the first direct measure of activity during a period of adult weight gain in a primate species and strongly indicates that activity is an important factor contributing to adult weight gain.

Our findings are also supported by studies that use accelerometry to objectively monitor physical activity and find that obese individuals have lower activity levels than individuals of normal weight (Ekelund et al. 2002; Janz et al. 2002; Trost et al. 2003; Abbott and Davies 2004; Ekelund et al. 2004; Treuth et al. 2004; Sternfeld et al. 2005), and by studies in rodents showing that obese mice have lower activity levels than their lean counterparts (Clark and Gay 1972; Levin 1991). However, it has been unclear from these studies whether low activity is a cause or consequence of obesity. Our findings suggest that individual differences in physical activity levels play an important causal role in adult weight gain.

Interestingly, we found large individual differences in nighttime activity (10–fold). This finding is supported by Higley and colleagues (Mehlman et al. 2000) who also found a 10-fold range in the duration of nighttime activity in free ranging male rhesus monkeys. This study also showed that nighttime activity was correlated with daytime activity suggesting that the mechanisms controlling activity at these two time periods are similar. A positive correlation between daytime and nighttime activity is supported by studies in children with attention-deficit hyperactivity disorder that show that these children are more active than controls during both the day and the night

(Cohen-Zion and Ancoli-Israel 2004). The mechanisms that regulate physical activity are currently not well understood. However, studies have shown that peptides that regulate food intake, such as leptin (Pelleymounter et al. 1995; Nagy et al. 1997; Salbe et al. 1997; Rowland 1998), ghrelin (Castaneda et al. 2005; Matsuda et al. 2005), pancreatic polypeptide (Lassmann et al. 1980; Uhe et al. 1992; Nakajima et al. 1994), cholecystokinin (Sei et al. 1999), and the neurotransmitters serotonin (Borer et al. 1988; Heisler et al. 1999; Nguyen et al. 1999; Nonogaki et al. 2003), glutamate (Donzanti and Uretsky 1983; Swanson and Kalivas 2000), dopamine (Fallon and Moore 1978; Zhou and Palmiter 1995; Swanson et al. 1997; Rowland 1998), norepinephrine (Rowland 1998), nitric oxide synthase (Dzoljic et al. 1997; Khedara et al. 1999) and B-endorphin (Hill et al. 2002), and the hormone estrogen (Wade 1972; Thorburn and Proietto 2000), all play potential roles in the regulation of physical activity. Additionally, several brain regions have been implicated in the regulation of activity, predominantly the reticular activating formation (Rowland 1998). Additionally, lesion studies have shown that areas of the basal forebrain, ventromedial hypothalamus, paraventricular nucleus, amygdala, and thalamus all play possible roles in activity regulation (Rowland 1998). The mechanisms that regulate nighttime activity have received much less attention. However, two recent studies begin to address this issue. Orexin A injection in the paraventricular nucleus increases activity during both the day and night in rats suggesting that orexin A may play a role in regulating both daytime and nighttime activity (Kiwaki et al. 2004). Also, CNS serotonin turnover has been shown to be inversely correlated with nighttime activity in male rhesus monkeys (Mehlman et al. 2000).

Activity associated energy expenditure was associated with weight gain, and monkeys that expended the most energy by being physically active gained significantly less weight than monkeys that expended the least energy by being physically active. On average, $18 \pm 3\%$ of total energy was expended by physical activity, however, individual differences in the percentage of total energy expenditure due to activity ranged from 8 to 43% of total energy expenditure. This is similar to findings in humans showing that the amount of total energy expenditure due to activity averages 30% (Weinsier et al. 1998), and ranges from 21 to 51% (Levine et al. 1999).

Individual differences in the number of calories consumed per day were great and ranged from 411–2210 kcal/day. The number of calories consumed by each monkey remained stable during the experimental period; however, food intake was not predictive of weight gain. This parallels previous studies in humans that failed to find an association between individual caloric intake and individual weight gain or body fat gain (Johnson et al. 1956; Stefanik et al. 1959; Maxfield and Konishi 1966; Ries 1973; Yearick 1978; Matter et al. 1980; Birkbeck 1981; Baecke et al. 1983; Bellisle et al. 1988; Guillaume et al. 1998; Lorenzo et al. 2003). This frequent failure to find an association between weight gain and caloric intake may reflect the large role that individual differences in activity level play in regulating body weight. Interestingly, in our study the number of calories excreted per gram of stool was correlated with energy intake such that the individuals with the highest caloric intake excreted twice as many calories per gram of stool. The fact that individuals absorb fewer calories when they consume more calories is well documented in the animal literature (Brody 1945; Blaxter 1989; Scotellaro et al. 1991) but is not generally considered in human studies. Individual

differences in energy absorption may contribute to the lack of association between caloric intake and weight gain.

Measurements of energy expenditure in this study were similar to what has been previously reported in rhesus monkeys (Lane et al. 1996; Blanc et al. 2003). Daily energy expenditure increased over the period of weight gain and was correlated with change in body weight, such that the monkeys that gained the most weight increased their energy expenditure the most. The finding that energy expenditure increases with increased body weight has been documented in studies with humans (Bandini et al. 1989; Nelson et al. 1992; Goran et al. 1995; Carpenter et al. 1999; Rosenbaum et al. 2003) and so it is not surprising that energy expenditure would increase as the volume of metabolically active tissue increased. Although there was a change in energy expenditure, the initial energy expenditure of each individual monkey was correlated with that monkey's final energy expenditure. However, we found that the weight gain of monkeys with the highest energy expenditure did not differ from the weight gain of the monkeys with the lowest energy expenditure. Additionally, when energy expenditure was normalized for lean body mass using regression analysis there was still no difference in weight gain between the monkeys with the highest adjusted energy expenditure and the lowest adjusted energy expenditure. We note that in no monkey did energy balance (intake – expenditure) equal zero. Energy that was excreted in the stool and thus not absorbed would account in part for this discrepancy. This has been reported to be 6.3% in rhesus monkeys (Lane et al. 1995). Energy excreted in urine, and energy in skin cells, hair and nails were also not accounted for.

We determined that the average basal metabolic rate accounted for 61% of total daily energy expenditure and ranged from 47–83% of total energy expenditure in individual monkeys. Similarly, basal metabolic rate of humans accounts for 60% of total energy expenditure (Nelson et al. 1992; Weinsier et al. 1998), ranging from 22-83% (Black et al. 1996; Levine et al. 1999). Basal metabolic rate also did not predict weight gain in this study. Additionally, when basal metabolic rate was adjusted for lean body mass by regression analysis there was still no difference in weight gain between the monkeys with the highest adjusted basal metabolic rate and the monkeys with the lowest adjusted basal metabolic rate. These findings are supported by previous studies in both humans and mice, showing that individuals with a low metabolic rate are not more susceptible to weight gain than individuals with a high metabolic rate (Seidell et al. 1992; Hambly et al. 2005). However, there are certain populations in which low metabolic rate predicts weight gain. For example, in Pima Indians, low metabolic rate is a risk factor for weight gain (Tataranni et al. 2003). Additionally, children with stunted growth because of poor nutrition (Grillol et al. 2005) and children with Downs Syndrome (Luke et al. 1994) have a lower basal metabolic rate and are more prone to obesity and weight gain than individuals in a control population. Basal metabolic rate accounts for the largest proportion of energy expenditure, however, the lack of relationship between low basal metabolic rate and weight gain suggests that differences in basal metabolic rate within the normal range are not as likely to underlie weight gain as differences in activity.

Ovariectomy (surgical menopause) is associated with changes in energy balance. We have previously shown that ovariectomy is associated with a rapid increase in caloric intake (29%) and weight (about 3%) in female rhesus monkeys (Sullivan et al. 2005). In

addition, it is well documented in small animals (mice, rats, cats; (Fettman et al. 1997; Chu et al. 1999; Ainslie et al. 2001; Geary et al. 2001) that ovariectomy leads to a 14–21% increase in weight within several weeks of ovariectomy. Also, it is well documented that along with weight gain, an increase in body mass index (BMI) and an increase in adiposity occur during the menopausal transition in women (Williamson et al. 1990; Haffner et al. 1991; Ley et al. 1992; Kotani et al. 1994; Lovejoy 1998; Tchernof and Poehlman 1998; Chang et al. 2000). Thus, it is important to note that the monkeys in this study were ovariectomized. It is possible that the relationships between energy balance parameters are different in the ovariectomized versus the ovary-intact state. Thus caution should be used in extending the findings we report here to all weight gain over adulthood. Future studies are needed to objectively measure activity in gonad-intact females and males over periods of adult weight gain.

In conclusion, this study shows that physical activity level is the best predictor of weight gain in adulthood, in ovariectomized female monkeys consuming a diet typical of that consumed in the Western world. This finding suggests that the best way to prevent weight gain over adulthood is to focus on living an active lifestyle, as opposed to only dieting. However, even though a high percentage of adults in Western countries are overweight and/or obese, there is little evidence that people are routinely opting for a more active lifestyle. More than 60% of American do not participate in the recommended amount of physical activity and 25% are inactive (Lee et al. 2000; Oguma et al. 2002). Although physicians routinely advocate that obese patients adopt a more active lifestyle, the results of this study suggest that this strategy deserves greater emphasis.

Chapter 5

INDIVIDUAL DIFFERENCES IN DIET-INDUCED WEIGHT LOSS IN ADULT

FEMALE RHESUS MONKEYS (*MACACA MULATTA*) ARE NOT RELATED TO

DIFFERENCES IN THE COMPENSATORY DECREASES IN METABOLIC

RATE OR ACTIVITY

5.1 INTRODUCTION

Moderate weight loss in obese and overweight individuals is associated with

significant health benefits, including a reduction in the risk of heart disease (Krauss et al.

2000), stroke (Krauss et al. 2000), type 2 diabetes (Lean et al. 1990; Tuomilehto et al.

2001; Knowler et al. 2002), hypertension (Eliahou et al. 1981; Oberman et al. 1990),

hyperlipidemia (Giovannini et al. 1990; Stern et al. 1995), hypercholesterolemia

(Goldstein 1992; Stern et al. 1995), cardiovascular disease (Goldstein 1992; Stern et al.

1995), osteoarthritis (Stern et al. 1995), and depression (Goldstein 1992; Stern et al.

1995). Thus, it is not surprising that at any time two-thirds of obese North American

adults are trying to lose weight (Serdula et al. 1999; Strychar 2006). Currently, American

consumers spend $33 billion annually on weight loss products and services (Cleland et al.

1997). Despite these efforts, the prevalence of overweight or obese adults has escalated

over the past several decades, such that 65% of adults in the United States have a Body

Mass Index (BMI) above the healthy range (Flegal et al. 2002). In part this is due to the

fact that many individuals attempting to loose weight fail to do so (Stunkard and

McLaren-Hume 1959; Stern et al. 1995). Dieting is currently the most common strategy

used to promote weight loss (Williamson et al. 1992; DiPietro et al. 1993; Horm and Anderson 1993; Keesey and Hirvonen 1997; Lappalainen et al. 1999). However, the success rates for diet-induced weight loss are very low, ranging from 2 to 20% (Wing and Hill 2001). Not surprisingly, there is significant interest in identifying factors that optimize diet-induced weight loss, and developing a clearer understanding of what factors impede diet-induced weight loss (Foreyt and Goodrick 1991).

Although, the majority of dieters are not able to successfully lose weight, there appears to be a subset of individuals who do lose weight when they diet (Keim et al. 1991; Van Gaal et al. 1992; Wadden 1993; Hainer et al. 2000; Fairfield et al. 2002). It would seem that much could be learned from identifying factors that differ between those who lose weight when dieting, and those who do not. A number of epidemiologic studies have looked to see if baseline characteristics of an individual affect subsequent weight loss (Stein et al. 1981; Wadden et al. 1992; Jeffery et al. 1998; Kiernan et al. 1998; Hoie and Bruusgaard 1999; Ogden 2000; Cuntz et al. 2001; Teixeira et al. 2005; Vogels et al. 2005; Wing and Phelan 2005). While some studies indicate that initial body weight predicts diet-induced weight loss (Garrow et al. 1978; Stein et al. 1981; Wadden and Stunkard 1986; Wadden et al. 1992; Hoie and Bruusgaard 1995; Foster et al. 1999; Packianathan et al. 2005), such that heavier individuals lose the most weight, others studies find no correlation between weight loss and initial body weight (Kreitzman et al. 1992; Karlsson et al. 1994; Astrup et al. 1995). It seems reasonable that both the food intake and the activity of an individual would influence their propensity to lose weight. However, the majority of studies have not measured food intake or physical activity, but rather have relied upon self-report of these measures (Hoie and Bruusgaard 1999; Ogden

2000; Vogels et al. 2005; Wing and Phelan 2005), and several studies have shown that self-reporting of food intake (Champagne et al. 1998; DeLany et al. 2002) and activity (Klesges et al. 1990; Matthews and Freedson 1995; Melanson and Freedson 1995; Treuth et al. 2004) can be very inaccurate. To more closely examine the predictive strength of initial body weight, food intake and activity in determining diet-induced weight loss we directly measured these variables in a group of 18 female rhesus monkeys while they ate a prescribed diet for a two–month period. We also measured metabolic rate throughout the 24-hour day to accurately assess individual differences in total metabolic rate, basal metabolic rate (BMR) and activity-associated energy expenditure.

Alternatively, it is possible that individual differences in the compensatory decrease in energy expenditure triggered by decreasing energy intake may underlie individual differences in diet-induced weight loss. Dieting leads to a compensatory decrease in energy expenditure in humans (Dauncey 1980; Bessard et al. 1983; de Boer et al. 1986; Henry et al. 1988; de Groot et al. 1989; Heyman et al. 1992; Leibel et al. 1995; Heilbronn et al. 2006), nonhuman primates (Lane et al. 1996), and rodents (McCarter and McGee 1989; Duffy et al. 1990; Dulloo and Calokatisa 1991; Even and Nicolaidis 1993; Levin and Keesey 1998). Much of the decrease in energy expenditure results from a decrease in basal metabolic rate [BMR (Webb and Abrams 1983; de Boer et al. 1986; Hill et al. 1987; de Groot et al. 1989; Garrow and Webster 1989; Foster et al. 1990; Fricker et al. 1991; Leibel et al. 1995; Ramsey et al. 1997; Dulloo and Jacquet 1998; Hainer et al. 2001; Friedlander et al. 2005; Heilbronn et al. 2006)]. Interestingly, there are large individual differences in diet-induced compensatory decreases in BMR (Leibel et al. 1995; Dulloo and Jacquet 1998; Hainer et al. 2001). However, the majority of

studies suggest that the magnitude of the decrease in BMR is dependent on the amount of weight lost (Heshka et al. 1990; Fricker et al. 1991; Van Gaal et al. 1992; Froidevaux et al. 1993; Leibel et al. 1995; Dulloo and Jacquet 1998; Foster et al. 1999; Blanc et al. 2003; Mueller-Cunningham et al. 2003; Barnard et al. 2005). This suggests that individual differences in diet-induced decreases in BMR reflect differences in the amount of weight lost rather than contributing to individual differences in weight loss. The decrease in energy expenditure that occurs during dieting also appears to be associated with a decrease in activity. Activity has been reported to decline during dieting in humans (Keys et al. 1950; de Groot et al. 1989; Leibel et al. 1995) and in nonhuman primates (Rana and Mehta 1991; Kemnitz et al. 1993). To more closely examine the issue of whether individual differences in diet-induced weight loss result, at least in part, from individual differences in the body's compensatory responses to dieting, we monitored body weight, metabolic rate, and activity throughout the two month period of dieting in this study.

5.2 MATERIALS AND METHODS

Animals

Eighteen adult female rhesus monkeys (*Macaca mulatta*), 9-13 years of age, were used in this study. They lived in individual stainless steel cages in a temperature-controlled room (24 ± 2 C), with lights on between 0700 and 1900 h. Two and a half years prior to the initiation of this study, these monkeys were ovariectomized and placed on a high fat diet (35% of calories from fat) to approximate the conditions experienced by many post-menopausal women in the Western world (Williams et al. 2003). The high fat diet was formulated according to a recipe developed by Clarkson and colleagues and was

designed to study diet-induced atherosclerosis (Shadoan et al. 2003; Williams et al. 2003). At the beginning of this study, monkeys continued to receive the high fat diet *ab libitum* during a 1-month baseline data collection period. After the baseline period monkeys were placed on a low fat diet (5% fat) which involved switching their food back to the standard feeding regimen at ONPRC in which they received high protein monkey chow biscuits (no. 5047, jumbo biscuits, Ralston Purina Co., St. Louis). During the first month of receiving the monkey chow diet the calories available at each meal were reduced by 30% of each animal's *ad libitum* calorie consumption during the baseline period. During the second month of the diet, the calories available were further reduced by 60% of each animal's *ad libitum* calorie consumption during the baseline period. Throughout the study, meals were provided at 0915 and 1515 h. All aspects of the study were reviewed and approved by the ONPRC Animal Care and Use Committee.

Experimental Design

The overall goal of this study was to identify individual differences in weight loss during a standardized diet, and determine whether weight loss was significantly influenced by initial characteristics of an individual or by their level of compensation to the decrease in calorie intake. The study tested two hypotheses: (1) that differences in the initial physiologic state of an individual would lead to individual differences in diet-induced weight loss, and/or (2) that differences in the compensatory response of an individual to dieting leads to differences in diet-induced weight loss. During the baseline period (1 month), initial measurements of body weight, BMI, food intake, activity, total energy expenditure, BMR, the thermic effect of food and activity associated energy

expenditure were made. Monkeys were subsequently placed on a diet for two months and a second measurement of BMI, total energy expenditure, basal metabolic rate, the thermic effect of food and activity associated energy expenditure was made at the end of the diet period. Throughout the study, food intake was measured at every meal, body weight was measured weekly, and activity was measured continuously via accelerometry.

Experimental Measures

Food Intake: Total food consumption at each meal was recorded daily throughout the study, by counting the amount of food remaining prior to the next meal.

Body weight: Body weight measurements were made weekly prior to consumption of the AM meal, at approximately 0800 h.

BMI: BMI was calculated as body weight (kg) divided by the square of crown-rump length (m^2) as previously described (see Chapter 2).

Metabolic Rate: The metabolic rate of each monkey was measured by placing the monkey in a sealed Lexan metabolic chamber (Columbus Instruments, Columbus, OH) and measuring the amount of carbon dioxide produced and oxygen consumed using a computer-controlled indirect open circuit calorimeter (Oxymax System, Columbus Instruments, Columbus, OH) and previously described methods (see Chapter 3). The twenty-four hour metabolic rate of each monkey was assessed during the baseline period, when monkeys were consuming high fat diet, and after two months of dieting. To determine total daily energy expenditure, monkeys were placed in the metabolic chamber at 1000 h and remained in the chamber until 0900 h the next morning. Before placement in the chamber monkeys were fed their standard meal at 0915 h. They were then fed a

banana (114 g ± 10g, 108 calories) at 1515 h while in the chamber. Water was available *ad libitum* through out metabolic testing. BMR activity associated energy expenditure and the thermic effect of food were calculated as previously described (Chapter 3).

Activity: Activity was measured continuously throughout the experiment using triaxial Actical accelerometers (MiniMitter, Bend, OR) and previously described methods (see Chapter 3).

Statistical Analyses

For all analyses, normality and homoscedacity were initially tested. If these criteria were met, a repeated measures ANOVA was utilized to look at differences in variables overtime. The assumption of sphericity was examined with Mauchly's Test. The Greenhouse-Geiser correction factor was used in cases where the assumption of sphericity was violated. Least Significant Difference post-hoc tests were used to determine time periods that were significantly different from each other. If the variables were measured twice then a paired t-test was used to look for differences in the variable before and after dieting. Correlations were determined using a Pearson product moment correlation. If data was not normally distributed, and could not be normalized by transformation (using a square root or log transformation) then nonparametric tests were utilized. To look for differences in non-normally distributed data over time the Friedman test was used, followed by the Wilcoxon Signed Ranks test. If variables were measured twice, then a Wilcoxon Signed Ranks test was utilized. A Spearman's rho correlation was used to analyze relationships between parameters that were not normally distributed. Data are presented as mean ± SEM. Alpha values are considered significant with $p \leq$

0.05. All statistical analyses were conducted using the SPSS software package, version 13.0 (SPSS Inc., Chicago, Illinois).

5.3 RESULTS

During the first month of dieting we fed monkeys 30% fewer calories than they were eating *ad libitum* during the baseline period. Also their diet was switched from a highly palatable high fat diet (35% fat) to low fat monkey chow (5% fat). During the first month of dieting some monkeys ate fewer calories than were provided so the actual decrease in calorie intake during the first month of dieting was 44±2.6% of calorie intake during the baseline period. At the beginning of the second month of dieting, calorie intake was further reduced so that monkeys were fed 60% fewer calories than they were eating during the baseline period. Again, some monkeys ate less food than was provided so that the actual reduction in calorie intake was 68±0.81% of baseline calorie intake.

In response to the decrease in food intake, body weight significantly decreased over the two months of dieting ($F_{1.85,31.5}$=19.41, p<0.0001; Figure 5.1). During the first six weeks of dieting body weight was not significantly different from weight during high fat diet consumption. However, by the seventh (p=0.003) and eighth (p<0.0001) weeks of dieting body weight was significantly lower. On average, monkeys lost 6.4 ± 1.7% of their initial weight over the two months of dieting. However, there were large individual differences in weight loss, ranging from a 22% decrease in body weight to a 4.5% gain in body weight. Mean body mass index (BMI) decreased from 32.2 ± 1.9 kg/m^2 to 30.0 ± 1.6 kg/m^2 after 2 months of dieting (t=3.9, df=17, p=0.001).
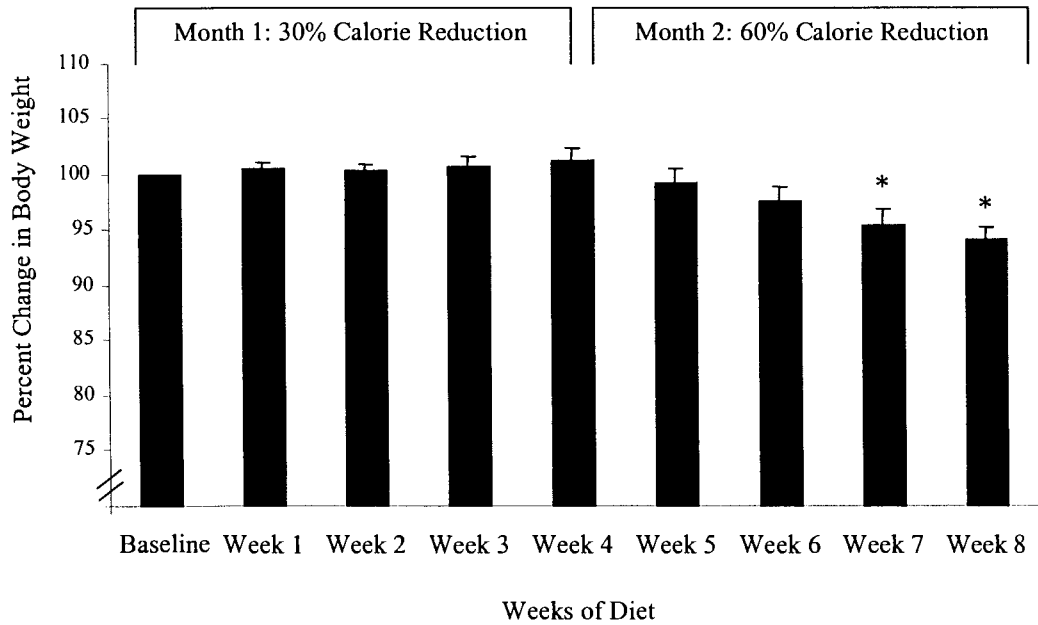
Figure 5.1 Body weight significantly decreased over the 2 months of dieting

$(F_{1.85,31.5}=19.41, p<0.0001)$. Body weight was significantly lower than baseline during

the seventh (p=0.003) and eighth (p<0.0001) weeks of dieting. Asterisks indicate a

significant difference from baseline measures.

The amount of weight lost was correlated with initial body weight ($r=0.59$, $p=0.01$) and initial BMI ($r=0.62$, $p=0.01$), such that the heavier, fatter monkeys lost the most body weight. Percent weight loss over the two month diet was negatively correlated with initial energy balance, such that the monkeys with the greatest energy balance (i.e. the monkeys that routinely ate more calories than they expended) prior to dieting lost the least amount of weight on the diet ($r=-0.70$, $p=0.001$; Figure 5.2A). Weight loss was also negatively correlated with initial food intake with monkeys that ate the least initially losing the most weight ($r=-0.66$, $p=0.003$, Figure 5.2B). There was no correlation between weight loss and initial activity ($r=0.18$, $p=0.49$), or weight loss and initial basal metabolic rate ($r=0.14$, $p=0.58$).

The two months of dieting caused a significant decrease in energy balance (caloric intake - energy expenditure; $t=8.5$, $df=16$, $p<0.0001$), with a greater decrease in energy intake than in energy expenditure. However, in response to the decrease in calories, total energy expenditure did decrease significantly (13% decrease; $t=5.3$, $df=15$, $p<0.0001$; Figure 5.3A). But total energy expenditure per kilogram of body weight did not significantly change ($t=0.82$, $df=16$, $p=0.94$). Both BMR (13% decrease, $t=5.5$, $df=15$, $p<0.0001$, Figure 5.4A) and BMR per kilogram of body weight (14% decrease, $t=5.2$, $df=15$, $p<0.0001$) significantly decreased in response to the calorie reduction. The thermic effect of an isocaloric meal did not change over the course of the experiment ($p=0.81$). Percent weight loss was not correlated with the percent decrease in total energy expenditure ($r=0.16$, $p=0.54$; Figure 5.3B). However, percent weight loss was correlated with the percent decrease in BMR ($r=0.52$, $p=0.04$; Figure 5.4B), such that the monkeys that lost the most weight decreased their BMR the most. Also, percent weight loss was
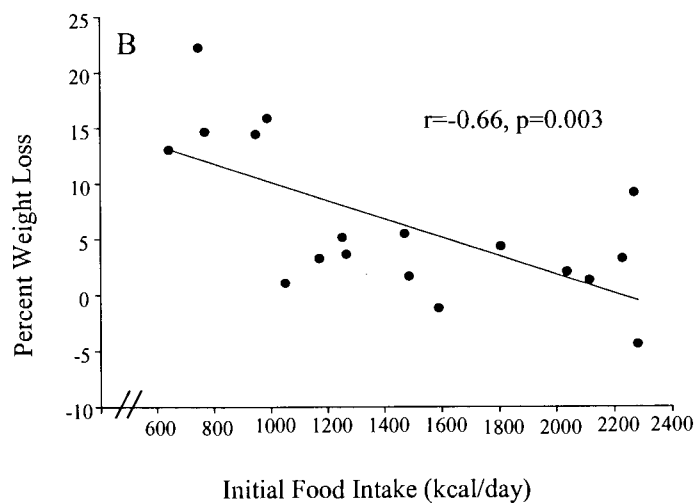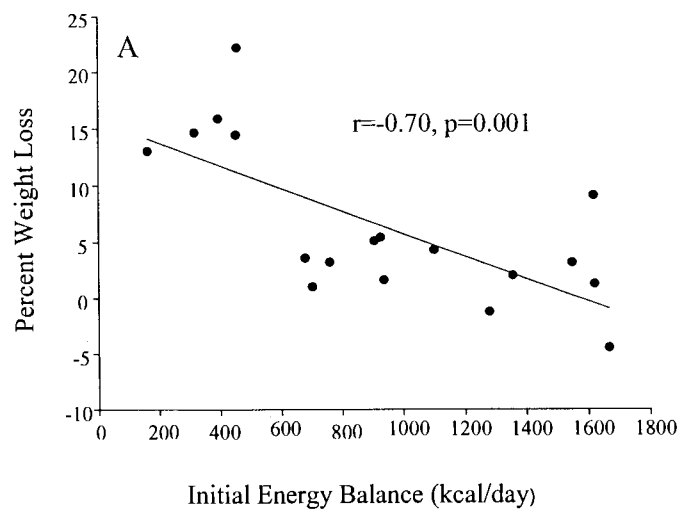
Figure 5.2 (A) Correlation between percent weight loss and initial energy balance (r=-0.70, p=0.001). (B) Correlation between percent weight loss and initial food intake (r=-0.66, p=0.003).
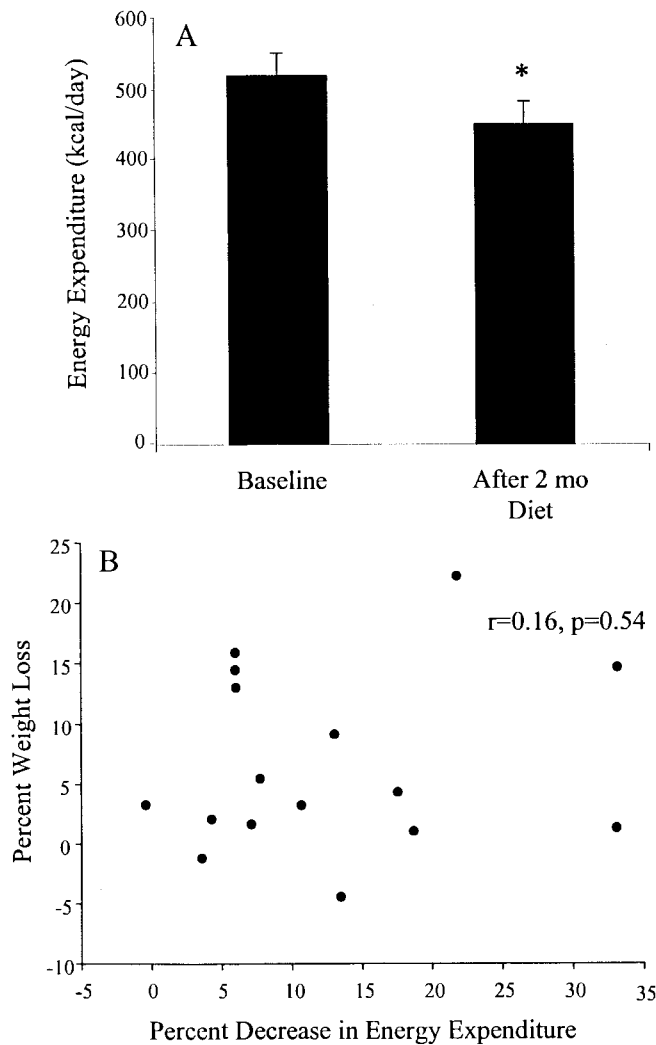
Figure 5.3 (A) Total energy expenditure decreased after 2 months of dieting (13%

decrease, t=5.3, df=15, p<0.0001). (B) Correlation between percent weight loss and the

percent decrease in total energy expenditure (r=0.16, p=0.54).
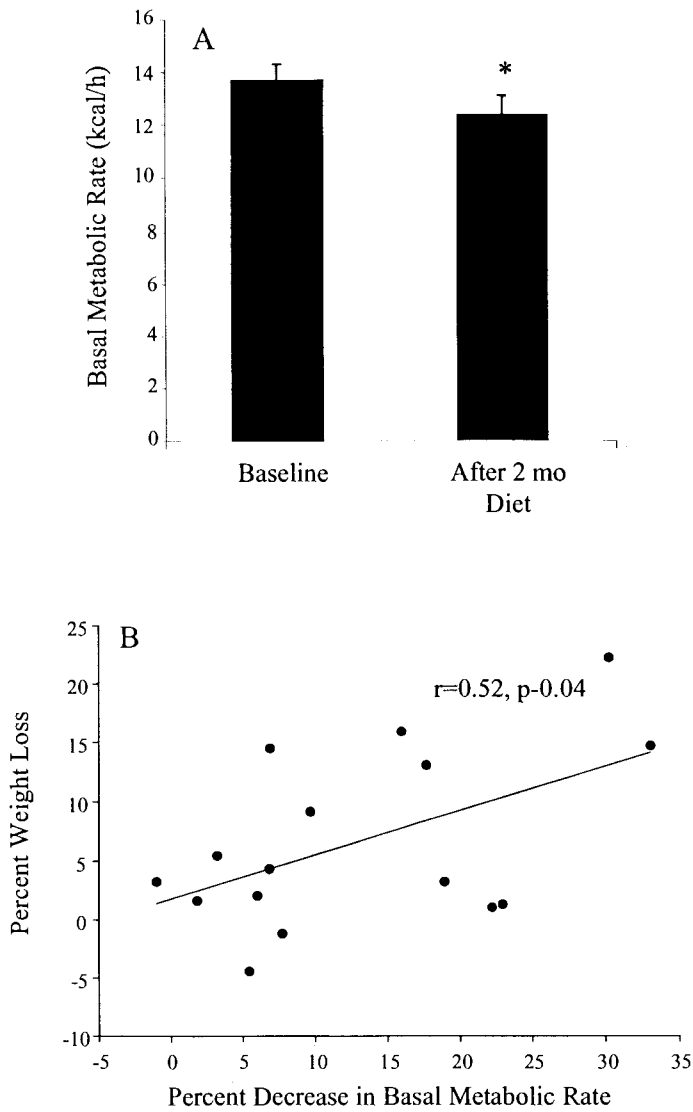
Figure 5.4 (A) Basal metabolic rate decreased after 2 months of dieting (13% decrease,

t=5.5, df=15, p<0.0001). (B) Correlation between percent weight loss and the percent

decrease in basal metabolic rate (r=0.52, p=0.04).

correlated with the percent decrease in BMR per kilogram of body weight (r=0.52, p=0.04), with the monkeys that lost the most weight having the largest percent decrease in BMR per kilogram of body weight.

Physical activity also decreased significantly over the course of the study ($F_{3.4, 47.4}$=5.13, p=0.03; Figure 5.5A). By the fourth week of dieting, activity levels were significantly decreased (18% decrease, p=0.02), and remained at a lower level than during the baseline period throughout the rest of the diet period. Activity-associated energy expenditure was also significantly lower than baseline levels after 2 months of dieting (31% decrease, t=-5.5, df=16, p<0.0001). However, weight loss was not correlated with the decrease in activity (r=0.05, p=0.84; Figure 5.5B) or the decrease in activity-associated energy expenditure (r=0.33, p=0.19).

5.4 DISCUSSION

In this study, we characterized individual differences in diet-induced weight loss, the initial weight, food intake, activity and metabolic rate of each monkey, and the decreases in activity and metabolic rate that accompanied diet-induced weight loss. We found that after two months of dieting monkeys lost a moderate amount of weight (6.4±1.7% of their initial weight). There were large differences in the percent of weight lost between individuals, ranging from one monkey that lost 22% of its initial body weight to another monkey that gained 4.5% of its initial body weight. This parallels humans studies which often report large differences in weight loss between individuals with the same degree of calorie reduction (Webb and Abrams 1983; Keim et al. 1991; Wadden 1993; Hainer et al. 2000; Hainer et al. 2001). It is noteworthy that during the
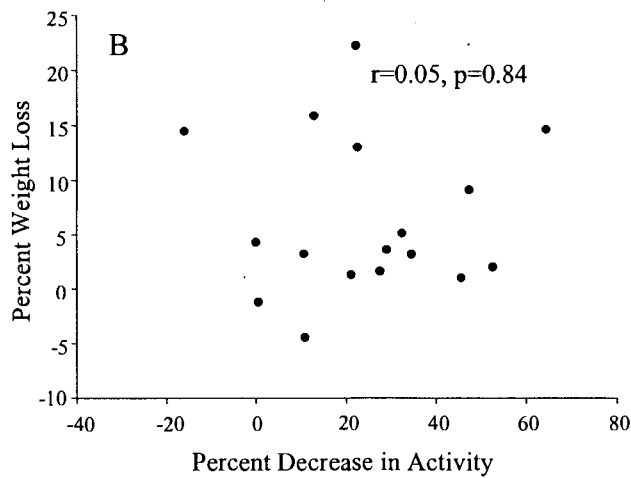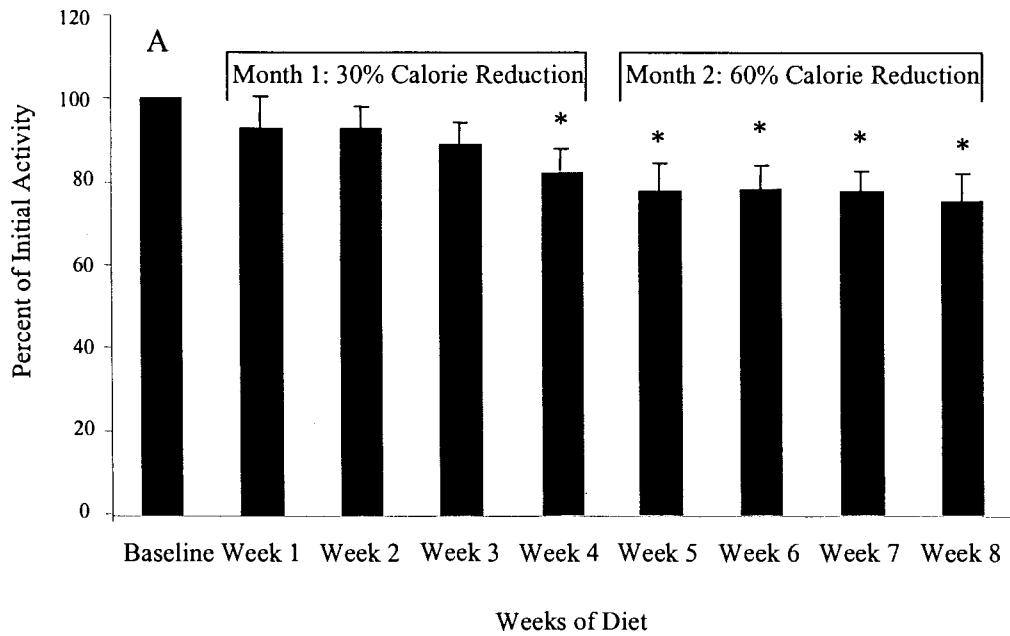
Figure 5.5 (A) Activity significantly decreased over the 2 months of dieting ($F_{3.4,}$

$_{47.4}$=5.13, p=0.03). By the fourth week of dieting, activity was significantly decreased

(18%, p=0.02) and remained lower than activity during high fat diet consumption

throughout the rest of the diet period. (B) Correlation between percent weight loss and

the percent decrease in activity (r=0.05, p=0.84).

first month of dieting the group as a whole did not significantly lose weight. Furthermore, it was surprising that after a second month of dramatic dieting average weight loss was only 6.4%. Dieting appeared to be fairly ineffective in causing weight loss, as there were rather large compensatory decreases in energy expenditure during the dieting period.

The initial characteristic that best predicted weight loss was the initial energy status of the monkeys. The monkeys that had the greatest positive energy balance (i.e. they ate more calories than they expended) were least likely to lose weight over the two months of dieting. This relationship was driven by differences in food intake, in that animals with the highest food intake were also significantly less likely to lose weight. However, neither initial activity level nor basal metabolic rate predicted how much weight an individual would lose on a diet. This finding suggests that dieting is most effective for individuals that are close to energy balance, and that dieting is least effective for individuals that most need to diet (i.e. individuals that consistently eat more calories than they expend).

Weight loss was also predicted by initial body weight and initial BMI. Studies in humans are conflicting, with some studies indicating that initial body weight predicts diet-induced weight loss (Stein et al. 1981; Wadden et al. 1992; Hoie and Bruusgaard 1995; Packianathan et al. 2005), such that heavier individuals lose the most weight on a diet. In contrast, other studies find no correlation between weight loss and initial body weight (Kreitzman et al. 1992; Karlsson et al. 1994; Astrup et al. 1995). These differences may well be related to varying durations of the dieting or varying intensity of dieting. In this study, in which monkeys experienced a diet that was strictly based on

their level of initial food intake (a decrease to 60% initial food intake followed by a dcrease to 30% initial food intake) it is perhaps not surprising that there was a strong correlation between initial body weight and BMI and the amount of weight lost.

There was a compensatory 13% decrease in daily energy expenditure in response to this diet. A decrease in energy expenditure of this magnitude has been previously reported in humans in response to a reduction in available calories (Webb and Abrams 1983; de Boer et al. 1986; de Groot et al. 1989; Leibel et al. 1995; Heilbronn et al. 2006). In this study, total energy expenditure was reduced due to decreases in both BMR and activity-associated energy expenditure, the two main components of energy expenditure. However, the thermic effect of an isocaloric meal did not change in response to dieting. Nevertheless, it is likely that the total daily thermic effect of food during the dieting period was lower than during the baseline period, as monkeys were consuming substantially fewer calories than during the baseline period. Thus it seems probable that a decrease in the daily thermic effect of food also contributed to the decrease in total daily energy expenditure.

Basal metabolic rate, which accounts for the majority (60%) of total daily energy expenditure in humans (Nelson et al. 1992; Weinsier et al. 1998) and nonhuman primates (Sullivan et al. 2006; see Chapter 4) was reduced by 13% after two months of dieting. This decrease is similar in magnitude to the decrease in BMR that has been previously reported in humans during dieting (Webb and Abrams 1983; de Boer et al. 1986; de Groot et al. 1989; Foster et al. 1990; Leibel et al. 1995; Friedlander et al. 2005; Heilbronn et al. 2006). In this study, the decrease in BMR was correlated with weight loss, which is not surprising considering the well established relationship between basal metabolic rate

and tissue mass (Ravussin et al. 1986; Ainsworth et al. 1993; Ainsworth et al. 2000), such that a decrease in BMR occurs when the mass of metabolically active tissue is decreased. Many other studies find a similar relationship between the decrease in BMR and weight loss (Heshka et al. 1990; Fricker et al. 1991; Van Gaal et al. 1992; Froidevaux et al. 1993; Leibel et al. 1995; Dulloo and Jacquet 1998; Foster et al. 1999; Blanc et al. 2003; Mueller-Cunningham et al. 2003; Barnard et al. 2005). BMR per kilogram of body weight also decreased, indicating that BMR was decreased further than would have been expected due to just the decrease in metabolically active tissue. This further decrease in BMR presumably helped compensate for the reduction in calorie intake. Evidence for a decrease in BMR beyond that expected due to the loss of tissue mass has been previously documented in humans (Bessard et al. 1983; de Boer et al. 1986; Hill et al. 1987; de Groot et al. 1989; Heshka et al. 1990; Fricker et al. 1991; Leibel et al. 1995; Valtuena et al. 1995; Dulloo and Jacquet 1998; Heilbronn et al. 2006). Furthermore, in this study the percent decrease in BMR per kilogram of body weight was correlated with percent weight loss such that the monkeys that lost the most weight decreased the amount of energy required to maintain a unit of tissue mass the most. This is likely because the individuals that lost the most weight have greater drive to maintain their remaining body weight.

The decrease in caloric intake (30% reduction) also caused a substantial decrease in physical activity level (18% decrease) after the first month of dieting, and was further decreased by 25% after the last four weeks of the diet (60% reduction). Activity-associated energy expenditure was also reduced after two months of dieting. Activity-associated energy expenditure was reduced due to both the decrease in movement (i.e.

amount and intensity of activity), and because it takes less energy to move a reduced

body weight (Ainsworth et al. 1993; Ainsworth et al. 2000). This finding supports

previous reports in rhesus monkeys (Rana and Mehta 1991; Kemnitz et al. 1993) and

humans (Keys et al. 1950; de Groot et al. 1989; Leibel et al. 1995) which have found that

a decrease in physical activity accompanies decreases in calorie intake. In contrast,

rodents show an increase in activity in response to calorie reduction (Russell et al. 1987;

Duffy et al. 1990; McCarter et al. 1997; Chen et al. 2005). It is hypothesized that rodents

increase their activity due to an increased drive to forage for food, as the elevated activity

is decreased when food is made available (Koubi et al. 1991). The differential regulation

of physical activity in response to calorie reduction has been hypothesized to be

dependent on whether an animal has sufficient stored energy to make it through a time of

famine metabolizing stored energy (and slowing activity would protect their energy

stores) or whether their stored energy is low and thus survival would be dependent on

finding food and increasing activity would facilitate foraging; (Perrigo and Bronson

1983; Perrigo and Bronson 1985). A study in emperor penguins provides further support

for this hypothesis, as the penguins decreased their activity in response to the first three

months of fasting but then once their energy stores were depleted they increased their

activity (Robin et al. 1998).

In this study, there was no association between weight loss and the compensatory

decrease in total energy expenditure, activity-associated energy expenditure or activity

level. Therefore, this study provides no evidence that the compensatory decreases in total

energy expenditure, activity-associated energy expenditure, and activity level drives

weight loss or that weight loss drives the compensatory changes. BMR was positively

associated with weight loss. However, if differences in BMR were causing differences in weight loss you would expect to see a negative association between these two measures, such that individuals who had the smallest compensatory decrease in basal metabolic rate would lose the most weight. Thus, it appears that in this study weight loss was causing the decrease in basal metabolic rate, as individuals that lost the most weight decreased their basal metabolic rate the most. A lack of association between an individual's weight loss and the compensatory decrease in energy expenditure of that individual in response to dieting has been previously documented in humans (Friedlander et al. 2005). In the current study, differences in the compensatory decreases in energy expenditure between individuals were not driving individual differences in weight loss, suggesting that differences in weight loss were primarily related to an individual's initial energy status. Individuals that had a very positive energy balance when the diet was initiated lost the least weight during the diet. These findings suggest that dieting will be fairly ineffective in promoting weight loss in an individual that overeats and that for these individuals a different weight loss strategy, such as increasing physical activity level, may be more effective.

In conclusion, two initial parameters predicted weight loss: initial food intake and body weight. Although initial energy balance also correlated with weight loss, it is likely that this relationship was driven by initial food intake, as the same individuals were driving both correlations of weight loss with initial food intake and initial energy balance. Similarly, initial BMI also correlated with weight loss but this appears to be driven by body weight as these variables were tightly related and the same individuals were

responsible for the significant correlation between initial BMI and initial body weight and weight loss.

We conclude that in general, it is difficult to promote weight loss by dieting alone, as the body compensates for a substantial reduction in calorie intake by markedly decreasing energy expenditure (both BMR and activity). However, we found no evidence for differences in the compensatory decreases in energy expenditure accounting for individual differences in weight loss. Several studies find that exercise is effective in preventing the diet-induced decrease in energy expenditure (Belko et al. 1987; Heymsfield et al. 1989; Mole et al. 1989; Frey-Hewitt et al. 1990), suggesting that combining exercise with dieting may be the best strategy for weight loss. As losing weight and then maintaining weight loss is rather difficult for most people, the findings of this study argue that increased emphasis should be placed on preventing weight gain over adulthood. Our previous studies indicate that the amount of physical activity that an individual undertakes is the best predictor of adult weight gain (Sullivan et al. 2006; see Chapter 4), suggesting that development of obesity in adulthood could be best prevented by maintaining elevated levels of physical activity in the adult years.

Chapter 6

GENERAL DISCUSSION

6.1 Factors Associated With Individual Differences In Weight Change

With the increasing prevalence of obesity in developed countries, and the accompanying obesity-related health problems, a large amount of effort has been put forth by the research community to improve our understanding of the mechanisms underlying the regulation of body weight. It is becoming increasingly important to understand the factors that make some individuals more susceptible to weight gain than others and to understand what is different about individuals who are able to effectively lose weight. Although it is well known that changes in body weight reflect the balance between energy intake and energy expenditure, it remains unclear what physiological mechanisms (i.e., differences in: food intake, basal metabolic rate, physical activity, and the thermic effect of food) contribute to the large individual differences in weight change.

The results of the studies that I have undertaken in my dissertation research suggest that the mechanisms that predict weight change differ depending on the circumstances under which weight change occurs. In some circumstances weight change is influenced primarily by food intake; however during other circumstances activity appears to play the most important role. Additionally, initial body composition often appears to play an important role in determining response to weight gain or weight loss regimens. It is important to note that both the initial physiologic state of the individual (i.e. initial weight, body fat, food intake, level of activity, basal metabolic rate), and changes in the functioning of metabolic regulatory systems over the course of weight

change, can contribute to individual differences in weight change. This dissertation examined these parameters in three circumstances that commonly cause weight change in women (menopause, slow progressive weight gain over adulthood and dieting). In the following sections I will look across the results from these various studies to see if it is possible to discern overarching principles regarding factors influencing individual differences in body weight regulation under various conditions.

6.2. The Influence of Initial Body Composition in Modulating Weight Change

Interestingly, in the two circumstances where weight changed in response to manipulation of ovarian hormones the body composition of the individual prior to the change in ovarian hormones predicted individual differences in weight change. In response to ovariectomy the leanest monkeys gained the most weight ($r=-0.68$, $p=0.004$), and when ovariectomized monkeys were given estrogen replacement therapy the fattest monkeys lost the most weight ($r=0.47$, $p=0.05$). Together these studies provide strong evidence that the amount of body fat an individual has initially plays an important role in determining the magnitude of the weight change in response to changes in ovarian hormones.

One possible mechanism underlying the dependence of the response to ovarian hormones on body fat is that individuals with more adipose tissue produce more estrogen under basal conditions, and have greater amounts of free estrogen, so they experience different degrees of change in circulating estrogen levels when the ovaries are removed or when exogenous estrogen is provided, compared to lean individuals. It is well documented that adipose tissue produces estrogen by converting androgens or inactive

127

forms of estrogen to active estrogenic compounds (Bolt and Gobel 1972; Siiteri 1987; Simpson et al. 1996; Belanger et al. 2002). After menopause, when the ovary stops producing estrogen, the concentration of circulating estrogen in women is directly proportional to their adipose tissue stores (Lukanova et al. 2004; Castracane et al. 2006). Furthermore, it is generally accepted that only the free or unbound fraction of estrogen is biologically active (Anderson 1974; Siiteri 1987). As a number of studies have shown that sex hormone-binding globulin (SHBG), a plasma protein that binds to estrogen, decreases with increasing BMI (Sulkes et al. 1984; Weaver et al. 1990; Lukanova et al. 2004), it is also likely that there is a greater percentage of circulating estrogen that is biologically active in obese individuals. Together, these findings suggest that the amount of biologically active estrogen is elevated in obese individuals not only because they produce more estrogen, but also because a greater percentage of the circulating estrogen is circulating in the unbound state. This suggests that the obese monkeys used in this dissertation had higher levels of biologically activity estrogen compared to the lean monkeys both before and after ovariectomy. Less of a decrease in circulating estrogen after ovariectomy would not surprisingly lead to less of an increase in body weight. Following similar reasoning, estrogen replacement therapy in obese monkeys would lead to higher circulating levels of bioactive estrogen compared to lean monkeys, making the obese monkeys more susceptible to estrogen-induced weight loss. Future studies could address this hypothesis by measuring the level of circulating free estradiol, estrone and SHBG after ovariectomy or after estrogen replacement therapy to determine if the level of total bioactive estrogen correlates with individual differences in weight change. As I collected blood samples from the monkeys in my dissertation studies after ovariectomy

and after estrogen replacement therapy, I plan to measure SHBG, estradiol, estrone and estriol to determine the level of bioactive estrogen and then look to see if this correlated with weight gain after ovariectomy and/or weight loss with estrogen replacement therapy. If this hypothesis were true it would have important implications for predicting how much weight an individual woman would gain over menopause and in determining which women would lose the most weight in response to hormone replacement therapy.

I also found that initial body composition was an important predictor of individual differences in diet-induced weight loss. The amount of weight lost during dieting was predicted by both initial body weight (r=0.59, p=0.01) and initial BMI (r=0.62, p=0.006), such that monkeys that weighed the most and had the highest BMI lost the most weight while dieting. Initial body composition has also been found to predict diet-induced weight loss in a number of human studies (Garrow et al. 1978; Stein et al. 1981; Wadden and Stunkard 1986; Wadden et al. 1992; Hoie and Bruusgaard 1995; Foster et al. 1999; Packianathan et al. 2005). Furthermore, several studies have documented that when obese adults lose weight, fat mass accounts for a greater proportion of the mass lost than it does in lean individuals (Dulloo et al. 1996; Forbes 1999; Forbes 2000). Both obese and lean individuals preferentially lose fat and protect lean tissue mass during weight loss (Espat et al. 1994; Dulloo and Jacquet 1998; Melchior 1998; Faintuch et al. 2000; Newman et al. 2005). Thus, it seems likely that obese and lean individuals lose lean tissue mass at the same rate but that the fat individuals lose more fat mass and thus lose more total body weight. Determining if lean and obese individuals protect their lean tissue to the same degree could test this hypothesis. One mechanism for protecting lean tissue mass, which is predominantly protein (Caster et al. 1956; Miller 1969), is the

release of growth hormone in response to metabolic stress (Manglik et al. 1998; Douyon and Schteingart 2002; Naranjo et al. 2002). Growth hormone stimulates production of IGF-1, primarily by the liver (Manglik et al. 1998), which suppresses protein degradation and enhances protein synthesis (Webb and Abrams 1983; Ward and Atkinson 1999). One could compare growth hormone and IGF-1 levels in obese versus lean subjects during calorie reduction to determine if they activate the growth hormone/IFG-1 regulatory system to the same degree in response to calorie reduction.

Initial body weight and body composition were not predictors of slow progressive weight gain, as neither initial body weight (r=-0.24, p=0.35), initial body fat (r=-0.19; p=0.45) or initial BMI (r=-0.27, p=0.28) predicted weight gain. One might conclude that changes in weight are influenced by initial body weight, if they are abrupt but not if they occur slowly over adulthood, as slow adult weight gain was the one circumstance in which initial body composition was not a predictor of weight change. Evidence that supports this hypothesis comes from a recent review which found that studies observing no association between initial body composition and weight loss were longer in duration and had more gradual weight loss than studies finding an association (Teixeira et al. 2005). This hypothesis requires further testing by setting up experiments in which the rate of weight change is manipulated, and then analyzing whether initial body weight or body fat influences weight change depending on the rate of weight change.

6.3 The Influence of Food Intake in Modulating Weight Change

There were large differences in the amount of food consumed between monkeys in each of the studies in this dissertation (a 3-5 fold difference across the various studies). Individual differences in weight change were correlated with initial food intake in some situations, or with change in food intake in other situations. Initial food intake was a strong predictor of diet-induced weight loss, with the individuals that ate the least prior to going on a diet losing the most weight. However, evidence from the other studies I undertook suggests that initial food intake is often not a strong regulator of weight gain. Initial food intake was not associated with slow progressive weight gain, weight gain due to high fat diet consumption (r=0.40, p=0.13; Appendix 1), or weight gain due to ovariectomy (r=-0.15, p=0.58). Together these studies suggest that initial food intake is an important regulator of diet-induced weight loss, but not weight gain, at least in the three weight gain circumstances studied in this dissertation. A lack of association between food intake and weight gain has been previously observed in number of human studies (Johnson et al. 1956; Stefanik et al. 1959; Maxfield and Konishi 1966; Ries 1973; Yearick 1978; Matter et al. 1980; Birkbeck 1981; Baecke et al. 1983; Bellisle et al. 1988; Guillaume et al. 1998; Lorenzo et al. 2003). The lack of association between food intake and body weight in many human studies, and in this dissertation, may be because individual differences in energy expenditure are able to override individual differences in food intake, at least during weight gain. Alternatively, data from this dissertation (see Chapter 4), and studies in other animals (Blaxter 1989) and humans (Murphy et al. 1993), have shown that there are differences in the amount of calories absorbed by the GI tract

between individuals. These differences seem to depend on the amount of food that an individual consumes, such that individuals that eat a large number of calories are less efficient at digesting these calories and thus excrete a higher percent of consumed calories in their stool and absorb a lower percentage of consumed calories than individuals who eat a small number of calories (Blaxter 1989; Noblet et al. 1994). It is possible that differences in absorption efficiency of consumed calories may be responsible for the lack of a correlation between weight gain and food intake. I began to address this hypothesis by weighing and collecting stool samples from each monkey used in my dissertation research and determining the caloric content of the stool samples from the 2 monkeys that ate the most and the 2 monkeys that ate the least. However, I only collected stool samples for a 24 hour period and I later learned that stool output is highly variable from day to day (Rendtorff and Kashgarian 1967; Wyman et al. 1978; Murphy et al. 1993), so I would have needed to collect samples for a 5 day period in order to be able to get an accurate estimation of stool output which would be needed to calculate the number of calories excreted in the stool per day for each monkey. Nevertheless, I was able to see a two-fold difference in the number of calories per gram of stool between the monkeys that ate the most and the monkeys that ate the least (see Chapter 4). Future studies could address this hypothesis by determining the total weight of stool produced over a 5 day period and then using bomb calorimetry determine the energy density of the stool for each individual and look to see if calories absorbed by an individual predict individual differences in weight change.

The change in food intake in response to changes in ovarian hormones plays a role in determining weight change, at least in some circumstances. When ovariectomized

monkeys were given estrogen replacement therapy individual differences in weight loss were associated with the decrease in food intake, such that the monkeys that lost the most weight decreased their food intake the most ($r=0.52$, $p=0.03$). However, weight gain 6 weeks after ovariectomy was not associated with the change in food intake ($r=-0.05$, $p=0.84$). It is possible that the short time frame of the ovariectomy study (i.e. 6 weeks) was not long enough to see the relationship between the change in food intake and body weight change, as there was not a correlation between the change in food intake and the change in body weight after only six weeks in the estrogen replacement therapy study ($r=0.37$, $p=0.13$). This hypothesis is supported by numerous other studies that also find that food intake and body weight increase simultaneously after ovariectomy in rodents (Grunt 1964; Hervey and Hervey 1965; Kakolewski et al. 1968; Wade and Zucker 1970; Mook et al. 1972; Leshner and Collier 1973; Tarttelin and Gorski 1973; Landau and Zucker 1976; McElroy and Wade 1987; Chu et al. 1999; Ainslie et al. 2001; Chen and Heiman 2001; Shinoda et al. 2002), and domestic animals (Fettman et al. 1997; Harper et al. 2001; Martin et al. 2001). However, unfortunately none of these studies looked for a correlation between the change in food intake and the change in body weight in individual animals. Thus, further studies could track changes in food intake and body weight for several months after ovariectomy and look for individual differences in the change in food intake and the change in body weight, and a correlation between these two parameters.

It appears that similar to the effects of initial food intake (discussed above) on weight change, the change in food intake appears to be important in predicting individual differences in weight loss but not weight gain. The change in food intake did not

correlate with weight gain due to ovariectomy (r=-0.05, p=0.84) or high fat diet feeding (r=-0.14, p=0.61). However, as I previously discussed above, the lack of correlation between food intake and weight gain after ovariectomy could be because the time frame of the study was not long enough to see the relationship. The relationship between change in food intake and change in body weight could not be looked at during slow progressive weight gain over adulthood, as food intake did not change during this study. However, the fact that the change in food intake did not correlate with the change in body weight due to high fat consumption, where there were large changes in body weight and food intake, suggests that other metabolic changes such as changes in energy expenditure may have been important determinants of individual differences in weight gain. During high fat diet-induced weight gain we did not measure any form of energy expenditure. However, this hypothesis is supported by a number of overfeeding studies in humans that show compensatory increases in energy expenditure (Miller et al. 1967; Apfelbaum et al. 1971; Sims et al. 1973; Dauncey and Ingram 1979; Dauncey 1980; Hill et al. 1983; Ravussin et al. 1988; Diaz et al. 1992; Leibel et al. 1995; Levine et al. 1999). This hypothesis is further supported by a study by Levine et al. (1999), which found that the change in NEAT (nonexercise activity thermogenesis) in response to calorie overconsumption predicted individual differences in weight gain. Thus, it seems likely that during weight gain the compensatory changes in energy expenditure are important in predicting weight change. It would be interesting to confirm the finding by Levine and measure physical activity and metabolic rate during a period of calorie overconsumption and determine if changes in energy expenditure are able to predict individual differences in weight gain.

Together these studies show that those individuals who eat little to start with or who decrease their food intake more are more likely to lose weight, indicating that decreasing food intake is important for promoting weight loss. As the monkeys that were overeating the most prior to dieting were least likely to lose weight on a diet these findings also suggest that individuals that overeat will not be very successful at losing weight on a diet and may need to decrease their food intake more than others and combine increasing activity with dieting to be able to effectively lose weight. However, during periods of weight gain neither initial food intake nor change in food intake appears to be important regulators of individual differences in weight gain. Furthermore, it is likely that during weight gain compensatory changes in energy expenditure and absorption efficiency by the GI tract play important roles in predicting differences in weight gain.

6.4 The Influence of Activity Level in Modulating Weight Change

Throughout the studies in this dissertation we observed consistently large differences in activity between individual monkeys (a 10-11-fold difference). The difference in activity that was observed between monkeys was striking, as it was much greater in magnitude than differences between individuals in any of the other variables that we measured including food intake and metabolic rate. The other interesting aspect of activity was that the activity level of an individual appeared to be stable over time (see Chapter 4). In fact, in a study that is not part of this dissertation, I found that when I monitored the activity of the same monkeys when they moved back and forth from group housing (a large pen with 3-4 other monkeys to interact with) to single cages (with

relatively little space and no other animals to interact with) that there was no significant change in their activity level (Sullivan et al. 2005). Thus, there are large individual differences in activity that remain stable over time and are not dependent on the space available for activity, suggesting that activity may be a very tightly regulated aspect of energy balance.

Initial physical activity was an important predictor of individual differences in slow progressive weight gain, such that the most active individuals gained less weight than the most sedentary individuals ($r=-0.52$, $p=0.04$). However, the initial activity level of an individual did not predict weight loss due to estrogen replacement therapy or diet-induced weight loss. It is possible that initial activity is important in predicting weight gain but not weight loss. However, I only measured activity in one of the three weight gain experiments that were undertaken in this dissertation. Alternatively, it is possible that activity predicts changes in weight when weight change is gradual and other metabolic regulatory parameters, such as food intake, remain stable, as was the case in the slow progressive weight gain study. When there are large changes in other mechanisms that influence body weight, such as food intake (as there were during high fat diet-induced weight gain and diet-induced weight loss) it is possible that the changes in food intake overshadow individual differences in activity. There are very few studies that measure activity during weight loss in humans (Keys et al. 1950; de Groot et al. 1989; Leibel et al. 1995), and none of these have looked to see if the initial level of activity predicts individual differences in weight loss. A number of epidemiologic studies have looked to see if baseline characteristics of an individual affect subsequent weight loss (Stein et al. 1981; Wadden et al. 1992; Jeffery et al. 1998; Kiernan et al.

1998; Hoie and Bruusgaard 1999; Ogden 2000; Cuntz et al. 2001; Teixeira et al. 2005; Vogels et al. 2005; Wing and Phelan 2005), however, none of these studies measured activity. Thus, it seems important that future studies be performed to measure activity during a period of gradual weight loss to determine if initial activity predicts individual differences in weight loss. This study would be relatively easy to conduct in monkeys as measuring the activity of monkeys is not hard and it would allow a controlled environment where the diet and the calorie restriction would be well-controlled and consistent between individuals.

Although activity was relatively stable in an individual over time, there were two circumstances in my dissertation research where I did observe a change in activity. There was a 25% decrease in activity in response to the dramatic reduction in available calories in the diet-induced weight loss study. And estrogen replacement therapy caused an increase in physical activity. However, in both of these studies the activity at the beginning of the study was highly correlated with activity at the end of the study, such that the monkeys that were most active to start with remained the most active even after a change in activity. In neither case was the change in activity predictive of weight loss. Perhaps if weight change had been more gradual in these studies activity may have played a greater role in modulating weight loss.

6.5 The Influence of Metabolic Rate in Modulating Weight Change

Neither basal nor 24-hour metabolic rate were important predictors of weight change under any of the circumstances evaluated in this thesis. It is possible that metabolic rate was not an important predictor of weight change in these studies because

the population of monkeys that I used in this dissertation was somewhat homogeneous (i.e. all female rhesus monkeys of Chinese origin), and thus did not have as much variability in metabolic rate as would be found in human populations with mixed ethnicity. There was a 2-6-fold difference in total metabolic rate between individuals across the various studies and a 2-3-fold difference in BMR. However, in these monkeys the main variable contributing to individual differences in metabolic rate was the amount of lean tissue mass that each individual had. This has been shown to be true for humans as well (Kreitzman et al. 1992; Keesey and Hirvonen 1997). Normalizing metabolic rate for lean tissue mass eliminated a large amount of variability between animals resulting in a two-fold difference in metabolic rate between individuals. In humans some, but not all studies find that individuals with a low metabolic rate are more susceptible to weight gain (Ravussin et al. 1988; Astrup et al. 1996; Astrup et al. 1999; Tataranni et al. 2003; Buscemi et al. 2005). Though the population of monkeys used in the study was somewhat homogeneous one could study rhesus macaques from different geographic origins to allow for a more diverse study population, more akin to having different racial ethnicities in a human study. Recent evidence suggests that though rhesus macaques from the same geographic region are genetically very similar that there are genetic differences between rhesus macaques from different geographic origins (Chinese, Indian and Nepalese) (Kanthaswamy et al. 2006; Kyes et al. 2006). Thus, it is possible that individual differences in metabolic rate may play a role in regulating individual differences in weight change in a more heterogeneous population of monkeys.

Changes in metabolic rate were looked for in three of the four studies in which I monitored weight change (slow progressive weight gain, estrogen replacement therapy

and diet induced weight loss). Metabolic rate did not change in response to estrogen replacement therapy, thus we could not look at the relationship between change in metabolic rate and individual differences in weight loss. Metabolic rate increased during the slow progressive weight gain, most likely due in response to the increase in mass of metabolically active tissue. A positive correlation between the increase in metabolic rate and weight gain is best interpreted to mean that the monkeys that gained the most weight increased their metabolic rate the most. If the change in metabolic rate was causing individual differences in weight gain you would expect the inverse relationship, that the monkeys that gained the most weight had the smallest increase in metabolic rate. Thus, in this experiment the changes in body weight appear to have caused the changes in metabolic rate. Metabolic rate also changed significantly in response to diet-induced weight loss. In this case there was a substantial decrease in both in total metabolic rate (13% decrease) and basal metabolic rate (14% decrease), that was correlated with weight loss such that the monkeys that lost the most weight decreased their basal metabolic rate the most ($r=0.52$, $p=0.04$). Similar to what was observed during slow progressive weight gain the change in weight seems to be driving the change in basal metabolic rate instead of differences in the change in metabolic rate predicting differences in weight change. Thus, again it appears that changes in metabolic rate occurred in response to the weight change, but were not causing the change in body weight.

6.6 Individuals that Do Not Follow General Trends

In the preceding discussion I have discussed how and when each of the several weight regulatory mechanisms may play an important role in body weight regulation.

However, in a number of circumstances there were a few individuals that did not follow the general trends. For example, in the diet-induced weight loss study there was a significant correlation between weight loss and initial body weight, such that the heavier monkeys were more likely to lose weight. However, in this study there were two lean and light monkeys that lost a large percent of their initial body weight over the two months of dieting. This may be because these monkeys were eating a low amount of food at the initiation of the study, and initial food intake was another parameter that predicted diet-induced weight loss. Looking at these outliers suggests that although initial body fat is an important regulator of diet-induced weight loss, that initial food intake is a stronger predictor of weight loss such that all individuals with low food intake (even those that are lean) are susceptible to diet-induced weight loss.

During slow adult weight gain there was a significant association between weight gain and initial activity level such that the least active monkeys gained the most amount of weight during this period. Although this held true for most monkeys, there was one monkey that was relatively sedentary but did not gain weight. This may be because this monkey had a high metabolic rate, which was able to prevent weight gain. It may be that a key factor determining weight gain is metabolic rate, and that in this rather homogenous population differences in activity generally contribute significantly to differences in metabolic rate.

In some studies all of the individuals did follow the general trend. For example, weight gain after ovariectomy was predicted by initial weight and BMI such that the lightest, lean monkeys gained the most weight after ovariectomy. In this case there were no outlier monkeys suggesting that there is a very robust relationship between initial

body composition and weight gain and that differences in other metabolic regulatory systems are not able modulate the role of body composition in determining the body's response to a change in ovarian hormones.

In conclusion, looking at outlier populations may allow one insight into the relative importance of various metabolic regulatory systems in controlling body weight changes in specific circumstances.

6.7 Summary and Conclusions

One of the main findings of this dissertation is that ovarian hormones are important regulators of body weight in a primate species suggesting that a decline in circulating hormones over menopause contributes, at least in part, to the increase in body weight that women experience over menopause. Also, I found that estrogen replacement therapy was able to reverse this weight gain, which suggests that HRT could aid obese postmenopausal women in losing weight and combating weight gain during the menopausal transition. As HRT is associated with a number of side effects including an increased risk of estrogen-dependent breast and uterine cancer, cardiovascular problems and stroke (Copeland et al. 2004; Stefanick et al. 2006), it would seem inappropriate for HRT to be prescribed for the sole purpose of limiting post-menopausal weight gain. Many aspects of a person's health and physiology should be taken into account when physicians consider prescribing HRT. Using such a personalized approach it may be appropriate to prescribe HRT to an obese postmenopausal woman who is having problems with bone loss but is generally healthy and has no family history of estrogen-dependent breast cancer, uterine cancer, heart disease or stroke. However, it would be

inappropriate to prescribe HRT to any women with a history of estrogen-dependent breast or uterine cancer, or with a family history of stroke, no matter how much she wanted to prevent weight gain associated with menopause. A better weight loss strategy for such a woman may include moderate dieting, while increasing her daily level of physical activity.

There were significant correlations between individual levels of physical activity and change in body weight during both a period of slow adult weight gain and in response to estrogen replacement therapy. Thus, level of physical activity appears to play an important role in maintenance of a healthy body weight over adulthood, suggesting that one of the best ways to prevent weight gain over adulthood, or to increase weight loss, would be to focus on living an active lifestyle. As the majority of Americans (60%) do not get the recommended amount of exercise, and 25% are largely inactive (Lee et al. 2000; Oguma et al. 2002) it appears that a large amount of effort needs to be put forth by the health care community to increase awareness in the health benefits of being active.

The findings of this thesis suggest that strict dieting is a fairly ineffective weight loss strategy, as it is accompanied by compensatory decreases in energy expenditure both in the form of a decrease in basal metabolic rate and a decrease in physical activity. Studies in rats find that a dramatic decrease in calories leads to a larger decrease in energy expenditure than a more moderate calorie reduction (Hill et al. 1985; Even and Nicolaidis 1993), suggesting that a more moderate calorie reduction which promotes gradual weight loss may actually be a more effective weight loss strategy, as the compensatory decreases in energy expenditure would be minimized. Another way to reduce the compensatory decreases in energy expenditure in response to calorie reduction

would be to combine dieting and exercise. This strategy is supported by a number of studies that have found that combining exercise with dieting is an effective strategy for preventing the compensatory decreases in energy expenditure that accompany dieting alone (Belko et al. 1987; Heymsfield et al. 1989; Mole et al. 1989; Frey-Hewitt et al. 1990; Wilmore et al. 1998). In regard to dieting, my results also showed that initial food intake was the most important predictor of diet-induced weight loss, with the individuals that overeat the most losing the least weight. As my data also showed a strong correlation between being sedentary and having a high percentage of body fat, one can see that the individuals who will most need to adopt a weight loss strategy to maintain a healthy body weight are sedentary individuals who eat many more calories than they expend. The results of my dissertation research suggest that a moderate diet coupled with regular exercise will be more effective than other options, such as undertaking a strict diet, in helping them achieve a healthy body weight.

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Chapter 8

Appendix

INDIVIDUAL DIFFERENCES IN WEIGHT GAIN

DURING HIGH FAT DIET CONSUMPTION

8.1 INTRODUCTION

In general, most species show an increase in weight when they are provided a diet that contains an increased percentage of calories derived from fat (Curb and Marcus 1991; Chalkley et al. 2002; Warwick et al. 2002; Estadella et al. 2004; Huang et al. 2004; MacLean et al. 2004). This is likely associated with the high palatability of many high fat nutrients, and with the fact that fat has more than twice the caloric density of carbohydrate or protein. In human studies weight gain has been promoted by giving people access to highly palatable food, much of which has a high fat content (Forbes et al. 1986; Bouchard et al. 1990; Diaz et al. 1992; Leibel et al. 1995; Levine et al. 1999). Just as in animal studies, groups that consume more calories, or have a larger increase in calorie consumption, show a larger mean increase in body weight, indicating that increased calorie intake causes weight gain (Forbes et al. 1986; Bouchard et al. 1990; Diaz et al. 1992; Larson et al. 1995; Leibel et al. 1995; Levine et al. 1999). However, there are relatively few studies that have looked at individual differences in weight gain in response to high fat or highly palatable diets (Sims et al. 1973; Forbes et al. 1986; Bouchard et al. 1990; Diaz et al. 1992; Larson et al. 1995; Levine et al. 1999). The results of these studies are somewhat surprising in that they indicate that there is generally a poor correlation between the increase in calorie intake and the amount of weight gain for a particular individual (Forbes et al. 1986; Larson et al. 1995). The lack

of correlation between increased caloric intake and weight gain is also observed in human populations where changes in food intake and weight have been tracked over several years (Johnson et al. 1956; Stefanik et al. 1959; Maxfield and Konishi 1966; Ries 1973; Yearick 1978; Matter et al. 1980; Birkbeck 1981; Baecke et al. 1983; Bellisle et al. 1988; Guillaume et al. 1998; Lorenzo et al. 2003). One reasonable explanation for the lack of correlation between individual differences in food intake and weight gain could be related to the fact that most of the human studies have relied on self-report of calorie intake, which has often been shown to be inaccurate (Champagne et al. 1998; DeLany et al. 2002). The study reported in this chapter was undertaken to more carefully examine the relationship between food intake and weight gain in the monkey model used in this dissertation.

To initiate studies of individual differences in weight regulation, adult female rhesus monkeys were transitioned from a diet of low fat monkey chow (5% fat) to a diet more typical of that consumed by humans in the Western world (35% fat; Williams et al. 2003). The use of monkeys for this study allowed accurate measurement of caloric intake across the 24 hour day throughout the study, in a species that, like humans, eat most of their daily calories as daytime meals (Oates 1987; Ding 2004).

8.2 METHODS

Animals

Sixteen ovariectomized adult female rhesus monkeys (*Macaca mulatta*), 7 - 11 years of age, were studied during the transition from a low fat diet of standard monkey chow (5% fat) to a higher fat diet typical of the diet consumed by humans in the Western

world (35% fat). Animals were used as their own controls, thus body weight and food intake measurements made throughout the study were compared to the baseline measures for each animal.

All monkeys were housed in individual stainless steel cages in a temperature-controlled room (24 ± 2 °C), with lights on for 12 h a day (0700 - 1900 h). Monkeys were fed *ab libitum* throughout the study. Initially, monkeys were fed two meals a day consisting of 12 high protein monkey chow biscuits (no. 5047, jumbo biscuits, Ralston Purina Co., St. Louis, MO) at 0930 and 1300 h. In addition, a half apple was provided at 1900 h. Monkeys had adapted to the diet and living conditions for at least 20 months prior to initiation of this study.

The diet was switched from a low fat chow diet (5% fat) to a high fat diet (35% fat) and meals were provided at 0915 and 1515 h. The high fat diet was formulated following the recipe developed by Clarkson and colleagues to study diet-induced atherosclerosis (Shadoan et al. 2003; Williams et al. 2003). Water was available *ad libitum* throughout the study. The study was reviewed and approved by the ONPRC Animal Care and Use Committee.

Measurement of body weight, caloric intake and body fat

Body weight assessments: Monkeys were weighed weekly throughout the study. Weight measurements were made before consumption of the morning meal, at approximately 0800 h.

Calorie intake assessments: Total food consumption at each meal was recorded throughout the study by counting the amount of food remaining prior to the next meal

presentation. Assessments of calorie consumption occurring at night (from 1900 – 0800 h) were made twice prior to high fat diet consumption and at weekly intervals during the 9 months of high fat diet consumption. The lights were off during this time period for 12 of the 13 hours (1900-0700). However, we were concerned that animals may show increased food consumption in the one hour period after lights came on (from 0700-0800 h), so prior to undertaking this study we recorded food consumption at 0700 and 0800 h for four days. We found that overnight food consumption measures were not significantly affected by measuring overnight food intake at 0700 h versus at 0800 h (t = 0.72, df = 15, p = 0.49; t = 1.0, df = 15, p = 0.33; t = 1.46, df = 15, p = 0.16; t = - 0.44, df = 15, p = 0.67) for days 1 - 4 respectively, thus for this study overnight food intake was measured from 1900 – 0800 h.

DEXA-measurements: Body composition (% body fat, total body fat mass, and lean body mass) was determined using dual energy X-ray absorptiometry (DEXA) and previously described methods before high fat diet consumption and 5 and 9 months after initiation of the high fat diet in experimental monkeys, using techniques described in Chapter 2.

Statistical Analyses

Changes in weight and calorie intake are expressed as percent of baseline levels. The assumptions of normality and homoscedacity were tested for all analyses. All measurements were normally distributed or were normally distributed after a square root or log transformation. Measurements made multiple times throughout the study were analyzed using a one-way repeated measures analysis of variance. The assumption of

sphericity was examined with Mauchly's Test. The Greenhouse-Geiser correction factor was used in cases where the assumption of sphericity was violated. If a significant difference was found, planned contrasts were utilized to compare measurements to baseline, using the least significant difference test. Overnight food intake before and after high fat diet consumption was analyzed using a paired Student's t-test. Correlations between parameters were determined using a Pearson product moment correlation. Comparisons between the top and bottom quartile of monkeys, based on percent increase in caloric intake or percent of calories consumed at night, were made with independent t-tests. For all tests, alpha values were considered significant with $p \leq 0.05$. Data are presented as mean $\pm$ SEM. All statistical analyses were conducted using the SPSS software package (SPSS Inc., Chicago, Illinois).

8.3 RESULTS

Upon initiation of the study, individual differences in daily calorie intake (360 to 975 calories/day) and body weight (4.8 to 9.0 kg) were substantial. However, there was no correlation between initial caloric intake and body weight ($r=-0.24$, $p=0.36$; Figure 8.1). Mean caloric intake ($F_{1.3,19.0}=21.2$, $p<0.0001$) and body weight ($F_{1.1,16.6}=5.70$, $p=0.03$) both increased significantly over the course of the study (Table 8.1; Figure 8.2). High fat diet consumption increased both caloric intake ($303\pm43\%$ of baseline levels; $p<0.0001$) and body weight ($113\pm5\%$ of baseline levels, $p=0.03$) within 5 months, and both remained elevated throughout the 9 months of high-fat diet consumption. There were large individual differences in weight gain in response to the high fat diet ranging
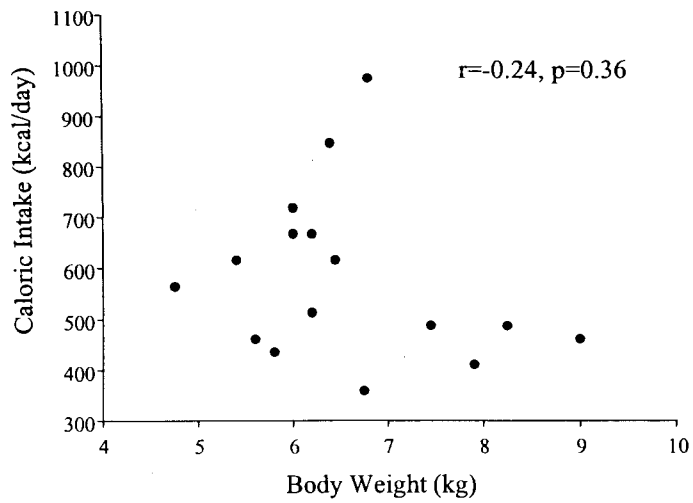
Figure 8.1 Body weight versus caloric intake before high fat diet consumption (r=-0.24, p=0.36).

Table 8.1 Changes in body composition (Mean ± SEM) during transition to high fat diet. Asterisks indicate a significant difference from baseline measures.

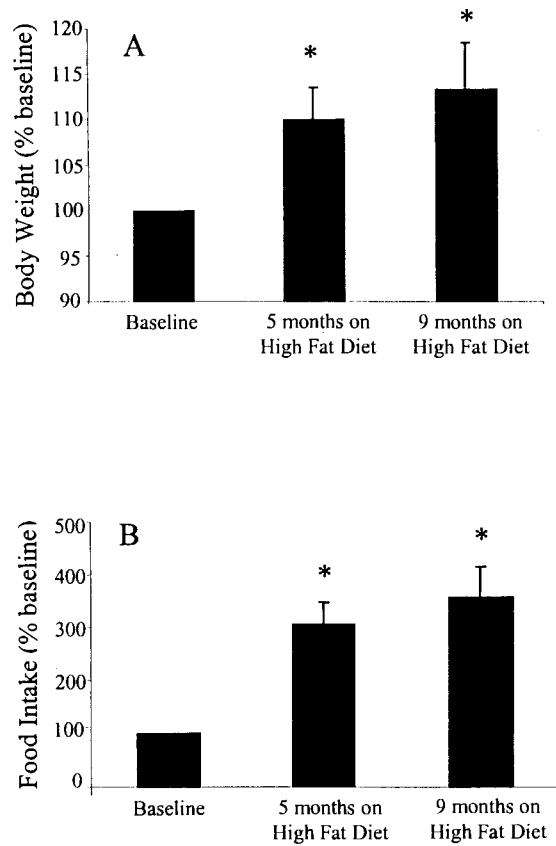|	Baseline	5 months high fat diet	9 months high fat diet
Body Weight (kg)	6.56 ± 0.28	7.23 ± 0.43 *	7.48 ± 0.52 *
Lean body mass (g)	5586 ± 301	5464 ± 275	5211 ± 221
Percent body fat (%)	13.29 ± 3.32	18.62 ± 3.86	20.98 ± 4.23*
Total body fat mass (g)	954 ± 286	1491 ±378	2160 ± 496*

Figure 8.2 Changes in (A) body weight ($F_{1.1,16.6}=5.70$, p=0.03), and (B) caloric intake ($F_{1.3,19.0}=21.2$, p<0.0001) after 5 and 9 months of high fat diet consumption. Asterisks indicate a significant difference from baseline measures.

from a monkey that lost 20% of it's initial weight to a monkey that increased it's weight by 50%.

Despite substantial increases in both mean caloric intake and mean body weight there was no correlation between the percent change in body weight and the percent change in caloric intake (r=-0.14, p=0.61, Figure 8.3). Moreover, there was no significant difference in body weight gain between the quartile of monkeys that increased their calorie consumption the most compared to the quartile that increased their calorie consumption the least (t=0.71, df=6, p=0.50).

High fat diet consumption significantly increased percent body fat ($F_{2,24}$=3.96, p=0.03), such that body fat was significantly higher than baseline levels after 9 months of high fat diet consumption (p=0.05) and was almost higher than baseline levels after 5 months (p=0.06). Fat mass also significantly increased with high fat diet consumption ($F_{2,24}$=3.87,p=0.04), such that fat mass was significantly higher than baseline levels after 9 months of high fat diet consumption (p=0.04), and there was a trend in this direction after 5 months (p=0.07). However, there was no significant difference in lean tissue mass ($F_{1.10,13.2}$=2.72, p=0.12) over the 9 months of consuming the high fat diet.

At the beginning of the study, the mean percent of total calories consumed at night was 37.4 ± 4.71%, however the percent of total calories that individual monkeys consumed at night varied from 4 to 63% (Figure 8.4A). For most monkeys, the percent of calories consumed at night decreased after 9 months of high-fat diet consumption (t=5.37,df=15, p<0.0001; Figure 8.4A). Nevertheless, the monkeys that consumed highest percent of their daily calories at night initially continued to consume the highest
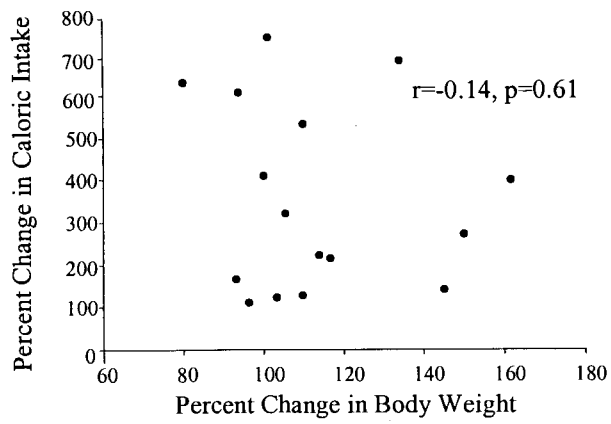
Figure 8.3 Percent change in body weight and percent change in caloric intake after 9 months of high fat diet consumption (r=-0.14, p=0.61).
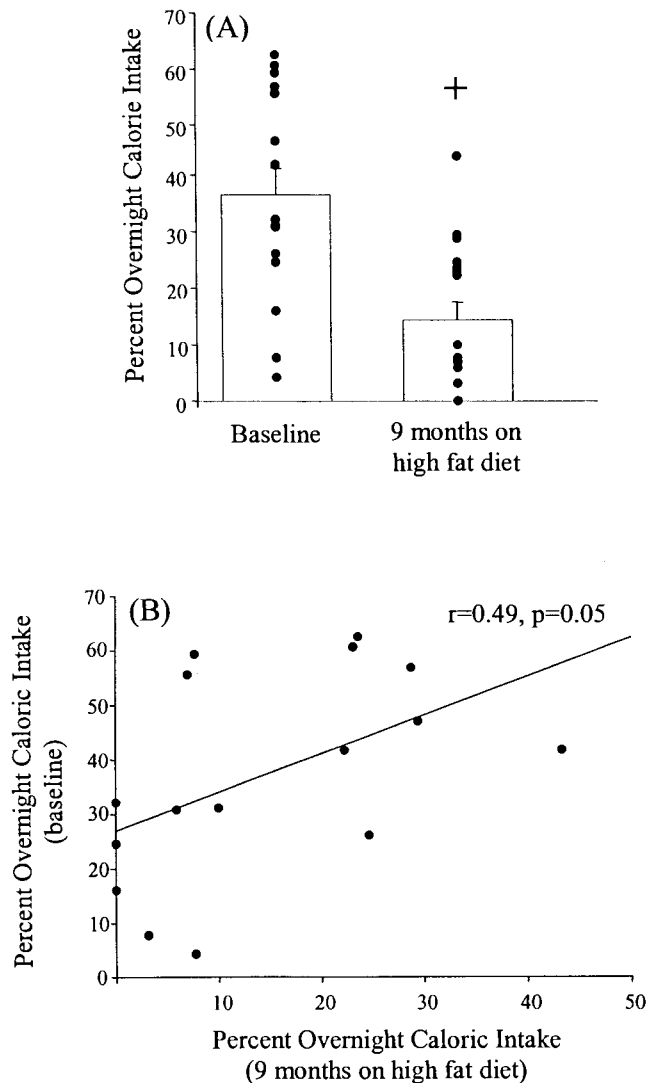
Figure 8.4 (A) Percent of total calories consumed at night during the baseline period and after 9 months on a high fat diet. Bars represent mean percent of calories consumed at night and the points represent values for individual monkeys. The cross indicates that significant difference between the percent of calories consumed at night during the baseline period and after high fat diet consumption (t=5.37, df=5, p <0.0001). (B) Correlation between the percent of calories consumed at night during the baseline period and after 9 months of high fat diet consumption (r=0.49, p=0.05).

percent of their daily calories at night at the end of the study (r=0.49, p=0.05; Figure 8.4B). The mean variability in the percent of calories consumed at night for individual monkeys over the last 12 weeks of the study was 3.2%. There was no indication that monkeys that consumed a greater proportion of calories at night were heavier, or gained more weight during any part of the study, compared to monkeys that ate a lower percent of their daily calories at night. Percent of calories consumed at night was not correlated with initial weight (r=0.05, p=0.87) or with weight change after 9 months on a high fat diet (r=0.06, p=0.84; Figure 8.5A). Additionally, there was no difference in body weight change in the quartile of monkeys that consumed the highest percent of their daily calories at night versus the quartile who consumed the lowest percent of calories at night (t=-0.74, df=6, p=0.94; Figure 8.5B).

8.4 DISCUSSION

As expected, based on the results of a number of studies in humans (Yao and Roberts 2001; Archer et al. 2003; Renzaho and C 2003) and animal species (Chalkley et al. 2002; Warwick et al. 2002; Estadella et al. 2004; Huang et al. 2004; MacLean et al. 2004), consumption of a palatable diet with a higher percent of calories from fat caused a dramatic increase in both caloric intake and mean body weight. Interestingly, there were large differences in the amount of weight gained between individual monkeys. Surprisingly, however individual differences in weight gain were not correlated with either initial caloric intake or the increase in caloric intake in response to a palatable diet. The lack of correlation between the change in caloric intake and weight gain has been
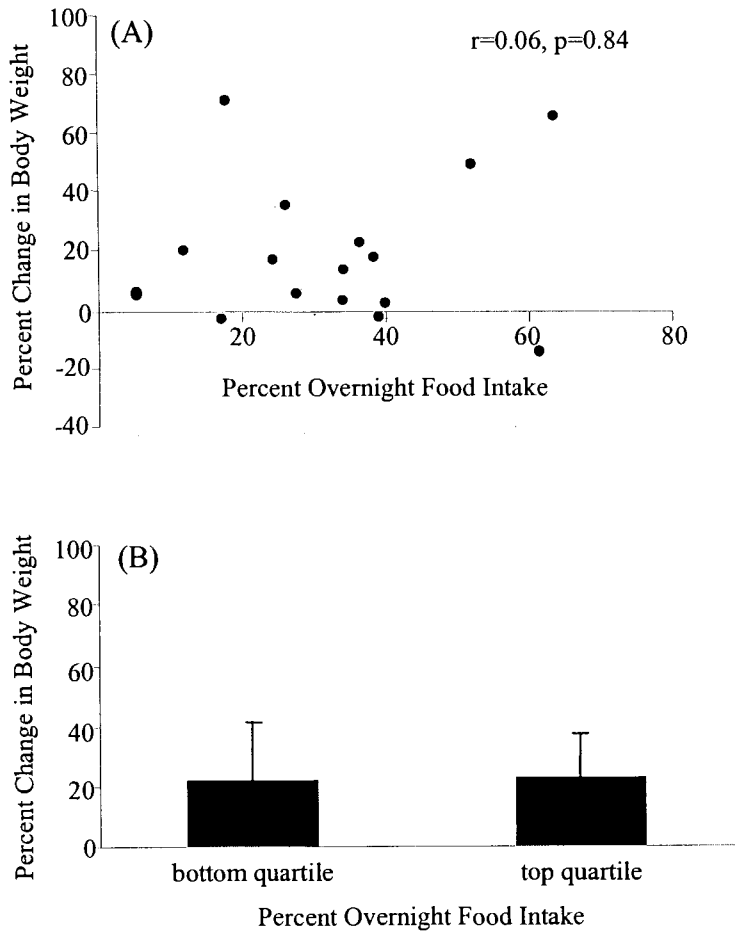
Figure 8.5 (A) Percent of calories consumed at night during the baseline period versus

weight change over the course of the study (r=0.06, p=0.84). (B) Body weight change

over the course of the study in the quartile of monkeys that consumed the highest percent

of total calories at night during the baseline period versus the quartile who consumed the

lowest percent of calories at night during the baseline period (t=-.74, df = 6, p = 0.94).

previously observed in human populations (Johnson et al. 1956; Stefanik et al. 1959; Maxfield and Konishi 1966; Ries 1973; Yearick 1978; Matter et al. 1980; Birkbeck 1981; Baecke et al. 1983; Bellisle et al. 1988; Guillaume et al. 1998; Lorenzo et al. 2003). The lack of correlation between caloric intake and body weight in many human studies, as well as in our monkey study, is likely to be due to differences in metabolic rate, basal metabolic rate, physical activity, the thermic effect of food and absorption of nutrients from the gastrointestinal tract between individuals. However, as these factors were not measured in this study their role in promoting individual differences in weight gain in this study is unclear. The findings of large individual differences in weight gain that were unrelated to individual differences in food intake or the change in food intake, led me to conduct the set of experiments that make up this dissertation in order to further examine the factors that predict individual differences in weight change under a variety of situations. However, as food intake did not predict weight gain in this study, in subsequent studies I measured total energy expenditure, basal metabolic rate, activity-associated energy expenditure and the thermic effect of food, in addition to food intake.

It was surprising that greater weight gain was not observed in response to the large and sustained increase in caloric intake (368% of baseline levels) that occurred in this study. The modest weight gain that occurred after maintaining monkeys on a high fat diet for a 9 month period, with a large increase in calories available, suggests that the monkeys' homeostatic systems were able to compensate, in part, for the increase in caloric intake. Recent evidence, in humans, suggests that non-exercise activity thermogenesis (NEAT) increases with positive energy balance and that there are large individual differences in the increase in NEAT that correlate with the amount of fat

225

gained, such that the individuals that increased NEAT the most gained the least amount of fat (Levine et al. 1999; Levine 2004). Thus, it is would be interesting to measure NEAT in monkeys consuming a high fat diet and determine if differences in changes in NEAT would account for the differences in weight gain that we observed.

Other types of activity could also compensate, in part, for the increased calorie consumption. A large body of epidemiologic data shows an association between low levels of physical activity and a higher rate of adult weight gain (Rissanen et al. 1991; Klesges et al. 1992; Williamson et al. 1993; Weinsier et al. 1995; Kyle et al. 2001; Di Pietro et al. 2004; Sternfeld et al. 2004; Brown et al. 2005; Hunter and Byrne 2005; Littman et al. 2005). However, most of these studies rely on self-report of physical activity which can be inaccurate (Klesges et al. 1990; Matthews and Freedson 1995; Melanson and Freedson 1995; Treuth et al. 2004). Additionally, other studies have found no association between low activity and weight gain (Rising et al. 1994; Weinsier et al. 1995; Kyle et al. 2001; Di Pietro et al. 2004; Kyle et al. 2004; Sternfeld et al. 2004; Ekelund et al. 2005; Hunter and Byrne 2005; Littman et al. 2005; Sternfeld et al. 2005). Thus, further studies are needed that objectively measure activity during a period of weight gain to determine that role that activity plays in causing differences in weight gain between individuals.

Low metabolic rate has also been found to be associated with increased propensity to gain weight (Ravussin et al. 1988; Astrup et al. 1996; Astrup et al. 1999; Luke et al. 2000; Tataranni et al. 2003; Buscemi et al. 2005). However, other studies show no association between low metabolic rate and weight gain (Weinsier et al. 1995; Brehm et al. 2005) and one study found that weight gain was higher in individuals with a

high basal metabolic rate (Seidell et al. 1992). Thus, the role that metabolic rate plays in promoting individual differences in weight loss also remains unclear.

Lastly, there are studies that suggest that individual differences in the amount of energy utilized to digest and absorb food (the thermic effect of food) predict susceptibility to weight gain such that individuals with lower thermic effect of food gain more weight (Horton 1983; Tappy 1996). However, other studies do not find differences in the thermic effect of food between individuals that gain weight and those that do not (Bandini et al. 1989; Tataranni et al. 1995; Astrup 1996; Brehm et al. 2005). Thus, further studies are needed that measure all of the components of energy expenditure during a period of weight gain to understand that role that each plays in contributing to individual differences in weight gain.

The notion that eating in the evening is more likely to promote weight gain than consuming food at other times of day is popular and many diets recommend limiting food intake during the evening hours (Gagliardi 1998; Atkins 2002; Agatston 2003). As metabolic rate decreases during periods of sleep in humans (Kreider et al. 1958; Kreider and Iampietro 1959; White et al. 1985; Palca et al. 1986; Fraser et al. 1989; Fontvieille et al. 1994; Mortola 2004), and animals (Rubal et al. 1992; Thompson et al. 1994; Seifert and Mortola 2002; Kalin et al. 2003; Power ML 2003), it seems logical to assume that eating during a time when metabolic rate is slowing may lead to less utilization of consumed energy and therefore, more storage of energy and ultimately to weight gain. However, evidence that nighttime eating causes greater weight gain than comparable calorie consumption at other times of day is limited and data from some studies do not support this conclusion. Thus, this study also assessed the percentage of daily calories

consumed at night during this period of weight gain to look for an association between increased calorie intake at night and increased propensity for weight gain.

The findings of this study contradicted the hypothesis that nighttime eating is associated with increased weight gain, as we found that monkeys eating a large proportion of calories at night did not show an increased propensity to gain weight and were not significantly heavier or fatter than monkeys eating the majority of their daily caloric intake during daytime hours. Before weight gain, there was a 10-fold variation in the percent of calories consumed at night between individual monkeys, with monkeys consuming from 4-63% of their total calories at night. A similar range of nighttime caloric intake, 24–65% of total calories, has been reported in humans (Baecke et al. 1983; Bellisle et al. 1988; Kant et al. 1995; Kant et al. 1997). Our findings agree with the 10 year study in over 7,000 people, by Kant et al. (Kant et al. 1997), which found that nighttime caloric intake was not associated with long term weight change, and several other studies that found no association between obesity and nighttime consumption of calories (Fricker et al. 1990; Kant et al. 1995). Our results differ from the positive correlation reported by Keim et al. (Keim et al. 1996) between weight gain and percent of calories consumed in the evening in women whom had recently lost weight. However this was a much shorter-term study (i.e., 2 weeks). Our results also differ from reports that individuals with nighttime eating syndrome, defined as consuming more than fifty percent of ones' daily calorie intake at night, gain weight more easily than individuals who consumed the majority of their calories during the day (Grilo and Masheb 2004). However, in addition to not controlling for total calorie consumption in this study, individuals with nighttime eating syndrome have an increased incidence of depression

and insomnia (Stunkard et al. 1955; Gluck et al. 2001), so differences in the propensity to gain weight associated with nighttime eating syndrome could result from factors other than nighttime eating. Therefore, the findings of this study indicate that eating at night is not associated with increased propensity to gain weight. Which suggest that individuals trying to lose weight should not rely on decreasing evening calorie intake as a primary weight loss strategy, but should focus on other strategies such as decreasing overall caloric intake and increasing activity.

In conclusion, consumption of a highly palatable diet with a greater percent of calories from fat led to increases in food intake and body weight in ovariectomized female rhesus monkeys. Interestingly, there were large individual differences in how much weight an individual gained that were not associated with neither initial food intake nor the change in food intake. Consequently, further studies are needed that measure the metabolic regulatory processes governing energy expenditure, in addition to energy intake and body weight, during a period of weight gain to determine the role that each play in contributing to individual differences in weight gain. Furthermore, the finding that monkeys only gained a small amount of weight in response to a large sustained increase in calorie intake suggest that compensatory changes in energy expenditure occur in response to increased food intake. Further studies could examine this issue by measuring each regulatory process governing energy expenditure during a period of increased calorie intake. In addition, the findings of this study suggest that eating a higher percent of calories at night is not associated with increased propensity to gain weight.

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