Body composition modulates the effect of a high-fat diet on learned eating behaviour in male rats

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ABSTRACT

Meal pattern and eating behaviour in animals and humans are learned over time and involve both innate/biological and environmental factors. It has been shown that animals can learn to anticipate certain outcomes following a behaviour (instrumental conditioning), i.e. anticipatory hunger/satiety. First reported by Jacques le Magnen in 1957, rats learn to eat a smaller amount of food that is followed by a short period of food deprivation than of a food followed specifically by a long fast. High-fat diet (HFD) and obesity directly affect the hippocampus and hence learning and memory processes. Mechanisms are elusive, but key mechanisms proposed are those involving insulin resistance, glucose intolerance and impaired neuronal plasticity. Other crucial factors involved in the relationship between HFD and learning are hormones such as ghrelin and leptin, which both have roles in food intake and energy homeostasis but also in mechanisms of learning and memory because of their ability to cross the blood brain barrier. Anticipatory eating is a hunger-reinforced instrumental behavior that is attenuated by the ingestion of HFD as a maintenance diet. The purpose of the present work was to investigate the results of an experiment investigating the effect of high-fat maintenance diet on food intake, learning of anticipatory eating, and body weight and composition in adult male rats. Thirty Sprague Dawley rats were randomly assigned to either a high-fat maintenance diet or Purina chow and were subjected to an anticipatory learning experimental paradigm for 10 cycles of 2 days including one deprivation period (8 h). HFD rats were grouped based on body weight and fat gain. Results indicated that pre-training and post-training, total body fat and abdominal fat did not differ in high

body-weight gainers and low weight gainers, but were significantly greater compared to controls. Because weight gain did not correlate with fat gain, HFD rats were grouped into high weight/high fat gainers, high weight/low fat gainers, low weight/high fat gainers, and low weight/low fat gainers. Learning of anticipatory eating was evident in all rats as but the proportion of first peaks in the earlier cycles was greater in the group maintained on the HFD as a whole, indicating greater speed of learning. However, within the HFD group, those who gained the most amount of fat (g) showed slower learning. Significant diet and cycle effects indicated numerical evidence of reward, with highest reliability in the low-weight, low-fat gainer group. This indicates that a specific type of body composition developed through HFD may influence certain learning processes. The idea that cognitive processes contribute to the control of food intake in rats is also present in human eating behaviour. Anticipatory eating reflects a capacity for managing hunger: that is, humans may learn subconsciously to eat more food before a period of hunger before the conventional time to eat. Impairments in this capacity may contribute to the development of obesity, as an issue primarily of the mental mechanisms organizing eating behaviour.

RÉSUMÉ

Les modèles de repas et de comportement alimentaire chez les animaux et les humains ont été appris au fil du temps et impliquent des facteurs environnementaux et biologiques/innés. Il a été démontré que les animaux peuvent apprendre à anticiper certains résultats suite à un comportement (conditionnement instrumental), par exemple la faim/satiété anticipée. Signalé pour la première fois en 1957 par Jacques le Magnen, des rats apprennent à manger une petite quantité de nourriture qui est suivie d'une courte période de privation de nourriture plutôt que d'un aliment suivi d'un long jeûne. Un régime riche en gras (RRG) et l'obésité influent directement l'hippocampe et donc l'apprentissage et les processus de mémoire. Les mécanismes sont insaisissables, mais les principaux mécanismes proposés sont ceux qui impliquent l'insulino-résistance, l'intolérance au glucose et la plasticité neuronale réduite. D'autres facteurs essentiels impliqués dans la relation entre le RRG et l'apprentissage sont les hormones telles que la ghréline et la leptine, qui toutes deux ont un rôle dans l'ingestion alimentaire et l'homéostasie énergétique mais aussi dans les mécanismes d'apprentissage et la mémoire compte tenu de leur capacité à traverser la barrière hémato-encéphalique. L'ingestion anticipatoire est un comportement instrumental renforcé par la faim qui est atténué par l'ingestion de RRG comme régime de maintien. Le but de ce travail était d'analyser les résultats d'une expérience sur les effets d'un régime alimentaire de maintien riche en graisses sur la prise alimentaire, l'apprentissage de l'ingestion alimentaire anticipatoire et le poids et la composition corporelle chez les rats mâles adultes. Trente rats Sprague Dawley ont été assignés au hasard à un régime de maintien riche en gras ou au Purina chow et ont été soumis à un modèle expérimental de l'apprentissage anticipée pendant 10

cycles de 2 jours, y compris une période de privation de nourriture (8 h). Les rats nourris avec le RRG on été groups selon leur gain de poids et de gras corporel. Chez les animaux qui ont gagné beaucoup ou peu de poids corporel, le gras corporal total ainsi que le gras abdominal avant et après l'entraînement de l'apprentissage anticipé n'étaient pas différents; cependant le gain de gras corporel total et le gras abdominal étaient significativement plus élevés que chez les animaux témoins. Comme le gain de poids n'était pas corrélé avec le gain de gras corporel, les animaux nourris avec le RRG ont été subdivisés en quatre sous-catégories comprenant ceux qui avaient gagné beaucoup de poids mais peu de gras corporel, ceux qui avaient gagné beaucoup de poids et de gras corporel, ainsi que les animaux qui avaient gagné peu de poids mais beaucoup de gras corporel et ceux qui avaient gagné peu de poids et peu de gras corporel. L'apprentissage de l'ingestion anticipatoire a été observé chez tous les animaux, mais la proportion des premiers pics d'ingestion au cours des premiers cycles d'entraînement était plus élevée chez les rats nourris avec le RRG, indiquant un apprentissage plus rapide. Cependant, chez les animaux nourris avec le RRG, ceux qui avaient gagné le plus de gras corporel (g) montraient un apprentissage plus lent. Les effets significatifs du régime alimentaire et du cycle indiquaient une évidence numérique de récompense, avec un effet plus robuste chez les animaux qui avaient gagné le moins de poids et de gras corporel. Cela indique qu'un type spécifique de composition corporelle développé par HFD peut influencer certains processus d'apprentissage. L'idée que les processus cognitifs contribuent au contrôle de la prise alimentaire chez le rat est aussi importante chez l'homme. L'ingestion alimentaire anticipatoire reflète une capacité de gestion de la faim : autrement dit, les humains peuvent apprendre inconsciemment à manger plus de nourriture avant une

période de faim, précédant l'heure conventionnelle à manger. Une déficience de cette capacité pourrait contribuer au développement de l'obésité, essentiellement comme une question de mécanismes mentaux organisant des comportements alimentaires.

CONTRIBUTION OF AUTHORS TO MANUSCRIPT

Dr. Louise Thibault, Dr. Hope Weiler, Dr. David Booth, Dr. Soghra Jarvandi, and Mavreta Vagenas are the co-authors of the Manuscript presented.

Dr. Louise Thibault, the candidate's supervisor and the principal investigator contributed to the study procedures and funded the experiment. Dr. Thibault developed the plan for the study and through the weekly meetings monitored the progress of the work and edited the Manuscript.

Dr. Booth and Dr. Weiler edited the Manuscript and provided invaluable comments and suggestions to the development of animal studies and interpretation of data through continuous feedback. In addition Dr. Booth provided very useful comments on statistical analyses pertaining to learning of anticipatory eating results.

Mavreta Vagenas, a Masters Applied graduate of McGill in the School of Dietetics worked alongside the candidate, while she worked on her own project relating to the Manuscript. Mavreta's work was essential in exploring aspects of animal body composition, body composition measurements and animal weight classifications.

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The candidate reviewed the literature, performed the statistical analyses in the Manuscript, prepared figures, and tables and wrote the first draft of the Manuscript. In

addition, the candidate prepared the Manuscript to be submitted to The International Journal of Obesity (IJO) and contributed to the revisions according to the comments from committee members.

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LIST OF ABBREVIATIONS

AG	Acyl-Ghrelin
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid
ANOVA	Analysis of Variance
BDNF	Brain-Derived Neurotrophic Factor
BMC	Bone Mineral Content
CR	Caloric-Restriction
CS	Conditioned Stimulus
DIO	Diet-Induced Obesity
DR	Diet Resistant
ER	Energy Restriction
g	Gram
GLUT4	Glucose Transporter 4
h	Hour(s)
kcal	Kilocalorie
L	Intake of test food having an odour predictive of the longer fast
LBM	Lean Body Mass

LSmean	Least Square Mean
MUFA	Monounsaturated Fatty Acid
n	Number
NMDA	N-methyl-d-aspartate
NPY	Neuropeptide Y
PI3K	Phosphatidylinositide 3-kinases
PUFA	Polyunsaturated Fatty Acid
r	Correlation Coefficient
S	Intake of test food having an odour predictive of the shorter fast
SEM	Standard Error of Mean
SFA	Saturated Fatty Acid
US	Unconditioned Stimulus

CHAPTER 1, INTRODUCTION

1.1 Background and rationale

Ingestive behaviour involves physiological and psychological mechanisms, and is therefore complex. Simply measuring daily food intake does not reflect behaviour, and is not able to capture anticipatory eating and the modulation by body composition. Meal pattern is something animals and humans have learned over time and involves both innate/biological and environmental factors. Learning is a "change in the organization of an individual's behaviour so that performance represents the external and internal environments" (Booth, 1987). First demonstrated by Ivan Pavlov, classical conditioning is involved in eating behaviour. In classical conditioning, a neutral stimulus is paired with an unconditioned stimulus (US), one that automatically creates an unlearned response. For example, most research done on eating behaviour has paired conditioned stimuli (CS), such as flavours or odours with a nutritive or non-nutritive outcome, e.g. glucose, amino acids, or triglycerides. If animals can learn to associate a CS with a US, it has been shown that animals can learn to anticipate certain outcomes following a behaviour, i.e. anticipatory hunger/satiety. Although animals show this learning ability, it is now being hypothesized whether high-fat foods or obesity itself attenuate this ability to learn anticipatory eating.

First described by Jacques Le Magnen in 1957 (1999), rats showed the ability to eat a smaller amount of food that was followed by a short period of food deprivation than of a differently flavoured food followed by a long fast. Subsequent work on anticipatory eating demonstrated that rats learned to increase their food intake before both short and

long fast lengths with differential cuing (i.e. odour or texture; Le Magnen, 1957; Thibault & Booth, 2006; White, Mok, Thibault et al., 2001). At the start of a series of papers by Thibault and Booth's research group (White, Mok, Thibault et al., 2001), evidence for anticipatory eating was present in a subgroup of rats that had enough intakes before the short fast to prevent hunger returning. This led to the conclusion that eating was reinforced by a subsequent rise of hunger under the discriminative stimulus of the texture of the diet (White, Mok, Thibault et al., 2001). A more recent experiment showed that although intake (with either carbohydrate- or protein-based food) was greatest before a long fast, intake also increased before a short fast and with a similar pattern (Thibault & Booth, 2006). This pattern became a consistent pattern of anticipatory eating, as was seen with single or choice tests foods, and both liquid and solid test foods (Jarvandi, Booth & Thibault, 2007; Jarvandi, Thibault & Booth, 2009). Furthermore, this substantiated pattern of anticipatory eating, which occurs for both long and short fast lengths demonstrated that there is no need for the contrast between either fast length (Jarvandi, Thibault & Booth, 2009). The difference between learned intake before both fast lengths is now being addressed with the same underlying mechanisms, thus confirming the rationale of experimenting with just one prolonged fast. Both fast lengths could reinforce intake negatively, thus avoiding later depletion. Alternatively, both fasts lengths could reinforce intake positively, thus creating a state of repletion (Jarvandi, Booth & Thibault, 2012). The idea that a state of deprivation can lead to unconscious reward has implications in human obesity, that is dieting could be a contributor to the extra eating, where humans who decrease their food intake at one meal are rewarded by the food eaten after, leading to extra eating of those foods (Booth, Jarvandi & Thibault, 2012).

It is now being hypothesized whether high-fat foods or obesity itself attenuate this ability to learn anticipatory. Preliminary work with inclusion of a high-fat diet as trial food showed the weakest amount of anticipatory eating (Jarvandi, Thibault & Booth, 2007b). These results may be connected to the extensive evidence that a high-fat diet yields increased obesity and decreased learning (Woods, D'Alessio, Tso et al., 2004). Evidence suggests impairments in learning and cognitive function in diet-induced obese mice (Winocur & Greenwood, 2005; Kanoski & Davidson, 2011); however, little has been shown comparing effects of obesity and diet composition as two separate entities. Valladoilid-Acebes, Stucchi, Cano et al. (2011) found that a high-fat diet impaired learning performance, and found that independent of important markers of obesity (e.g. hypertension/hyperinsulinemia), dietary intake could impair learning in its own. With the notion that HFD and obesity directly affect the hippocampus and hence learning and memory processes, the purpose of this study is to examine whether learned intake before an 8 h fast is affected by adaptation on a high-fat maintenance diet. Differentiation has been made in the current study between weight gain and fat gain from a high-fat diet.

1.2 Objectives of planned research

The main objectives of this thesis were to provide vast evidence of learning of anticipatory eating and furthermore to report on the results of an experiment investigating the effect of high-fat maintenance diet on eating behaviour, learning of anticipatory eating, and body weight and composition in adult rats. In order to reach such an objective, the study questions of this thesis were:

1. Is anticipatory eating evident in rats regardless of differential cuing and with one fast length?
2. Is learning impaired in rats fed a HFD compared to animals fed a standard diet?
3. Is there individual variability in terms of weight gain and various aspects of body composition within and between HFD and standard diet fed animals?
4. Is learning impaired more as a function of HFD induced obesity or HFD feeding without obesity (e.g. in rats resistant to HFD obesity)?

CHAPTER 2, REVIEW OF PUBLISHED RESEARCH FINDINGS

2.1 Introduction

The following literature review will cover various topics related to my proposed thesis of analyzing the effects of a maintenance HFD on flavour-specific anticipatory eating and body composition in Sprague-Dawley rats. Obesity and high fat diets impair performance on various learning tasks, such as anticipatory eating. It is important to review the possible mechanisms and factors playing a role in such learning. The study of ingestive behavior is complex because simply measuring absolute intake of food doesn't yield much information about the learning process of eating as a behavior. While meal patterns and eating behavior have innate qualities, they also imply learned responses through experiences involving internal (physiological) and external (i.e. social, physiological and sensory) factors (Booth, 1987). To investigate the effects of maintenance on a HFD on the learning of flavour-specific anticipatory eating, various factors will be considered such as aspects that may affect learning and memory performance independent of the adverse effects of obesity.

2.2 Learning and Memory

In both humans and animal models of learned eating, a relationship has been found between obesity and cognition. More specifically, obesity and HFD are associated with impairments in learning and memory. Research has been done involving the hippocampus and its associated neurons in order to assess the effect of HFD on synaptic activity in the hippocampus. In understanding the activity of the hippocampus, it is important to understand the receptors involved. A decreased number of hippocampal

neurons leads to memory impairment and therefore decreased performance in certain memory and learning tasks (Goodman, Trouche, Massou et al., 2010).

2.2.1 High-Fat Diets

N-Methyl-d-aspartate receptors (NMDARs) are linked to hippocampal-dependent learning and memory; and therefore have been studied specifically as possible factors in the impairment of learning and memory in obese subjects. Several studies have shown that HFD impair hippocampal function resulting in impairment in learning and memory tasks. HFD (energy content of 5.35 kcal/g and contained fat mostly from lard, 59.28%, for a 12 wk period) consumption can cause significantly more weight gain than a normal diet of standard laboratory chow (energy content of 4.02 kcal/g, and 19.77% of total energy from fat). Additionally, HFD can cause peripheral insulin resistance as well as neuronal insulin resistance, which may both contribute to cognitive impairment (Pipatpiboon, Pratchayasakul, Chattipakorn, et al., 2012). In a study investigating the attenuation of cognitive deficits induced by HFD (58% of energy from fat, 25% of energy from protein, and 17% of energy from carbohydrate) feeding, adult male Sprague-Dawley rats weighing 150-190 g gained more weight on the HFD than control rats and had increased levels of plasma glucose, triglycerides, cholesterol and insulin although it was not indicated if these rats were obese. HFD fed rats also performed worse than controls on a Morris water maze task, a paradigm testing spatial-based learning and memory by measuring the time taken to locate a hidden underwater platform. The fact that HFD fed rats showed impaired memory could be attributed to mechanisms involving the development of peripheral glucose intolerance and/or insulin resistance (Pathan,

Gaikwad, Viswanad, et al., 2008).

The mechanisms by which HFD affect the brain, specifically the hippocampus, have been investigated (Kanoski & Davidson, 2011). A high intake of saturated fat and simple carbohydrates may contribute to the development of metabolic syndrome. Metabolic syndrome, being a condition involving a collection of risk factors for cardiovascular disease and type II diabetes, involves insulin resistance and glucose intolerance. The latter two conditions have direct effects on the hippocampus, such as increased decline in cognitive function. For example, poor glycemic control (i.e. insulin resistance) has been consistently associated with impaired memory performance such as tests of delayed verbal memory. A study showed that 6-week old male Sprague-Dawley rats fed a HFD (31.8% of energy from fat from butter and corn oil) rich in saturated fatty acids (SFA) were impaired on their performance in a spatial memory task after 20 weeks of feeding. Furthermore, rats on the HFD were divided into groups based on their development of obesity- weight gain in the top tertile of HFD animals were considered diet-induced obese (DIO) and in the bottom tertile of HFD animals were considered high-fat diet-resistant (DR). DIO rats were significantly more hyperglycemic, hyperinsulinemic and memory-impaired than controls and DR rats. The evidence showed that insulin resistant rats, who were injected with insulin (via artificial ECF) into their hippocampus, improved in spatial memory performance in an inverted U-dose related manner (100 μU and 1 mU insulin significantly improved performance); however, this was not the case for DIO rats (McNay, Ong, McCrimmon et al., 2010). McNeilly and colleagues conducted a study, which yielded similar results (McNeilly, Williamson,

Sutherland, et al., 2011). The authors randomly assigned male Wistar rats (age not indicated) to either a regular chow or HFD (45% of energy from crude fat, 20% of energy from crude protein and 35% of energy from carbohydrate) for a 12-week period. Similar to what was confirmed in previous studies, rats fed a HFD had increased insulin resistance and adiposity, making them overweight. Most importantly, HFD fed rats had impaired behavioral flexibility in that they had a more difficult time switching from a delayed matching to position (DMTP) task to a delayed non-matching to position (DNMTP) task than chow-fed rats. This study proposed that the impairments in learning were correlated with peripheral insulin resistance, but not with weight gain.

Other studies suggest that HFD has an effect on the central nervous system's normal development. Lindqvist, Mohapel, Bouter et al. (2006) investigated the influence of HFD on hippocampal neurogenesis, a process exhibited throughout adult life (Åberg, Åberg, Hedbäcker, et al., 2000). Both young male and female Sprague-Dawley rats (age not indicated) were divided into two groups, those who were fed HFD (42% of energy from fat from coconut butter and corn oil) and those who were fed low-fat diets (standard chow, 10% of energy from fat) for 4 weeks. Results showed that aside from no differences in weight and fat accumulation between groups (i.e no obesity development in HFD group), hippocampal neurogenesis was impaired in males fed HFD as a result of a reduced number of newly born cells in the dentate gyrus of the hippocampus. There failed to be an effect of HFD on neurogenesis in females, which can possibly be attributed to their unaltered corticosterone levels due to HFD. Corticosterone is said to inhibit neurogenesis; therefore the increasing amounts in male rats had a negative effect.

The authors proposed the absence of impairment on neurogenesis in female rats fed HFD might also be due to the effects of estrogen, which is said to enhance neurogenesis via stimulation of insulin-like growth factor; however estrogen was not measured in this study. Several studies have demonstrated impairments in learning and memory due the ingestion of HFD with and without the development of obesity. Since HFD impairs hippocampal neurogenesis at a young age, this may be a mechanism by which learning and memory are impaired. This study by Lindqvist, Mohapel, Bouter et al. (2006) is important because unlike other similar studies, which involve obesity as a result of HFD, this study's results are independent of obesity. This point is crucial because it suggests that hippocampal impairment was not resulting from obesity, but it may be a co-incident result of high-fat food intake (Lindqvist, Mohapel, Bouter et al., 2006). In contrast, the absence of obesity could be due to the young age of the rats and the use of adult rats or a more prolonged HFD may have yielded different results.

The effect of high-fat food on the brain has been studied. Greenwood and Winocur (1996) specifically proposed that dietary SFAs were responsible for deficits in cognitive impairment. This hypothesis spawned from their previous work's findings that 1 month old male Long-Evans hooded rats (n=8) fed HFD (40% of energy from fat high in either SFAs or polyunsaturated fatty acids) for 3 months experienced markedly impaired cognitive function on learning and memory tasks such as Olton's radial arm maze, a variable-interval delayed alternation task, and the Hebb-Williams maze series (Greenwood & Winocur, 1990) and those consuming the diet high in SFAs performed the worst (Winocur & Greenwood, 1993). In a study conducted in 1996, 40 one-month-old

male Long-Evans rats (60-80 g) were randomly assigned to one of five diets varying in their fat composition. Diets 1-4 were composed of 20% by weight of fat and diet 5 was standard rat chow (4.5% by weight of fat). The rats were trained to perform on a variable-interval delayed alternation (VIDA) task, which was then used to assess performance post-diets. In general, rats fed diets highest (diet 1- 9.42 g/100g and diet 2- 7.18 g/100g) in SFAs performed worst. Interestingly, the level of SFAs was the only component showing a significant effect on performance. Two out of the five diets contained either monounsaturated fatty acids (MUFAs; 13.56 g/100g mostly from olive oil) or polyunsaturated fatty acids (PUFAs; 12.08 g/100g mostly from soybean oil), which did not appear to be contributing factors to performance because rats on both these diets performed similarly, but better than those on diets 1 and 2. In the study, SFAs were seen to be the only predictor of cognitive performance because of the rats' stage of brain development. Studies have confirmed that PUFA and MUFA levels are most important during brain development. Once full brain size is attained, SFAs, not MUFAs and PUFAs, are directly correlated with cognitive impairment. For example, in rats fed safflower oil diets, levels of docosahexaenoic acid (DHA) in whole-brain phospholipids were reduced by 80-90% and showed fewer exploratory behaviors compared to soybean-oil-fed control rats (Neuringer, Anderson, & Connor, 1988). Greenwood and Winocur (1993) proposed various mechanisms in analyzing their results. Observed changes in neural membrane composition were observed at varying levels of dietary fatty-acid intake; however, these changes were not associated with SFA intake or changes in behavior. Therefore the changes in behavior may not be a reflection of SFA intake but with the associated essential fatty acids deficiency (below the recommendations of

12g/kg of linoleic acid and 1.3 g/kg of linolenic acid for adult rats). However, results showed that changes in behavior were independent of essential fatty acid intake. Other mechanisms may be related to the different oxidation rates of SFAs and PUFAs, PUFAs undergoing preferential oxidation compared to SFAs. In other words, behavior may be in part modulated by the type of energy supply to the brain, i.e. that coming from PUFAs is preferred (Greenwood & Winocur, 1996).

Studies have shown that the brain adapts to various kinds of fatty acids, which results in changes in neuronal function (Kaplan & Greenwood, 1998). Although the mechanisms for this are elusive, dietary fat influences cognitive performance due to the effect it has on brain development. Even though brain membrane changes and brain fatty acid profile changes were seen in conjunction with cognitive impairment, the two were not correlated suggesting that these brain changes themselves were not causing the cognitive impairment (Greenwood & Winocur, 1996).

Molteni, Barnard, Ying et al. (2002) further investigated the mechanisms by which a HFD impairs cognition. They randomly assigned adult female Fisher rats to a diet high in saturated fat (39% of energy mostly from lard plus a small amount from corn oil) and refined sugar (40% of energy from sucrose) or to a diet low in fat and high in complex carbohydrates (control diet) for 2 months, 6 months, or 2 years. Rats were tested on a water maze task, which assessed spatial memory. Rats on the high-fat and high-refined sugar diet took longer to perform the task than the rats on the diet low in fat and high in complex carbohydrates at all time points. Along with decreased performance, rats on the

high-fat and refined sugar diet showed decreased neuronal plasticity (defined by the authors as the capacity to compensate for challenges, involving cellular and molecular mechanisms of synapse formation and function, neurite growth, and behavioral adaptation) via decreased regulation of brain-derived neurotrophic factor (BDNF), which is normally increased in the hippocampus of animals learning spatial memory tasks. The reductions on BDNF were specific to the hippocampus, which would explain why spatial memory is affected (Molteni, Barnard, Ying, et al., 2002).

2.2.2 Energy Restriction

It is clear that a HFD can have detrimental effects on brain health and function, while also leading to the development of obesity. Yilmaz, Viral, Yilmaz et al., (2011) showed evidence that obesity causes adverse effects on higher-order processing in their study assessing the effects of energy restriction (ER), or seen in the literature as caloric-restriction (CR) by giving rats a 60% reduced standard rat chow diet for 10 weeks. In their study conducted with 38 four-month old male Wistar albino rats, results showed that ER in DIO (fed HFD, 30% of energy from fat) rats improved the efficiency of hippocampal receptors, reduced oxidative stress, and decreased peripheral membrane lipid concentration compared with obese rats on a normal diet. ER also decreased insulin, triglyceride and glucose levels in addition to significantly reducing body weight (Yilmaz, Viral, Yilmaz et al., 2011).

Energy restriction also appears to ease age-related cognitive decline. The effects of age and ER on key synaptic proteins in the CA3 region of the hippocampus have been

determined and it has therefore been investigated whether these changes were related with variations in behavior on a hippocampal-dependent learning and memory task (Adams, Shi, Linville et al., 2008). A group of F1 male F344 × BN hybrid rats (animals widely used for the study of age and ER) were exposed to incremental energy reduction of 10% per week for 4 weeks, reaching full 60% energy restriction by week 17, while others were fed ad libitum. All rats were then evaluated on a Morris water maze task. In terms of body weight, both groups differed significantly. The ER group maintained a stable body weight throughout the study whereas the ad libitum group progressively gained weight, which was expected in this animal species. NMDA and AMPA subunit proteins in the CA3 hippocampal region were all significantly decreased in the ad libitum group compared to the ER group between young (10-12 months) and middle age (18-20 months) and between middle age and old age (29-32 months). ER actually protected against age-related declines in NMDA and AMPA subunits. Both age and diet also had significant effects on the hippocampal-dependent task. Both groups experienced declined performance from young to middle to old age; however the ER group remained stable in their performance from middle to old age whereas the ad libitum group declined. The consistency of performance in the ER group was explained by stabilization of AMPA receptors in the CA3 region due to ER, suggesting that hippocampal activity may depend on the activity of these specific receptors.

Learning and memory are highly affected by both developmental and aging processes. That is, aging is accompanied by a loss of synapses and a reduction in NMDA receptors. The proposition is that ER may protect against these effects by reducing or

slowing aging effects. In a study done by Fontan-Lozano, Sáez-Cassanelli, Inda et al., (2007), 8-week old male Swiss mice were divided into two groups both fed on a standard NIH-07 diet, one of which was fed ad libitum and the other which was fed on alternate days (i.e. ER) for 6-8 months. The rats were assigned to a motor learning, operant conditioning, classical conditioning or object recognition task, which assessed behavioral learning. Similar to other findings, the researchers confirmed that ER restored synaptic plasticity and improved cognitive performance. Rats on ER showed an increase in the NR2B subunit of the NMDA receptor, which mimicked the activity of the NR2B subunit in young mice suggesting that aging was slowed (Fontán-Lozano, Sáez-Cassanelli, Inda et al., 2007).

2.2.3 Ghrelin

Ghrelin, a molecule of 28 amino acids and of blood-brain barrier crossing capacity (Banks, Tschöp, Robinson, et al., 2002; Diano, Farr, Benoit et al., 2006), is an endogenous ligand that stimulates the release of growth hormones from the pituitary (Kojima, Hosoma, Date et al., 1999). Ghrelin is expressed mainly in the stomach and its levels have a relationship with hunger. Ghrelin is an orexigenic hormone that is characterized as an appetite-stimulating hormone; therefore has rising plasma levels before mealtime and lower ones postprandially (Patterson, Bloom, & Gardiner, 2011). The ghrelin system is involved in several functions such as control of food intake, regulation of body weight, insulin release and beta-cell survival, adiposity, and the control of energy homeostasis. Acyl-ghrelin (AG), the natural ligand of the ghrelin receptor has been studied as a participant in the mechanisms of learning and memory.

Studies have shown that AG may alter specific molecular intermediates involved in memory acquisition/consolidation through processes that could include the promotion of synaptic plasticity (Gahete, Córdoba-Chacón, Kineman, et al., 2011). For example, a study by Diano, Farr, Benoit et al. (2006) showed that ghrelin knock-out (compared to their c57/Bl6 wild-type) mice show lower numbers of spine synapses in the CA1 region of the hippocampus and impaired performance in memory tasks, two deficits which were reversed by ghrelin administration (Diano, Farr, Benoit et al., 2006). This study emphasizes the importance of elevated ghrelin levels on the formation of synapses in the hippocampus, thereby improving memory performance. In the same study, ghrelin-administered Sprague-Dawley rats showed improved memory performance while increasing their exploration time of a novel object when 10 μg/kg ghrelin was subcutaneously injected before testing. Davis, Choy, Clegg et al. (2010) studied ghrelin's role in the hippocampus as well. The researchers hypothesized that ghrelin was needed for hippocampal-dependent learning as well as habituated responding for food, which was confirmed by their study comparing ghrelin receptor null mice (GHSR-/-) with their wild type counterparts (age and gender not indicated). The authors assessed fear potentiated learning testing for passive avoidance behavior, spatial learning testing behavior in a Morris water maze, and conditioned locomotor activity testing meal anticipation. Firstly, mice lacking a functional ghrelin receptor were significantly leaner than the wild types. Secondly, both groups learned to avoid foot shock in the fear potentiated learning task. Thirdly, GHSR-/- mice showed impairments in Morris water maze performance in that they took longer to locate the platform, as seen by a reduced number of entrees into the paired quadrant. Lastly, the GHSR-/- mice were unable to

acquire anticipatory locomotor activity associated with restricted feeding. This suggested and confirmed that the hippocampus regulates learning and memory as well as feeding activity, specifically inhibitory control of feeding, which may be based on evidence that food sated animals with hippocampal lesions show increased appetitive responding relative to controls (Davidson & Jarrard, 1993). The fact that GHSR-/- mice were impaired on the hippocampal-dependent spatial task suggests that ghrelin promotes efficient hippocampal activity such as long term potentiation. It has been shown that other than physiological cues that promote feeding, meal anticipation in the context of learning can stimulate feeding as well. In the present study, both groups were able to habituate to the meal-feeding regimen; however, anticipatory increases in locomotor activity antecedent to the habituated feeding response were only present in the wild type group. This suggests that ghrelin participates in food anticipatory activity but not feeding initiation (Davis, Choi, Clegg, & Benoit, 2011).

Diets high in SFAs and refined sugars impair hippocampus-dependent plasticity and spatial memory. In contrast, ghrelin increases spatial memory and learning as well as long term potentiation. It would therefore be plausible to hypothesize that infusion of ghrelin into the hippocampus would enhance synaptic plasticity and spatial memory in animals fed on diets high in SFAs and refined sugars. Chen, Xing, Wang et al. (2011) found that a single infusion (using an Ultra Micro Pump) of ghrelin (5 μL, 1 nM) into the hippocampus of adult male Wistar rats enhanced synaptic plasticity through presynaptic and postsynaptic mechanisms involving the PI3K signaling pathway. In addition, ghrelin also strengthened hippocampus-dependent learning and memory, specifically spatial

memory (Chen, Xing, Wang et al., 2011). Since an infusion of ghrelin demonstrated positive effects on actions that are seen to decline with aging, ghrelin may have therapeutic uses for cognitive impairments due to aging.

2.2.4 Leptin

Leptin has been extensively studied in the context of its key role in the hypothalamus as being involved in energy expenditure and food intake. Leptin is characterized as a peptide hormone that plays a role in the modulation of food intake and energy balance (Farr, Banks, & Morley, 2006). However leptin's role goes beyond the hypothalamus and its primary action of controlling food intake is only one of its many effects (Morrison, 2009). Leptin is well known to be secreted by adipocytes and is also known to have a role in the hippocampus, where it facilitates long-term potentiation and synaptic plasticity by facilitating the NMDA receptor function (Shanley, Irving, & Harvey, 2001) and hence is important for memory processing. Leptin improves memory processing, which has implications for the causes of decreased memory efficiency in obese individuals. Farr, Banks & Morley (2006) showed that leptin improved retention in 4 and 12 month old male SAMP8 mice. The mice were tested on two different avoidance paradigms, the T-maze footshock avoidance and step down inhibitory avoidance. Results showed that injecting 0.5 μg leptin per litre of saline injection into the hippocampus improved memory processing on both test performances. Although the mechanisms were unclear, 12-month aged mice, who had a greater level of impaired learning and memory, required less leptin than 4-month young mice to improve memory suggesting that response to leptin is age-dependent. A reason for this may be that leptin only has beneficial effects at

older ages when leptin receptor deficiencies due to obesity are high enough to produce detrimental memory impairment (Farr, Banks & Morley, 2006). This study indicates that the amount of leptin in the hippocampus after crossing the blood-brain barrier by a saturable transport system has an effect on cognitive processes, specifically memory. In an experiment by Kanoski, Hayes, Greenwald et al., (2011), adult male Sprague-Dawley rats were injected with 1 μg, 2 μg, or 4 μg leptin intra-parenchymally (volume, 100 nl) either to the ventral hippocampal region or dorsal hippocampal region. Leptin administration into the ventral but not dorsal region increased the latency to respond for food in an operant runway paradigm, but suppressed spatial memory consolidation for the spatial location of food (Kanoski, Hayes, Greenwald et al., 2011). Other studies have shown that administration of leptin improves performance on spatial memory tasks, where the target object is an escape platform (Oomura, Hori, Shiraishi et al., 2006). Similarly, a study using 2 leptin receptor-deficient rodents (7 week old male Zucker rats and db/db mice) demonstrated impaired spatial memory performance as well as impaired long-term potentiation thus confirming leptin's role in regulating hippocampal functions of learning and memory (Li, Aou, Oomura et al., 2002).

2.3 Anticipatory Eating

Anticipatory eating is a phenomenon first described by Jacques Le Magnen in 1957. It highlights that simply measuring food intake doesn't tell us anything about eating behavior. Learning is a component of eating behavior in that the pairing of sensory cues from foods with the subsequent nutritional consequences of eating can induce conditioned, or learned responses of food choice and intake. Le Magnen proposed the

term anticipatory satiety- that rats learn to eat a lesser amount of a flavoured food when it is followed by a short fasting period (3.5 h) and preceded by a long fasting period (13 h) than when a different flavoured food is followed by a longer fasting period and preceded by a short fasting period. These findings supported the idea that an acquired meal anticipates the expenditures during the time consistently following the meal (Le Magnen, 1999). The mechanism behind these results is surely one that involves physiological cues. Seeing as the rats most likely didn't learn the difference between different time durations, the rats likely ate more or less depending on their feelings of hunger, thus giving "anticipatory satiety" a more appropriate name- "anticipatory hunger" (White, Mok, Thibault et al., 2001). Although two following attempts failed to replicate Le Magnen's results (Ackroff & Sclafani, 1995; Revusky & Garcia, 1970), that rats increase food intake before deprivation, perhaps because the duration of the short fast was much shorter than that used by Le Magnen. White, Mok, Thibault et al., (2001) further examined Le Magnen's findings. They evaluated intake of a HF (40% of energy from fat) or low fat (4% of energy from fat) test diet in one texture (powder or pellet) offered for 1 h before a long (12.5 h) fast and in the other texture before a short (3 h) fast in male Sprague-Dawley rats. Results indicated evidence for anticipatory eating, in that like in Le Magnen's study, rats learned to eat more before a long fast than before a short fast. These results are interesting because in a classical conditioning context, if the long fast created an aversive feeling, one would expect the rats to eat less of the food preceding such a fast, i.e. conditioned aversion. In White's study, no significant amount of anticipatory hunger was observed in the HFD or low fat group. However a subgroup of rats trained on the HFD, those who ate the most during the first 4 days of training showed significant

signs of anticipatory eating. A possible reason for this could be that these rats gained conditioned preference for the food presented prior to the short fast, i.e. if they ate enough prior to the short fast it would prevent the rise in hunger during the longer fast. In a subsequent study, rats actually learned to eat more of the food predicting the long fast, thereby decreasing the aversive feeling of hunger (Jarvandi, Thibault, & Booth, 2009). This idea of avoidance learning and anticipatory hunger was further examined. Thibault and Booth (2006) evaluated intake before a short fast (3 h) or long fast (10 h) as well. However, in addition, they examined whether anticipatory hunger was reinforced by either carbohydrate or protein-rich test food. The basis behind this study was that different macronutrients caused different physiological effects and thus may have an effect on eating behavior (Thibault & Booth, 2006). For example, glucose provides memory-enhancing effects (Gold, 1986) and that protein is more efficient than carbohydrates at providing satiating effects so as to delay the rise of hunger (Booth, Chase, & Campbell, 1970). Results further substantiated anticipatory hunger- that rats were able to prevent the rise in hunger by learning to eat more of a test food paired with sensory characteristics preceding a long deprivation period than the same test food with different sensory characteristics preceding a short fast. However, the results failed to support a significant difference in learning behavior between the protein- and carbohydrate-rich test foods. The latter studies examined anticipatory satiety/hunger in a context without any component of choice, i.e. the rats had access to a single test food. However, in a natural human environment, individuals are always faced with different food choices and our learning outcomes are a result of the choices made.

Jarvandi, Booth & Thibault (2007) sought to examine anticipatory hunger while including this element of choice, where the test food was either a single mixed food or two foods simultaneously, one protein-rich and the other carbohydrate-rich (Jarvandi, Booth, & Thibault, 2007). Each food was paired with a conditioned stimulus (grape or cherry odorized flavour) and with either a long fast (10 h) or a short fast (3 h). Throughout the 8 cycles of 4 training days, an interesting pattern of results was observed. Early on, the rats displayed a conditioned preference for the flavour paired with the shorter fast because of the less aversive post-ingestion effects of the shorter versus longer deprivation period. However later on, learned anticipatory eating occurred due to the strength of the physiological effects of the longer deprivation period. Because the behavior of increased eating prior to the longer fast diminishes the aversive feelings of hunger, the rats ceased their intake of greater amounts, resulting in self-extinction until hunger returns. This behavioral pattern of extinguished anticipatory hunger improves on negative-feedback homeostasis with a feed-forward hyper-homeostatic mechanism i.e. a refined mechanism that regulates energy exchange (Jarvandi, Booth & Thibault 2007). Additionally, there was no difference between the choice and single test group, indicating that macronutrient selection had no effect. In summary, the findings have evolved to show a distinct learning pattern that can be observed and measured as "L – S", where L is the intake (g) before the long fast and S is the intake (g) before a short fast. A higher L – S score is indicative of learned anticipatory eating.

While fat-rich food may increase satiation in rats, various carbohydrates have been found to have similar effects (Booth, 1972; Booth & Jarman, 1976) Injection of

glucose in the duodenum or hepatic portal vein reduces the amount of food a rat consumes at a subsequent meal. Furthermore, just 0.5 to 1.5 g of carbohydrates (fructose, glucose, or starch) depresses food intake at the first meal after restoring access to food. This evidence suggests that glucose increases the intensity of satiation. However, even though fat-rich food and glucose may share this satiating effect, test-food rich in carbohydrate has not been shown to affect learning in an anticipatory learning paradigm.

2.3.1 Anticipatory Eating and High-Fat Diet

A HFD was introduced into anticipatory eating experiments in White, Mok, Thibault et al.'s study (2001), where rats were fed either a high-fat or low-fat test food in one texture prior to a short fast and in another texture prior to a long fast. The study set out to test for better learning given a HFD as a test food because sufficient caloric intake may be crucial to preventing hunger during a fast. However learned eating could not be differentiated based on the fat content of the test food. On the other hand, a maintenance HFD may weaken learning in anticipatory eating experiments (Jarvandi, Booth & Thibault 2007b). This possible trend allowed for further examination on the effects of HFD on eating behavior. In an investigation of the effect of maintenance on a HFD on the learning of anticipatory eating and food preference, Jarvandi, Booth & Thibault (2007b) found that the overall pattern of learning showed a trough of conditioned preference at cycle 3, a peak of anticipatory eating at cycle 5 and an approach to another peak at cycle 10. Interestingly, unlike previous studies done with regular maintenance chow, standardized peak values were lower, indicating that the HFD attenuated the learning of anticipatory eating in rats. The effects of the HFD may be explained by several possible factors.

Firstly, it may be that HFD reduced the reinforcing effect of hunger on anticipatory eating, which would have resulted in decreased motivation to increase food intake prior to the long fast. Secondly, one must question the state of hunger experienced by the rats. It may be that rats fed the HFD were less hungry and therefore did not show a significant learned response. Food containing a fat content delays gastric emptying (Collier, McLean, & O'dea, 1984) and rats fed a meal of pure fat reduced food intake after a delay due to prolonged satiating effects (Horn, Tordoff, & Friedman, 1996). A possible mechanism behind the increased satiating effects of HFD feeding is fat oxidation, a metabolic response possibly increased in reaction to fasting. It is known that fats, when combined with carbohydrates, are mostly stored whereas the carbohydrates are oxidized for fuel. In contrast, a fat-rich, low-carbohydrate meal shifts oxidation towards the fats and results in a more satiating effect. For example, when rats were fed HFD, they displayed an enhanced capacity to oxidize dietary fat and showed little or no increase in energy intake compared to rats fed mixed diets that were high in fats and carbohydrates (Friedman, 1998). This could also explain why some rats tend to become obese on a HF maintenance diet and why others do not, i.e. some rats may display higher levels of fat oxidation versus storage. Finally, Jarvandi's experiment (2007b) measured plasma leptin levels as well. Interestingly, the learning score for anticipatory eating was not correlated with the level of leptin or weight gain; however leptin was correlated with final weight. The mechanisms behind this are unclear; however, if leptin has been shown to enhance learning scores without HFD as a factor, then perhaps it is the HFD that is attenuating leptin's effects.

2.4 Glucose and Memory

Glucose is the main energy source of the brain. If the brain needs its glucose supply to maintain efficiency, it would be plausible to suggest an increased amount of glucose to the brain would increase the brain's efficiency. More specifically, as discussed above in section 2.2, the hippocampus is an important structure associated with the modulation of memory. Studies have shown that animals tend to perform better on a memory task when the task is associated with a sweet taste, glucose being the most effective (Ackroff, 2008). The research on glucose and memory has been extensive for both animals and humans. Hall and Gold found that injecting glucose (100-1250 mg/kg) subcutaneously into memory-deficit Sprague-Dawley rats (300-450 g) restored memory (Hall & Gold, 1990) and Benton and Owens found the consumption of a 50 g glucose drink was associated with better memory in young adults (Benton & Owens, 1993). Although these results are generally stated, the effect of glucose on memory is rather specific in terms of time, task and dose (Yiin, 2004). In terms of timing, in elderly adults (61-80 years) 50 g glucose enhances memory when administered pre- and post-training as well as prior to memory retrieval (Manning, Parsons, Cotter, et al., 1997). Glucose-administrated memory-enhancement is also task-specific. Since glucose has a direct effect on the hippocampus, glucose facilitates memory tasks associated with the hippocampus. For example, Manning, Pearson, Carter et al., (1997) found that glucose administration enhanced declarative memory in humans, but not nondeclarative. Humans are not alone in the effects of glucose seen on memory, rodents experience memory-enhancement as well whether on a spatial memory or inhibitory avoidance conditioning retention task (Gold, 1986; Korol & Gold, 1998). Dose is also an important factor that has been considered

when looking at glucose and memory. The memory-enhancement is seen to follow an inverted-U dose-response curve with optimal amounts being 100 mg/kg and 2 mg/kg (Messier, 2004; Parsons & Gold, 1992).

Mechanisms underlying the enhancement of memory via glucose administration have been extensively examined and still remain unclear; however a range of mechanisms have been considered. In a review by Messier (2004), the two leading hypotheses are examined: that of a central mechanism as well as a peripheral one. The evidence that injection of glucose into the brain improves memory is demonstrative of a central mechanism. This has been explained by the possibility of the glucose injections targeting specific brain areas or pathways, i.e. via cholinergic mechanisms by increasing acetycholine synthesis (Kopf & Baratti, 1996). Alternatively, peripheral mechanisms are also explored in terms of the importance of the liver being the controller of blood glucose levels. Glucose-responsive neurons located in the liver send messages to the brain to stimulate glucose's memory-enhancing actions. Taken all together, there is substantial evidence that glucose is influential to the brain both centrally and peripherally (Lieberman, Kanarek, & Prasad, 2005).

2.5 Insulin and the Brain

Glucose cannot be discussed without mentioning its association with insulin. If an increase in blood glucose has effects on the brain then the associated release of insulin may have a neurological effect as well. Insulin has been show to be found in high levels in the brain, contain its own receptor systems distributed within the brain, mainly in the

olfactory bulbs, hypothalamus, hippocampus, cerebellum, and cerebral cortex, be associated with cognition and cognitive deficits when changed in level or sensitivity, and be involved in glucose uptake in the brain via insulin-sensitive GLUT 4 receptor (Lieberman, Kanarek & Prasad, 2005; Park, 2001).

The impact of insulin on the brain has been studied; however, the effect is not clear and has been seen to have contrasting evidence. On one hand, it has been shown that the injection of insulin impaired retention of an inhibitory-avoidance task (Kopf & Baratti, 1996; Schwarzberg, Bernstein, Reiser, et al., 1989). On the other hand, this effect was not seen on the performance on a radial arm maze task (Blanchard & Duncan, 1997). Additionally, insulin improves performance on a Morris water maze spatial task and prevents memory loss and impairment of synaptic plasticity in rats (Biessels, Kamal, Ramakers et al., 1996; Biessels, Kamal, Urban et al., 1998; W. Zhao, Chen, Xu et al., 1999). Therefore, it seems as though the effects of insulin on performance may be dependent on the type of task (Park, 2001). Another factor that may play a role is the dose of insulin administered. Perhaps only some tests yielded positive effects of insulin because these contained a large-enough dose to cross the blood-brain barrier. Doses provided in the study by Kopf & Baratti (1996) for example, provided enough insulin to induce hypoglycemia, which was attenuated by an injection of glucose. Therefore, it may be the hypoglycemia that is causing the negative effect on performance. For example, previous results showed that insulin-induced hypoglycemia impaired T-maze learning in rats (Clayson, 1971).

It remains unclear why administrations of glucose have been demonstrated to enhance memory, while injection of insulin may or may not have this same effect. The

answer may lie within the proposed mechanisms by which insulin may modulate memory function. Insulin injections lower blood glucose concentrations, which could alter brain cholinergic functions and acetylcholine synthesis in the hypothalamus (Gibson & Blass, 1976). Whenever learning and memory are being investigated, the hippocampus is inevitably involved. Increases in insulin have been shown to correlate with an upregulation in synaptic plasticity, enhancement of NMDA receptor activity, and modification of neurotransmitter release processes (Zhao & Alkon, 2001). Furthermore, if glucose infusion has been seen to enhance learning and memory performance, and HFD has been shown to impair it, then perhaps the negative effect of HFD on learning is due to insulin resistance, because in an insulin resistant state, glucose uptake is decreased and cellular function is impaired due to neuronal insensitivity to insulin.

2.6 Reward

Learning is involved in the reward process of eating behavior. Instrumental behavior is learned by a response being associated with a reward, i.e. a positive reinforcement. In other words, the response predicts the positive reinforcement, thereby strengthening the response-reward contingency. Anticipatory eating is a hunger-reinforced instrumental behavior. When rats lack food for a long deprivation period, they experience hunger as a form of negative reinforcement. Rats then learn to eat more prior to a long fast to avoid these feelings of hunger. As a result, food has the potential to be seen as a reward. The reasons for eating are vast. Aside from eating due to energy needs, food availability, time of day, boredom and stress, the reward/palatability aspect of food plays a role as well (Levine & Billington, 1997). Preferences are also a driving factor for eating and it is possible that preferred foods are those that provide pleasure or positive reward value. In

other words, eating may not always be driven by nutritional needs, but by the hedonic value of food (Lieberman, Kanarek & Prasad, 2005) or the motivational value attributed with conditioned incentive stimuli (Berridge & Robinson, 2003). Although tempting to refer to the hedonic value of food as "food reward", it may not be the most appropriate. Berridge and Robinson (2003) explain that sensory facilitation or hedonic value [of food] is evidence only of incentive, which may be a result of classical conditioning, where an animal learns to associate unconditioned stimuli with a positive reinforcement.

2.6.1 Neuropeptide Y

Neuropeptide Y (NPY) is one of the earliest peptides identified and is a 36-amino-acid peptide found throughout the central nervous system. NPY, part of pancreatic polypeptide-fold family of regularity peptides, may be involved in the control of feeding behavior. Data shows that an increase in NPY produces an increase in food intake initiating meals. For example, Lynch, Grace, Billington et al. (1993) showed that lateral ventricle injection of NPY stimulated the consumption of normally-palatable sucrose and saccharin solutions and Sprague-Dawley male rats (225-340 g) preferred one of two flavours that was associated with NPY during training (Lynch, Grace, Billington, et al., 1993). Consistent with these results, NPY injection into the hypothalamic paraventricular nucleus (100 pmol/0.3 μl) also increased carbohydrate intake in size and duration of the meal in adult male Sprague-Dawley rats (350-375 g) (Leibowitz & Alexander, 1991). Together the evidence indicates that while NPY injections stimulate feeding behavior, carbohydrate intake is specifically increased and preferred over a high-fat or high-protein diet (Morley, Levine, Gosnell, et al., 1987). NPY has been shown to increase fat storage

and decrease energy expenditure (Woods, Seeley, Porte, et al., 1998). In other words, if NPY increases carbohydrate intake versus fat intake, then fat storage will likely increase, as seen with the effects of mixed meals described above. However, a review paper has shown that NPY does not consistently stimulate the intake of carbohydrates (Thibault & Booth, 1999). The hedonic effect of NPY may be an important aspect in terms of the development obesity in that it has a significant effect on energy homeostasis and metabolic response. Furthermore, data suggests that NPY may increase the rewarding aspect of food (Adam & Epel, 2007) and thus can produce obesity by increasing total daily food intake, specifically with a preference for carbohydrates (Stanley, Kirkouli, Lampert et al., 1986).

2.6.2 Opioids

Endogenous opioids represent another family of peptides related to the reward aspect of food, which affects food intake. The relationship between opioids and the ingestion of sweet foods has been a popular field of study and evidence suggests that opioids increase intake of sweet solutions (Levine, Weldon, Grace, et al., 1995). Evidence also shows that opioids may be involved in protein appetite, i.e, given an opioid (e.g. morphine) there is a greater intake of protein after a period of protein-deprivation but not after carbohydrate-deprivation (Thibault & Booth, 1999). Much evidence investigating opioid-related feeding has been done using the opioid antagonists naloxone and naltrexone. For example, a study that measured the hedonic properties of a food in Sprague-Dawley rats found that based on results from a taste reactivity test (assesses the palatability of a tastant), naltrexone reduced the hedonic properties of a sucrose solution (Parker, Maier,

Rennie, et al., 1992). Furthermore, Levine, Weldon, Grace et al. (1995) found that 0.3-3mg/kg doses of naloxone decreased intake of sweet chow in food deprived male Sprague-Dawley rats much more effectively than normal chow, indicating the specificity of opioids and sweet-tasting foods. Additionally, extremely low doses (0.03 mg/kg) of naxolone were associated with decreased sweet-food intake by 50% in satiated rats. The targeted action of opioid antagonists to sweet-tasting foods suggests that opioids affect intake of preferred diets (Lieberman, Kanarek, & Prasad, 2005). Opioid-related reward feeding may be so strong that even with paired NPY-induced feeding, opioid antagonists are able to reduce feeding of preferred foods while not altering the feeding of non-preferred foods (Glass, Grace, Cleary et al., 1996). These results support the role of opioids in the reward aspect of feeding independent of macronutrient composition. Not only do opioid peptides increase intake for preferred foods, the ingestion of palatable/preferred foods alters peptide levels and opioid receptor gene expression (Lieberman, Kanarek, & Prasad, 2005). Levine and Billington (1997) report evidence suggesting that prolonged exposure to palatable foods increases β-endorphin release in the hypothalamus of rats.

Any abnormalities in the opioid peptide system may be linked to obesity due to elevated preference and intake for highly fatty and sweet foods (Drewnowski, Krahn, Demitrack, et al., 1992). Furthermore, opioids may influence energy intake by mediating the pleasure or reward response to foods (Le Magnen, 1990). Thus efforts to counter the opioid system with antagonists, e.g. naloxone, could have importance in decreasing obesity.

Bridge

The literature shows that HFD impairs learning and memory performance on various learning and memory tasks. Furthermore, HFD affects body composition by increasing weight gain and risks for metabolic disorders. A wide array of hormones are involved in HFD's effect on the body and brain, i.e. insulin, leptin, ghrelin as well as various peptides relating to the reward system.

The body of evidence on anticipatory eating includes preliminary evidence that a HFD maintenance diet may attenuate learning of such a paradigm. However no work to date has tested anticipatory eating using a maintenance HFD, with one fasting length and without differential cuing. Therefore, the main objective the Manuscript was to address this question, while also looking at the effects of excess body weight and fat on learning performance.

CHAPTER 3, MANUSCRIPT

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Food reward is attenuated in HFD induced obesity with high gain in weight and fat mass

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3.1 Abstract

Objective: To investigate the effect of high-fat maintenance diet on body composition and learning of anticipatory eating in rats.

Methods: Thirty young adult (8 wk) male Sprague-Dawley rats were obtained from Charles River Laboratories. During a 16-day adaptation period, rats were adapted to the environment and given ad libitum access to standard ground Purina chow. Rats were then randomly assigned to one of two experimental groups, and received ad libitum of either a normal fat (control), or a high-fat diet (HFD) for a 37-day, pre-training period. Rats were then trained for anticipatory eating over a 20-day period. Whole body composition and abdominal region composition was determined using dual-energy x-ray absorptiometry (DXA) at three time points: baseline, pre-training and post-training. Daily body weight, body composition variables and learning of anticipatory eating were tested using repeated measures ANOVA.

Results: HFD rats were grouped based on body weight and fat gain post training. Pre-training and post-training, total body fat and abdominal fat did not differ in high body-weight gainers and low weight gainers, but were significantly greater compared to controls. Because weight gain did not correlate with fat gain, HFD rats were grouped into high weight/high fat gainers, high weight/low fat gainers, low weight/high fat gainers, and low weight/low fat gainers. Learning of anticipatory eating was evident in all rats as indicated by the upward linear and peak-trough-peak (cubic) pattern. Speed of learning, as indicated by the proportion of first peaks in the earlier cycles was greater in the group maintained on the HFD as a whole. However, within the HFD group, those who gained the most amount of fat (g), regardless of amount of weight gained, showed slower

learning. Numerical evidence of reward was indicated by significant diet and cycle effects, with highest reliability observed in the low-weight, low-fat gainer group. ***Conclusions:*** These data suggest that body fat mass classification enhances the interpretation of learned behavior of food intake in rats fed a HFD. The group that gained the least amount of body fat and weight were similar in body composition to controls, indicating the HFD may be a factor involved in learning patterns. In contrast, the low weight/low fat gainers learned quicker than their HFD counterparts.

Keywords: Anticipatory eating; High-fat diet, Long-delay, Learning, Food reward, Obesity

3.2 Introduction

First described by Jacques Le Magnen in 1957 (1999) rats showed the ability to learn to eat more before protracted withholding of food (Jarvandi, Thibault & Booth, 2009; Jarvandi, Booth & Thibault, 2007a; Thibault & Booth, 2006; White, Mok, Thibault et al., 2001), an effect called anticipatory eating. Previous pilot work displayed preliminary evidence that a high-fat diet (HFD) impaired learning of anticipatory eating (Jarvandi, Thibault & Booth, 2007b).

In a reanalysis of the raw data from previous experiment, it was proposed that this anticipatory eating was reinforced positively by the effects of nutritional repletion when access to maintenance food is restored, rather than negatively by effects of deprivation as was suggested earlier, and also that the acquired eating was instrumental rather than classically conditioned responding (Booth, Jarvandi & Thibault, 2012; Jarvandi, Booth & Thibault, 2012). This interpretation of food as reward over long delays has been advocated on the grounds that is mechanically simpler and fits better to other types of evidence. The reanalysis also indicated that learning may be poorer when fat is added to the maintenance food (Jarvandi, Thibault & Booth, 2007b; Booth, Jarvandi & Thibault, 2012).

During the deprivation-induced deficit in flow of energy to lean tissues (Toates & Booth, 1974), fat becomes a major fuel (Horowitz, Kaufman, Fox et al., 2005). Chronically high intakes of fat, and possibly the resulting insulin resistance, enhance the oxidation of fat (Schrauwen, Van Marken Lichtenbelt, Saris et al., 1997; Spriet, Dyck, Cederbald et al., 1992). In addition, there is a positive association between fat oxidation and satiety (Friedman, 1998; Le Magnen, Devos, Gaudilliere, et al., 1973). Therefore,

high-fat feeding potentially alters the metabolic response to fasting and suppresses the depletion-related rise in hunger, the motivation to eat.

Metabolism of fat may also affect the process itself of learning to eat anticipatorily. Rats chronically fed a HFD are impaired relative to rats fed standard laboratory chow in performance on a wide range of memory and learning tasks (Chambers, Heiman, Clegg et al., 2006; Greenwood & Winocur, 1990; Winocur & Greenwood, 1993). Such adverse effects on brain function are attributable in part to central insulin resistance resulting from high-fat feeding (Gerozissis, 2004; Greenwood & Winocur, 2005), even without the development of obesity (Woods, D'Alession, Tso, et al, 2004). Feeding on a HFD plays a role in the development of insulin resistance and brain mitochondrial dysfunction, which may cause impairments of synaptic plasticity (Pipatpiboon, Pratchayasakul, Chattipakorn et al., 2012). The "stress" that a high-fat, or Western type, diet places on the hippocampus has been proposed via various mechanisms including impaired glucoregulation, reduced levels of neurtotrophins, neuroinflammation, and changes in the structural integrity of the blood-brain barrier (Kanoski & Davidson, 2011). Valladoilid-Acebes, Stucchi, Cano et al. (2011) found that a HFD impaired learning performance, and found that independent of important markers of obesity (e.g. hyperglycemia/hyperinsulinemia), dietary intake could impair learning on its own. Consequently, the question whether a HFD impairs learning regardless of the development of obesity is approached in the current study.

Learning of anticipatory eating depends on the demanding predictive processes of associating the eating of a distinctive diet with metabolic processes occurring some hours later. However, the effects of HFD on the learning processes that control food intake remain largely unexplored. This paper presents data derived from a new experiment that

investigated the effect of maintenance on a HFD on the learning of anticipatory eating and body composition.

3.3 Method

3.3.1 Animals and Diets

Thirty, eight-week old male Sprague-Dawley rats weighing 226-246 g were obtained from Charles River Laboratories (St-Constant, Quebec). Rats were housed individually in wire-mesh cages maintained under controlled temperature (22-25^{0}C) and humidity (40-67%), on a 12:12 dark-light cycle with lights off from 11:00 h to 23:00 h for the duration of the study. During an initial 16-day adaptation period, rats were adapted to the environment and given ad libitum access to standard ground Purina chow (3.4 kcal/g; Charles River rodent chow 5075, St-Constant, Quebec) and tap water.

Following the 16-day adaptation period, rats were randomly assigned to two experimental groups, and received either a normal fat (control), or a high-fat (HFD) maintenance diet. Rats assigned to the normal fat diet (n=10) were given ground Purina chow (described above) ad libitum. Rats assigned to the HFD (n=20) were given 80% (g/100g) ground Purina chow and 20% (g/100g) butter ad libitum (Lactantia My Country Unsalted Cultured Butter, Parmalat Canada, Victoriaville, Quebec); 40% of energy from SFA rich fat. The control diet (12% of energy from fat [0% from SFA], 21% from protein, 67% from carbohydrates) and the high-fat diet (42% of energy from fat [23% from SFA], 14% from protein, 14% from carbohydrates) contained 3.4 kcal/g and 4.2 kcal/g, respectively. Both groups were given 24 h access to their maintenance diet and tap water ad libitum for 37 days. Food intake and the weight of each rat were measured daily

at 09:00 h using a digital balance with animal feature (Mettler PJ 1600 balance, Zurich, Switzerland).

3.3.2 Body Weight and Composition

Throughout the 37 days of the maintenance period, body weight (g) was recorded daily for all the rats. These data provided three measures of gain in weight: total weight gain (g), mean daily weight gain (g/day), and percent weight gain. A fourth measure was final weight, measured on day 53 or the last day preceding conditioning.

On the twelfth or thirteenth day of the adaptation period, the maintenance diet was removed from 06:30 to 09:30 h, rats were anaesthetized using isofluorane gas (Baxter International, Inc., Mississauga, Ontario) and their baseline whole body and abdominal composition was then determined in the prone position using dual-energy x-ray absorptiometry (DXA; day 12 or 13 of adaptation period; baseline) (Hologic 4500A QDR Version 11.2, Hologic Inc., Bedford, Mass., USA) using small animal software. Duplicate or triplicate DXA scans were conducted at each time point on each rat. Length from tip of nose to anus was also measured in the anesthetized state. The other variables used for analysis were total mass (g), total body fat (g and % of total mass), total lean body mass (g LBM), total bone mineral content (g BMC), total abdominal mass (g), abdominal fat (g and % of total abdominal mass), abdominal BMC (g), total lean mass (g), abdominal lean mass (g) and abdominal fat (% of total body fat) each measured by DXA at three time points: baseline (day 12 or 13 of adaptation period), pre-training (day 33 or 34 of maintenance diet feeding), and immediately following the end of training

cycles (day 59 or 60 of post-training). Fat pad mass (i.e. abdominal, retroperitoneal, and epididymal) was measured in grams when rats were terminated (day 61 and 62).

Rats in the HFD group were then categorized as high weight gainers and low weight gainers based on weight gain in grams per day over the 37 day maintenance period. Rats that gained between 4.1 to 5.1 g/day of body weight were categorized as low weight gainers (n=8) and rats that gained between 5.4 to 6.4 g/day of body weight were categorized as high weight gainers (n=12). Rats that gained between 26.9 to 34.0 g/day of abdominal fat were categorized as low-fat gainers (n=10) and rats that gained between 36.6 to 47.4 g/day of abdominal fat were categorized as high-fat gainers (n=10). With these four categorizations, the HFD group had 4 subgroups: high weight/low fat gainers (n= 4), low weight/low fat gainers (n= 6), low weight/low high fat gainers (n= 6), and high weight/high fat gainers (n= 4).

Following the last DXA analysis animals were killed at 11:00 h in a CO_2 chamber. Abdominal, retroperitoneal and epididymal fat was excised then weighed using a digital Mettler PJ 1600 balance to nearest gram.

3.3.3 Training for Anticipatory Eating

Rats were trained to eat in anticipation of food deprivation over ten cycles of two days each. On day 1 of a cycle, the maintenance food and water were withheld for 3 h at the start of the dark cycle (11:00 to 14:00). Rats were then given the experimental food of carbohydrate solution presented in standard water bottles for 1 h. This solution consisted

of maltodextrin (Bio-Serv, Frenchtown, New Jersey) at a concentration of 33 g /100 ml of solution, dissolved in distilled water. Experimental food intake was measured by weighing the test food before and after presentation (Mettler PJ 1600 balance). The bottle of maltodextrin solution was replaced with a bottle of water and all food was then withheld for an 8-h fasting period. At the end of the fast, the weight of each rat was recorded and rats were given ad libitum access to their respective maintenance diets and the intake of the maintenance diet was recorded 1 h after presentation. On day 2, rats were given ad libitum access to their respective maintenance diets for 24 h.

3.3.4 Intake Measures

Daily measures of food intake were used to compute mean intakes (LS means) and standard errors for each cycle for each group (control and HFD). Also the initial intake of maintenance diet at the end of the 8 h fast following the training period was measured after access for 1 h.

To compare the energy consumption of both groups, the amounts of maintenance food consumed in grams were converted to kilocalories (kcal) by multiplying intake (g) by 3.4 kcal per gram for the control diet and 4.2 kcal per gram for the HFD.

3.3.5 Analysis of data

3.3.5.1 Food Intake

The statistical analyses were carried out using SAS version 9.2 and 9.3. A probability level less than 5% was considered significant.

The amounts of maintenance food consumed (g) during the 37 days maintenance period prior to training, intakes (g) of test food during access for 1 h prior to the 8 h fast and initial intake of maintenance diet during 1 h access after fasting were compared between the control group and HFD group across days by repeated measures analysis of variance (ANOVA) using the PROC MIXED procedure. Orthogonal polynomial contrasts were used to analyze test food intake before the fast across cycles for linear, quadratic, cubic and quartic trends. Multiple pairwise comparisons were evaluated for statistical reliability using Scheffé's test.

The relationship between food intake (g and kcal) and body weight gain (g) was evaluated first using a model for a completely randomized design. Then an analysis of covariance was conducted, fitting two regression coefficients and a classification variable, and their interaction.

3.3.5.2 Body Weight and Composition

The weight recorded during the 37 days maintenance period prior to training as well as each body composition variable recorded at baseline, pre-training and post-training were analyzed identically to the food intake data described in section 3.3.5.1.

The Shapiro-Wilk (S-W) test was conducted to assess departure from normality of each measure. $P > 0.01$ was taken to mean that the data were not reliably different from normally distributed. All variables were found to be normal, therefore no data transformation were required.

A one-way analysis of variance (ANOVA) was used to compare data for each body composition variable (i.e. body fat, abdominal fat, total, total lean body mass, total bone mineral content, total and abdominal mass), fat pad mass, initial weight, final weight, percent weight gain, total weight gain, and weight gain in grams per day between all 30 rats, at different time points (i.e. baseline, pre-training, post-training).

3.3.5.3 Learning for Anticipatory Eating

The hypotheses about the strengths of learning were tested by various chi-squared tests to check for differences in peak intakes across cycles and groups. Peak intakes during the training period were identified from cycle 3 to 10 using a threshold of 2.0 g, i.e. the logically minimum criterion for a peak was at least 2.0 g greater than two successive preceding days. Statistical significance of the peaks was analyzed using Bonferroni's pairwise comparisons.

To test the hypotheses about the speed of learning, first peaks and troughs that occurred over the eight cycles were counted. This analysis was computed twice, once using the criterion of being greater than 2 grams and again using a criterion of being greater than 1 gram for an analysis of sensitivity. Peaks and troughs were counted as significant if they fell under the criteria and if they were followed by an inflection in intake (i.e. a fall after a peak or a rise after a trough). Data was analyzed using survival analysis, i.e. PROC LIFETEST to analyze the timing of peaks for each rat (control of HFD) over eight cycles.

To test the hypotheses for how the learning occurs, intakes and correlations at every cycle were observed, not just at peaks. For the 'reward' hypothesis, the variables entering the analysis were rats' intakes of maintenance food at the 1st hour of refeeding on one trial and intake of test food on the next trial, while adjusting for cycle. For the 'satiety' hypothesis, the variables entering the analysis were rats' intakes of maintenance food at the 1st hour of refeeding and the intake of test food earlier that same day/trial, while adjusting for cycle. All the latter correlations were computed across cycles using PROC CORR. Means, medians and numbers of counts (k) were computed for each group and for the total. Identical analysis was computed to obtain a correlation value for each cycle (3-10) while adjusting for rat. Finally, identical analysis was computed in each cycle across each subset of rats, i.e. obtaining a correlation value for each rat in each cycle. Statistical significance of differences was evaluated using ANOVA with the PROC GLM procedure.

All latter analyses testing learning of anticipatory eating were computed to compare five groups: control, high weight gainers/low fat gainers, low weight gainers/low fat gainers, low weight gainers/high fat gainers, and high weight gainers/high fat gainers.

3.4 Results

3.4.1 Food Intake During the Maintenance Period

The control group consumed significantly more chow (g) relative to the HFD group's consumption (30.64 ± 0.87 g, 28.59 ± 0.67 g, respectively), main effect $F(36, 937) = 4.92$, $P<0.0001$. However, the HFD group consumed significantly more energy (kcal) relative to the control group (120.08 ± 2.80 kcal, 104.17 ± 2.97 kcal, respectively),

main effect F (36, 937) = 5.13, P<0.0001. The significant interactions between diet and day indicated, that the dietary intakes differed, but that the size of the difference was not the same across all days.

When looking at the relationship between total food intake (g or kcal) and total weight gain over 37 days, the effect of diet on weight gain was significant, main effect F= 15.46, P= 0.0005 (g) and F=12.85, P= 0.0013 (kcal) indicating that there was a significant difference in weight gain between the control and HFD group, i.e. the type of diet consumed (control or HFD) had a statistically significant effect on weight gain or how much weight a rat gained at the end of the maintenance period (Figure 3.1).

Changes in rat weight over time are not the same for all diet treatments (i.e. chow or HFD; P=0.0006). There was no significant difference in initial weight and weight up to the 21st day, between the control group (LS mean =373.8 ± 5.5) and HFD group (LS mean= 366.4 ± 3.9); P= 0.2816. Body weight was significantly different between the control group (LS mean= 527.4 ± 8.9) and the HFD group (LS mean= 559.2 ± 7.9) within the 22nd day and everyday thereafter until the 37th day of the maintenance period (P= 0.0192), main effect F (36, 1007) = 1.97, P= 0.0006. The majority of this extra weight gain on the high fat diet was in fat: their percent gain in body fat was 28.4% ± 1.1 g, whereas fat gain in controls was 23.4% ± 1.5 g.

3.4.2 Body Composition

Changes in each body composition variable were not the same for all groups of rats (i.e. chow vs. HFD low gainers vs. HFD high gainers).

At baseline, total BMC (g), was significantly lower in HFD fed rats (LS mean= 7.92 ± 0.10) than control rats (LS mean= 8.30 ± 0.15), P= 0.0464. At pre-training, following 37 days feeding with control diet or HFD, total body fat, total mass, abdominal fat (% of total abdominal mass and % of total body fat), and total abdominal mass were significantly greater in the HFD group compared to the control group (Table 3.1). The body composition variables with significant differences between the control group and HFD group reported at pre-training were repeated post-training, with greater differences.

At pre-training, the variables, total lean mass (g), total BMC (g), total mass (g), abdominal lean mass (g), and total abdominal mass (g) were significantly greater in the high gainers compared to low gainers (Table 3.1). Additionally, the variables, total body fat (g), total BMC (g), total mass (g), abdominal fat (g, % of total abdominal mass, and % of total body fat), and total abdominal mass (g) were significantly greater in the high gainers compared to the control group (Table 3.1). Post-training, total lean mass (g), total BMC (g), total mass (g), and abdominal BMC (g) were significantly greater in the high gainers compared to the low gainers (Table 3.1). Post-training, total body fat (g and %), total BMC (g), total mass (g), abdominal fat (g, % of total abdominal mass, and % of total body fat), total abdominal BMC (g), and total abdominal mass (g) were significantly greater in the high gainers compared to the control group (Table 3.1). As well, total body fat (g and %), and abdominal fat (g, % of total abdominal mass, and % of total body fat) were significantly greater in the low gainers compared to the control group (Table 3.1). Overall, there was a highly statistically significant Pearson correlation coefficient of 0.910 ($P < 0.0001$), which indicated a strong association between abdominal fat

determined by DXA post-training (40.6 ± 4.9 for controls and 66.3 ± 3.4 for HFD) and abdominal area fat pad mass (37.8 ± 3.3 for controls, 55.3 ± 2.3 for HFD).

3.4.3 Learning for Anticipatory Eating

3.4.3.1 Effects on test-food intake of subsequent deprivation

Previous experiments on anticipatory eating have shown that the learning of extra intake goes through peaks and troughs: an acquired increase in intake appears to reduce the effect of the subsequent period of food deprivation, including a decline in intake which reverses when intake becomes low enough to restore the full force of the deprivation. This oscillation in test intake was replicated across the ten cycles of training in the present experiment, main effect of cycle $F(9,199) = 27.85$, $n = 30$, $P<0.0001$ (Figure 3.2). However, this overall measure did not give a reliable effect of maintenance diet $F(1,28) = 1.5$, $P= 0.221$, nor of interaction between Diets and Cycles $F(9,199) = 1.4$, $P= 0.4124$.

Significant peaks were present at cycle 3 and cycle 4 for both groups (Table 3.2) and again at cycle 5 for the control group (Table 3.2), whereas the HFD group peaked again at cycle 6 and again at cycle 10 (Table 3.2). Additionally, both groups displayed a negative trough (negative peak), i.e. decreased intake at cycle 8 (Table 3.2). This pattern of intake may be the reason for the quadratic [$F(1, 89) = 48.49$, $P < 0.0001$] and cubic trends [$F(1,151) = 6.10$, $P = 0.0146$].

When the HFD group was separated into high-gainers and low-gainers in weight, and into high weight gainers/low fat gainers, low weight gainers/low fat gainers, low weight

gainers/high fat gainers, and high weight gainers/high fat gainers, there was still no statistical interaction of groups with cycles in the intake of test food before the 8h fast. Hence the same general increase over cycles was seen $F(9,192) = 27.99$, $P<0.0001$ (Figure 3.2), with the linear [$F(1,153) = 165.96$, $P<0.0001$], quadratic [$F(1,85.9) = 48.49$, $P<0.0001$) and cubic trends [$F(1,145) = 6.10$, $P= 0.0067$]. After the peaks at cycles 3 and 4, the high-weight gainers showed only one peak, at cycle 6 (Table 3.2), whereas the low-weight gainers show peaks at cycles 6, 8, and 10 (Table 3.2). A trough present and is needed to explain a cubic trend, $F(1, 145) = 6.10$, $P = 0.0067$, which is the pattern for learning and extinction of anticipatory eating.

3.4.3.2 Compensation, reinforcement and extinction by initial maintenance intake

The intake (g) of maintenance diet during 1 h of access following the 8 h fast after testing increased for the control and HFD groups as indicated by the significant effect of cycle and the linear trend upwards [$F(9,205) = 8.66$, $P<0.0001$ and $F(1,64.4) = 36.26$, $P<0.0001$, respectively; Figure 3]. No statistically significant main effect of diet [$F(1,28) = 0.04$, $P= 0.8446$] or diet by cycle interaction [$F(9,205) = 1.14$, $P= 0.3363$] was seen. There was a quartic trend, attributable to increases in intake at cycles 2 and 9 in each group.

When energy intake was considered, there were main effects of diet [$F(1,28) = 5.95$, $P= 0.0213$] and cycle [$F(9,205) = 7.79$, $P<0.0001$] on initial intake after the 8 h deprivation, with the HFD group (LS mean= 39.45 ± 2.05 kcal) ingesting more energy than the control group (LS mean= 32.41 ± 2.90 kcal). Besides the linear trend [$F(1,64.6) = 33.70$, $P<0.0001$], there was a quartic trend [$F(1,228) = 4.36$, $P= 0.0374$]. This was

attributable to the peaks and trough(s) present. The control group had only one significant peak in energy intake, at cycle 9 (Table 3.2). The HFD group had a trough at cycle 3 and peaks at cycles 5, 8 and 9 (Table 3.2). That is, whereas the control group on plain chow showed a steady increase in initial repleting energy intake over cycles, the HFD group's energy consumption fluctuated considerably.

Initial intake of maintenance diet showed no main [F (2,27) = 3.35, P= 0.05] or interaction effects [F (18,21) = 0.94, P= 0.534] between high-gainers and low-gainers in the HFD group, except a main effect of group in energy intake [F (9,198) = 9.37, P<0.0001], as expected of a difference in weight gain. Same significant effects were seen when the HFD was classified into their subgroups varying in weight and fat gain.

The number of peaks over ten cycles is a measure of the speed of learning. The timing of the first peak is a more direct measure and so may be more sensitive. The proportion of first peaks in the earlier cycles (3 to 5) was greater in the group maintained on high-fat diet as a whole, X^2 (7) = 16.60, P<0.0202 (Table 3.3) and separately in both low and high weight gainers. Survival analysis of time to first peak displayed the same numerical differentiation between the control group and the whole HFD group (Figure 3.3) and high-gainers and low-gainers separately; however, these differences were not reliable, X^2 (1) = 0.3225, P= 0.5701.

3.4.3.3 Satiating effect of test food intake on initial refeeding

Satiety r values, i.e. correlations between rats' intakes of maintenance food at the 1st hour of refeeding and the intake of test food earlier that same day/trial were reliable across trials in the low weight/low fat gainers, 0.616 ± 0.1244 g, P < 0.0001, and the high

weight/high fat gainers, 0.4331 ± 0.1244 g, P= 0.0015. The low weight/low fat gainers showed significant differences in satiety r values from the control (P= 0.0295) and the low weight/high fat gainers (P= 0.0026). There was no statistically reliable effect of cycle as a whole, F (8)= 1.98, P= 0.0818); however, cycles 2 (LS mean= 0.503 ± 0.167 g), 3 (LS mean= 0.432 ± 0.167 g) and 4 (LS mean= 0.583 ± 0.167 g) displayed numerical differentiation (P=0.0050, 0.0144, 0.0014, respectively).

3.4.3.4 Rewarding effect on test food intake from initial intake on refeeding

Each of the high weight gain groups (LS means= 0.3394 ± 0.1189 g and 0.3897 ± 0.1189 g) and the group of low weight gainers with low fat gain (LS mean= 0.6643 ± 0.1189 g) had highly reliable correlations between refeeding intake and the subsequent trial's test food intake (P= 0.0075, 0.0025, <0.0001, respectively). This index of reward, i.e. correlations between rats' intakes of maintenance food at the 1st hour of refeeding on one trial and intake of test food on the next trial from refeeding was numerically substantial in the control group (r = 0.124) but not reliable (P>0.3). Again, the low weight/low fat gain group showed significant differences from the control (P= 0.0298) and low weight/high fat gainers (P= 0.0014), i.e. showing the most differences from other groups than any one other group. Correlations were reliable across cycles, F (8) = 3.58, P= 0.0045, while specific effects were seen in cycles 1 (LS mean= 0.618 ± 0.160 g, P= 0.0005), 2 (LS mean= 0.365 ± 0.160 g, P= 0.0290), 3 (LS mean= 0.584 ± 0.160 g, P= 0.0009), and 4 (LS mean= 0.714 ± 0.160 g, P<0.0001).

3.5 Discussion

Anticipatory eating has been documented repeatedly in experiments using various fast lengths (short vs. long) as well as different cued test/maintenance foods (Jarvandi, Booth

& Thibault, 2012). The current study hypothesized that anticipatory eating can also be seen with just one fast length, regardless of differential cuing. The upward linear trend in test food seen with both the control and HFD group's intake is consistent with the learning of anticipatory eating. However, the increase could have arisen from other processes as well or instead, such as adaptation to the test food or schedule or to growth (if also seen in daily maintenance intake). Early increases in test food intakes in particular could be adaptation, with persisting increase indicating a longer-term process.

A reliable pattern of a peak followed by a trough, i.e. a decrease followed by another increase, is a clear sign of learning, in that it is hard to explain except as learning, extinction and re-learning. Furthermore the peak-trough-peak (cubic) pattern has been seen repeatedly in experiments on anticipatory eating (Jarvandi, Booth & Thibault, 2012). Although this pattern was seen, there was no reliable difference between the control and HFD group, i.e. the strength of learning was the same across groups. Similar results were seen when the HFD group was classified further into subgroups based on weight and fat gain, i.e., there was no difference in learning patterns between controls, high weight/high fat gainers, high weight/low fat gainers, low weight/low fat gainers, and low weight/high fat gainers.

The number of peaks over ten cycles is a measure of the speed of learning. The timing of the first peak is a more direct measure and so may be more sensitive. To date, the one experiment that looked at a HFD as a maintenance diet, yielded evidence that animals on such a diet may learn less strongly than animals maintained on a balanced diet (Jarvandi, 2007b). In this study, The HFD animals were expected to learn slower than the controls, i.e. display later first peaks. In contrast, the proportion of first peaks in the

earlier cycles (3 to 5) was greater in the group maintained on high-fat diet as a whole. However, a subgroup in the HFD- the low weight/low fat gainers, displayed the greatest proportion of first peaks (%) when compared to their other HFD counterparts. This was a first sign of evidence that body composition, specifically weight and fat gain, may have an effect on learning of anticipatory eating.

The low fat/low weight gainers continued to differentiate from other groups when the aspect of reward was analysed. It was hypothesized that the more that the rat ate in the first hour of refeeding, the greater the reward and hence the greater the intake of test food at the next trial. While these effects were expected to be weaker in the HFD group as a whole, this was only seen within the HFD group when comparing the 4 subgroups. The low weight/low fat gain group showed significant differences from the control and low weight/high fat gainers, i.e. showing the most differences from other groups than any one other group.

HFD impairs hippocampal related cognition, regardless of any metabolic disorders related to obesity (Valladolid, Stucchi, Cano et al., 2010; Greenwood & Winocur, 2005; Kanoski, Hayes, Greenwald et al., 2007; McNay, Ong, McCrimmon et al., 2010; Lindqvist, Mohapel, Bouter et al., 2006; Woods, D'Alession, Tso et al., 2004). In agreement with the latter evidence, this study showed that the subgroup that gained the least fat and weight while maintained on the high-fat diet showed least impairments in learning compared to other HFD subgroups. The low weight/low fat gainers are similar in body type to the control animals, with the sole difference being their type of maintenance food. Therefore, the significant differences in learning patterns between those two groups could be attributed to diet.

Alternatively, obesity leads to greater impairment of hippocampal-dependent learning and memory function (Kanoski & Davidson, 2011). In comparison to the other HFD subgroups, the low weight/low fat gainers did show a higher proportion of earlier first peaks, indicating quicker learning. These results suggest that body weight/fat gain may impair learning of anticipatory eating and classifying the rats into further weight/fat categories was an effort to evaluate potential differences in learning in these different subgroups. The present evidence that the low weight/low fat gainers experience greater reward questions their differences in body composition when compared to fatter rats. Rats that gained more weight and/or fat may have had higher levels of fat oxidation and circulating metabolites, e.g. glucose, and therefore would have gotten less depleted than lean rats after a period of food deprivation. For the same reason, fatter rats may be less hungry after a fast and so the food they receive after is less rewarding than it would be for the lean rats. Alternatively to reward being less present, it could be that the negative reinforcement, i.e. eating more before the fast to avoid negative effects of depletion, was not as present in the fat animals as well.

The main findings from this experiment together with the possible explanations stated above, attempt to distinguish between two possible mechanisms. First that the duration without food could reinforce intake negatively, avoiding later depletion; or second that the fast could reinforce positively, i.e. reward intake as behaviour that leads eventually to a state of repletion (Booth, Jarvandi & Thibault, 2012). The latter suggestion describes that eating before the fast is reinforced by the food eaten after the fast resulting in the loss and regain of potentiation of the positively reinforcing power of repletion. The vast evidence that high-fat food influences metabolism in the body and the

brain (e.g., Greenwood & Winocur 2005; Woods, D'Alessio, Tso et al., 2004) could be a direction for future exploration into reward systems in high-fat fed or obese animals. A possible mechanism that has been explored is the decreased motivation to eat after a fast or before a subsequent fast due to altered metabolic response from a high-fat maintenance diet (Jarvandi, 2008).

In this experiment, the decreased reward or decreased negative reinforcement in some HFD animals raises questions about human learning processes that may contribute to possible behavioural mechanisms of obesity. Questions that arise are ones involving diet-breaching, namely if the associative process seen in rats is also evident in humans (Booth, Jarvandi & Thibault, 2012). Does food deprivation or meal skipping, that overweight people impose make the food they eat more rewarding therefore increasing daily energy intake and ruining any positive effects of their diet? Such a behavioural process, anticipatory eating, and the impairments that a HFD may impose on it implies a possible explanation underlying obesity that merits further exploration.

3.6 Acknowledgements

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Table 3.1 Comparison of body composition variables (LSmeans ± SEM) between rats fed a control diet (n=10) and HFD (n=20) and according to weight gain in HFD rats pre-training and post-training

Panel B-Variable	Control	HFD	HFD	
	n=20	n=20	High-gainers n=10	Low-gainers n=10
Pre-Training				
Total Body Fat (g)	122.9 ± 10.3	157.9 ± 7.3**	152.1 ± 9.4	166.5 ± 11.5*
Total Body Fat (%)	23.4 ± 1.6	28.4 ± 1.1*	28.6 ± 1.4	28.1 ± 1.7
Total Lean Mass (g)	386.7 ± 7.1	381.9 ± 6.3	364.9 ± 6.5	407.4 ± 8.0†
Total BMC (g)	13.00 ± 0.28	13.59 ± 0.23	13.07 ± 0.26	14.37 ± 0.32* †
Total Mass (g)	522.7 ± 9.0	553.4 ± 8.3*	530.1 ± 8.3	588.3 ± 10.1* †
Abdominal Fat (g)	35.1 ± 3.9	51.0 ± 2.8**	48.3 ± 3.6*	55.0 ± 4.4*
Abdominal Fat (% of Total Abdominal Mass)	25.6 ± 1.6	33.2 ± 1.1**	33.1 ± 1.5*	33.3 ± 1.8*
Abdominal Fat (% of Total Body Fat)	28.4 ± 0.9	32.1 ± 0.6**	31.5 ± 0.8*	33.0 ± 1.0*
Abdominal Lean Mass (g)	100.1 ± 2.4	99.8 ± 2.0	94.8 ± 2.2	107.3 ± 2.7†
Abdominal BMC (g)	0.93 ± 0.03	0.95 ± 0.02	0.92 ± 0.03	1.00 ± 0.03
Total Abdominal Mass (g)	136.2 ± 5.4	151.7 ± 4.1*	144.0 ± 4.9	163.3 ± 6.0* †
Post-Training				
Total Body Fat (g)	138.3 ± 12.0	195.7 ± 8.5**	30.7 ± 1.4*	32.2 ± 1.7*
Total Body Fat (%)	23.9 ± 1.6	31.3 ± 1.1**	397.1 ± 8.1	429.9 ± 9.9 †
Total Lean Mass (g)	425.5 ± 9.7	410.2 ± 6.8	15.12 ± 0.25	16.50 ± 0.31* †
Total BMC (g)	14.76 ± 0.33	15.67 ± 0.23	595.8 ± 9.1	660.4 ± 11.1* †
Total Mass (g)	578.6 ± 12.9	621.5 ± 9.1*	61.6 ± 4.3*	73.3 ± 5.3*
Abdominal Fat (g)	40.6 ± 4.9	66.3 ± 3.4**	34.8 ± 1.5*	36.8 ± 1.8*
Abdominal Fat (% of Total Abdominal Mass)	24.9 ± 1.6	35.6 ± 1.2**	33.5 ± 0.7*	33.9 ± 0.9*
Abdominal Fat (% of Total Body Fat)	29.0 ± 0.8	33.6 ± 0.6**	113.2 ± 2.6	121.3 ± 3.2
Abdominal Lean Mass (g)	119.6 ± 3.0	116.4 ± 2.1	1.11 ± 0.03	1.27 ± 0.04* †
Abdominal BMC (g)	1.12 ± 0.04	1.17 ± 0.03	175.9 ± 5.5	195.9 ± 6.7*
Total Abdominal Mass (g)	161.3 ± 6.4	183.9 ± 4.6**	30.7 ± 1.4*	32.2 ± 1.7*

Comparisons made between control and HFD; * $P < 0.05$ versus control rats, ** $P < 0.01$ versus control rats
Comparions made between control and high and low gainers; * $P < 0.05$ versus control rats, † $P < 0.05$ versus high-gainers

Table 3.2 Peak intakes (g) during the training period for the control, HFD, high-weight gainers and low-weight gainers: before the 8 h fast and 1 h after the fast

Peak intakes (intake – average of 2 previous days' intakes) during training period. Pairwise Comparisons												
	Control			HFD			High-Gainers			Low-Gainers		
Cycle	*Est.	**SE	P	Est.	SE	P	Est.	SE	P	Est.	SE	P
3	2.25	0.78	0.0045	2.48	0.56	<0.0001	2.29	0.89	0.0105	2.61	0.73	0.0004
4	2.49	0.78	0.0017	1.43	0.56	0.0105	1.90	0.89	0.0350	1.13	0.73	0.1205
5	2.54	0.78	0.0014	0.61	0.56	0.2748	0.34	0.89	0.7121	0.79	0.73	0.2748
6	1.42	0.78	0.0722	1.69	0.56	0.0026	1.92	0.89	0.0314	1.53	0.73	0.0357
7	0.68	0.78	0.3899	0.53	0.56	0.3396	0.82	0.89	0.3597	0.34	0.73	0.6379
8	-1.54	0.78	0.0502	-1.54	0.56	0.0059	-0.99	0.89	0.2658	-1.91	0.73	0.0090
9	0.66	0.78	0.3984	0.93	0.56	0.0949	1.05	0.89	0.2405	0.85	0.73	0.2400
10	0.277	0.78	0.7245	1.22	0.56	0.0289	0.82	0.89	0.3597	1.49	0.73	0.0410
Peak intakes (g) during 1 h access following 8 h fast. Pairwise Comparisons												
3	-0.78	0.54	0.1463	-0.80	0.38	0.0358	-0.79	0.60	0.1936	-0.81	0.49	0.1011
4	-0.26	0.54	0.6313	-0.15	0.38	0.6995	-0.00	0.60	0.9959	-0.24	0.49	0.6231
5	0.87	0.54	0.1061	1.39	0.38	0.0003	1.52	0.60	0.0123	1.31	0.49	0.0087
6	-0.59	0.54	0.2706	0.43	0.38	0.2556	0.28	0.60	0.6452	0.54	0.49	0.2780
7	0.53	0.54	0.3223	-0.53	0.38	0.1684	-0.33	0.60	0.5885	-0.66	0.49	0.1841
8	0.22	0.54	0.6898	0.99	0.38	0.0096	1.49	0.60	0.0145	0.66	0.49	0.1811
9	1.30	0.54	0.0164	0.94	0.38	0.0141	0.74	0.60	0.2245	1.08	0.49	0.0301
10	0.17	0.54	0.7586	-0.24	0.38	0.5206	-1.37	0.60	0.0246	0.50	0.49	0.3085
Peak intakes (kcal) during 1 h access following 8 h fast. Pairwise Comparisons												
3	-2.67	2.16	0.2173	-3.37	1.52	0.0278	-3.31	2.42	0.1724	-3.41	1.98	0.0853
4	-0.88	2.16	0.6838	-0.62	1.52	0.6857	-0.01	2.42	0.9957	-1.02	1.98	0.6059
5	2.97	2.16	0.1699	5.85	1.52	0.0002	6.41	2.42	0.0086	5.49	1.98	0.0059
6	-2.02	2.16	0.3496	1.82	1.52	0.2333	1.17	2.42	0.6288	2.25	1.98	0.2549
7	1.81	2.16	0.4008	-2.21	1.52	0.1488	-1.38	2.42	0.5701	-2.76	1.98	0.1633
8	0.73	2.16	0.7348	4.17	1.52	0.0066	6.26	2.42	0.0103	2.78	1.98	0.1604
9	4.42	2.16	0.0414	3.95	1.52	0.0101	3.09	2.42	0.2023	4.52	1.98	0.0229
10	0.56	2.16	0.7942	-1.03	1.52	0.5006	-5.74	2.42	0.0184	2.12	1.98	0.2851

* Estimate, ** Standard Error

3.7 Captions to Figures

Figure 3.1. Relationship between total intake (Panel A: kcal; Panel B: g) and total weight gain (g) at the end of the 37-day maintenance period

Figure 3.2. Intake of test food (g, LSmean ± SEM) over ten successive cycles for control group, HFD group, high-weight gainers and low-weight gainers before the 8 h fast

Figure 3.3. PROC LIFESTEST survival analysis testing time to first peak for control group (treatment 1) versus HFD group (treatment 2) using criteria of greater than one gram (Panel A). Proportion (%) of rats in their respective group experiencing a peak using criterion of greater than one gram. Classification within the HFD group is labeled as HG/L for high-weight/low-fat gainers and LG/L for low-weight/low-fat gainers, LG/H for low-weight/high-fat gainers and HG/H for high-weight/high-fat gainers (Panel B).

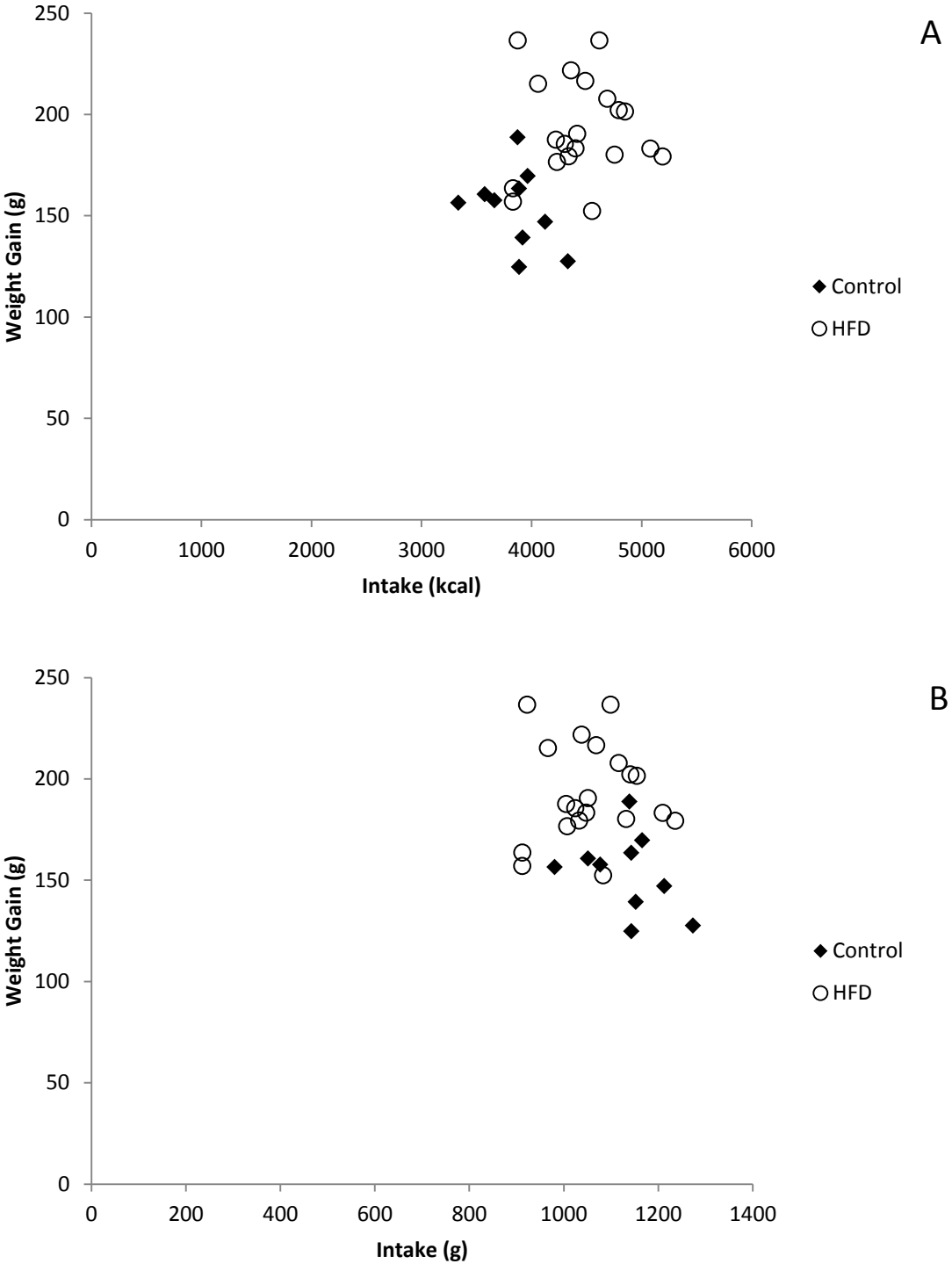

A
250
200
150
100
50
0
Weight Gain (g)
0
1000
2000
3000
4000
5000
6000
Intake (kcal)
Control
HFD
B
250
200
150
100
50
0
Weight Gain (g)
0
200
400
600
800
1000
1200
1400
Intake (g)
Control
HFD

Figure 3.1

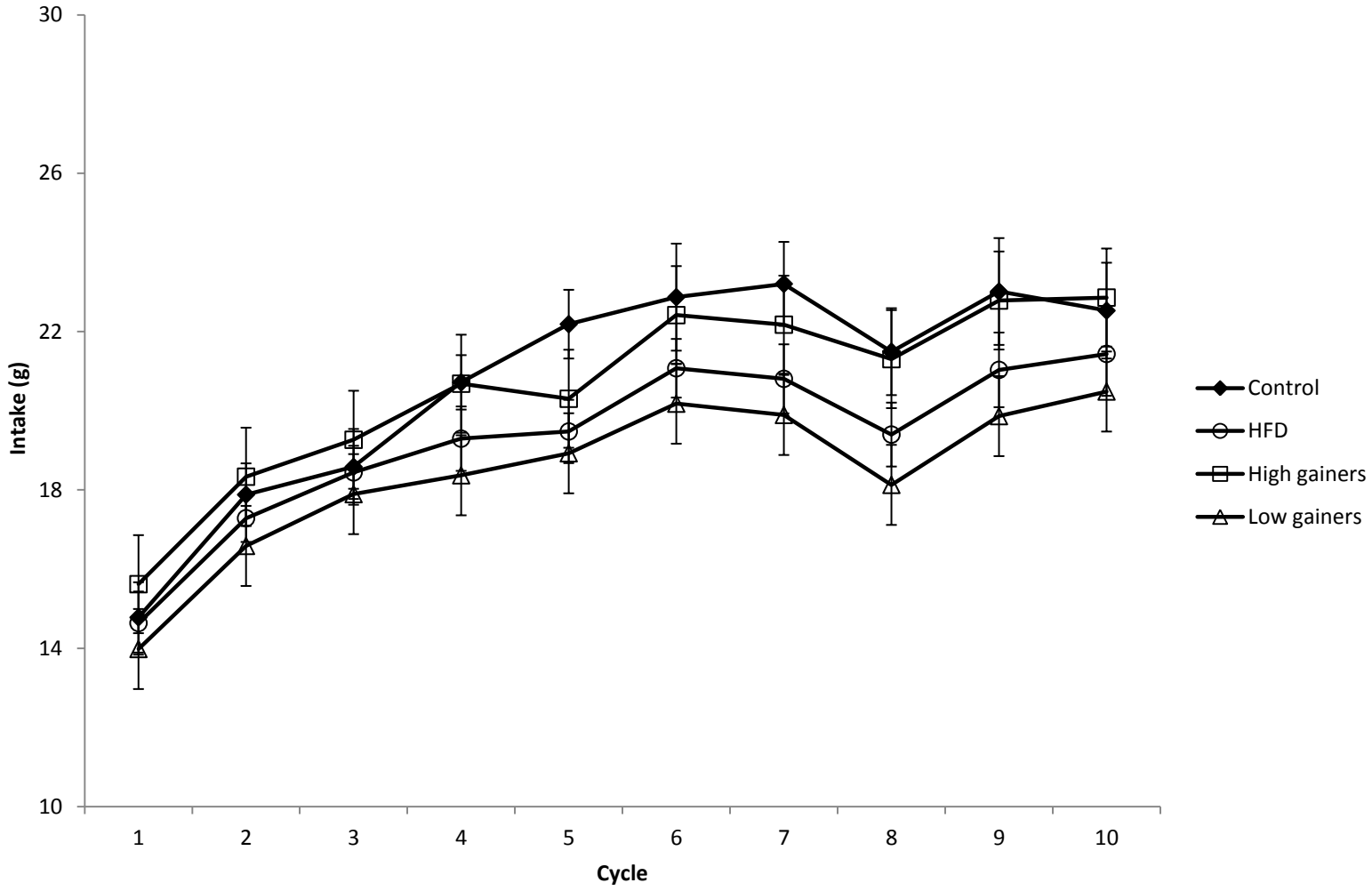
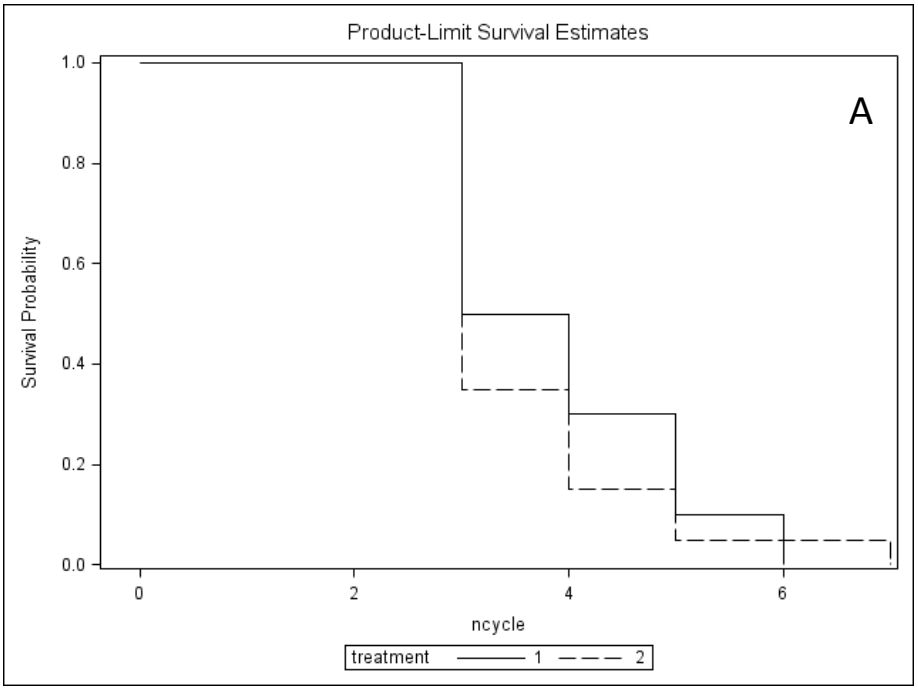

30
26
22
18
14
10
Intake (g)
1
2
3
4
5
6
7
8
9
10
Cycle
Control
HFD
High gainers
Low gainers

Figure 3.2.


Product-Limit Survival Estimates
A
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
ncycle
treatment 1 2

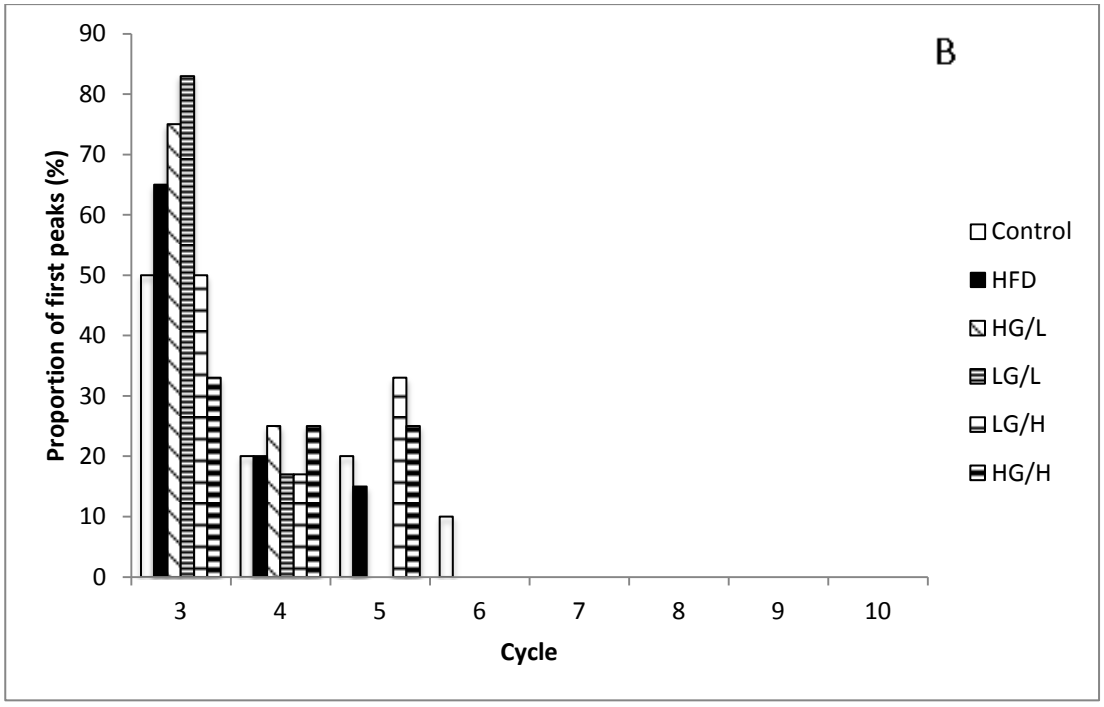

B
Proportion of first peaks (%)
90
80
70
60
50
40
30
20
10
0
3
4
5
6
7
8
9
10
Cycle
Control
HFD
HG/L
LG/L
LG/H
HG/H

Figure 3.3.

CHAPTER 4, OVERALL SUMMARY AND CONCLUSIONS

Despite much work done on anticipatory eating, little has been done on looking at the effects of a high-fat maintenance diet on such a learning paradigm. Throughout the series of 9 papers published on anticipatory eating (from collaborations by Drs Louise Thibault and David Booth), one by Jarvandi, Booth, and Thibault has manipulated the nutrient contents of the maintenance food to include fat, which led to animals showing least amounts of learning (Jarvandi, Booth & Thibault, 2007b).

A review of the literature showed that a high-fat diet may be connected to poor learning in various paradigms and thus the metabolism of fat may also affect the process itself of learning to eat anticipatorily. The consumption of high-fat food is associated with changes in brain reward circuitry (Sharma & Fulton, 2012; Davis, Tracy, Schurdak et al, 2008). Mechanisms proposed for patterns seen with anticipatory eating involve the motivating effect of the longer fast, thus eating more prior to it. With the attenuating effect of a maintenance HFD, this motivating effect may be impaired, and thus less anticipatory eating will be seen.

The current work confirmed patterns of anticipatory eating seen in previous work. That is, the peak-trough-peak (cubic) pattern is evidence of learning, extinction and re-learning. Furthermore, in response to the first study question, anticipatory eating was present in rats regardless of differential cuing and with one fast length. In answering the second study question, although weaker learning was not substantiated in the HFD group as a whole as expected, subgroups of the HFD group showed less learning. The classification of the HFD group into four weight/fat-gain subgroups enabled the

examination of the relationship between HFD and learning in rats of different body compositions. The findings that only certain HFD subgroups displayed less learning questions whether a HFD had a detrimental effect on learning per say. Rats that learned better in this experiment were those that gained the least amount of body fat and weight; however were still maintained on the HFD.

In this experiment, similar to previous literature, rats on the high-fat maintenance diet were ranked based on their body weight gain (g/day) and were then grouped as either obesity-prone (high-weight gainers) or obesity resistant (low-weight gainers). As expected, control rats differed significantly from the HFD rats in that the variables body weight gain (g and g/day), total body fat (g and %), abdominal fat (g and % of total body fat), and fat pad mass (i.e. abdominal, retroperitoneal and epididymal weighed at termination) were all significantly greater in the latter group. However, no significant differences in total body fat (g and %), abdominal fat (g and % of total body fat), and fat pad mass were observed between high-weight gainers and low-weight gainers following high-fat feeding. Based on this main finding, the weight-based categorization of rats may be inaccurate. Due to the poor correlation between weight and fat gain it is proposed that, when possible, body fat mass be used to classify rats as obesity-prone or obesity-resistant similar to a study by Dourmashkin, Chang, Gayles et al. (2005). Classifying rats further based on body fat gain was an attempt to settle this inconsistency, which may be more accurately related to the definition of obesity. This method also informs the third study question, that there is individual variability in terms of weight gain and various aspects of body composition within and between HFD and standard diet fed animals.

Significant differences between high-weight gainers and low-weight gainers were not only lacking in terms of body fat composition variables, but for learning performance as well. The low fat/low weight gainers continued to differentiate from other groups when the aspect of reward was looked at. It was expected that the more maintenance food the rat ate in the first hour of refeeding, the greater the reward and hence the greater the intake of test food at the next trial. While it was expected that these effects would be weaker in the HFD group as a whole, this was only seen within the HFD group when comparing the 4 subgroups. The low weight/low fat gain group showed significant differences from the control and low weight/high fat gainers, i.e. showing the most differences from other groups than any one other group.

The subgroup that gained the least fat and weight while maintained on the high-fat diet showed better learning compared to other HFD subgroups. The low weight/low fat gainers are similar in body type to the control animals, with the sole difference being their type of maintenance food. Therefore, the significant differences in learning patterns between those two groups could be attributed to diet. In comparison to the other HFD subgroups, the low weight/low fat gainers did show a higher proportion of earlier first peaks, indicating quicker learning.

Therefore, while earlier experiments in our laboratory attributed weaker learning amongst high-fat fed animals to the diet-induced obesity based on final weight (Jarvandi, 2008; Jarvandi, Booth & Thibault 2007b), the current results and classification of rats allowed for a potential conclusion that weaker learning could be due to excess body fat and not to the HFD per say. This distinction between the effect of diet and body composition, regardless of diet composition, on learning merits further work as it has

implications for human obesity and its behavioural processes. Therefore the fourth study question remains to have a definite answer. Questions that arise are ones involving diet-breaching and self-deprivation (Booth, Jarvandi & Thibault, 2012). The mechanisms seen with anticipatory eating in rats may also be present with humans and therefore a proposition for a human-based experiment was appropriate. Previous work on anticipatory eating set out to explore the mechanisms controlling the sizes of meals in day-to-day life and an unconscious/automatic mechanism occurring in the human brain to control hunger may shed light on attempts to reduce and explain obesity. Novel from previous work, the present study has shown that certain aspects of body composition, i.e. excess body fat, could attenuate anticipatory eating. Such automatic reward seen with anticipatory eating could be a major factor in the difficulty of decreasing energy intake in overweight and obesity (Booth, Jarvandi, & Thibault, 2012). However, the ability to learn such an automatic reward process could be less apparent in those with specific body composition characteristics, i.e. body fat may attenuate a specific learning ability. Current American trends in obesity and fat intake show that there is a reduced fat and calorie intake yet a paradoxical increase in the prevalence of obesity (Heini & Weinsler, 1997). It is the latter that merits much concern as indicated by the negative effect of body fat/weight gain seen in the current study. Such a behavioural process, anticipatory eating, and the impairments that a HFD or body fat/weight content may impose on it implies a possible explanation underlying obesity.

As with all research, the experiment included in this thesis has limitations. First, the literature reviewed in this project highlights important relationships between eating behaviour and various hormones e.g. leptin and ghrelin. Because the experiment focused

on learning and behavior patterns, it did not explore any biochemical measurements, such as blood leptin and ghrelin levels. Second, although 30 rats may seem an adequate sample size, it is difficult to tell if using an uneven number of control (n=10) and HFD (n=20) animals caused any disadvantage to the data analysis. Furthermore, once rats were separated into their various weight/fat classifications, sample sizes were quite small, which may have affected the significance of the results.

Aside from sample size perhaps being limiting, a major strength the current study is the age of the rats. Using adult rats was important for the current study because any confounding factors in relation to development or sexual maturation can be disregarded. Furthermore, the types of fatty acids included in the diet are important as some may have more an effect in developing animals, i.e. PUFA and MUFA, whereas others play a more significant role on the adult brain, i.e. SFA (Greenwood & Winocur, 1996; Winocur & Greenwood, 1993). Therefore it was appropriate for this study to use a SFA-rich diet to assess its effects on learning in male rats. Most of the literature reviewed in Chapter 2 concerning the effects of HFD on learning highlights results from studies using adult rats; however there are few exceptions, where studies do not indicate the age of animals or use young rats (e.g. Lindqvist, Mohapel, Bouter, et al., 2006; Pipatpiboon, Pratchayasakul, Chattipakorn, et al., 2012). The current model teased out what may happen in adult learning when fed a high-fat diet, while making sure that rats met other nutrient requirements.

The increasing trend of obesity in adulthood is not only a concern for various metabolic co-morbidities, but for behavioural and neurological aspects as well, e.g. learning. The rising prevalence is indication that current interventions to reduce obesity

may be failing, thus a mechanism occurring outside awareness is attractive, that is the effects of food consumed after a period of deprivation may unconsciously reward subsequent extra eating (Booth, Jaravndi & Thibault, 2012). Therefore, further work on learning processes involved in managing hunger in humans would be suitable.

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APPENDICES

Appendix 1. Supplementary Tables

Appendix 2. Supplementary Figures

Appendix 3. Statistical models and descriptions

Appendix 1. Supplementary Tables

Table 1. Chemical Composition of Purina Chow (control) and High-Fat Diet.

Diet composition (g/100 g)	Purina chow [a]	High-fat diet
Total protein	18.1	14.5
Total carbohydrate (CHO)	57.3	45.8
Total fat:	4.5	19.6
From chow	4.5	3.6
From butter [b]	-	16.0
Saturated Fatty Acids (SFA)	-	10.9
Monounsaturated Fatty Acids (MUFA)	-	4.5
Polyunsaturated Fatty Acids (PUFA)	-	0.6
Fibre	3.4	2.72
Vitamins and minerals	3.7	2.96
Energy (kJ/g)	14.2	17.5
Energy (kcal/g)	3.4	4.2
Percent of energy from fat	12	42.2
Percent of energy from SFA	-	23
Percent of energy from protein	21	14
Percent of energy from CHO	67	44

[a] Charles River rodent chow 5075, St-Constant, Quebec.
[b] My Country, Lactantia, Canada.

Table 2. Covariance Parameter Estimates

Cov Parm	**Subject**	**Estimate**
Food intake (g) during maintenance period. Repeated Measures ANOVA		
rat(diet)		5.4488
AR(1)	rat(diet)	0.2841
Residual		3.2000
Food intake (kcal) during maintenance period. Repeated Measures ANOVA		
rat(diet)		5.4488
AR(1)	rat(diet)	0.2841
Residual		3.2000
Test food intake (g) prior to 8h fast. Repeated Measures ANOVA		
rat(diet)		8.0710
AR(1)	rat(diet)	0.2341
Residual		4.5977
Test food intake (g) prior to 8h fast- High-gainers vs. Low-gainers. Repeated Measures ANOVA		
rat(diet)		7.5817
AR(1)	rat(diet)	0.2267
Residual		4.6830
Maintenance food intake (g) during 1 h access following 8 h fast. Repeated Measures ANOVA		
rat(diet)		3.1967
AR(1)	rat(diet)	0.1928
Residual		2.1035
Maintenance food intake (kcal) during 1 h access following 8 h fast. Repeated Measures ANOVA		
rat(diet)		50.5579
AR(1)	rat(diet)	0.1925
Residual		33.7562
Maintenance food intake (g) during 1 h access following 8 h fast- High-gainers vs. Low-gainers. Repeated Measures ANOVA		
rat(diet)		3.2416
AR(1)	rat(diet)	0.1869
Residual		2.1173
Maintenance food intake (kcal) during 1 h access following 8 h fast- High-gainers vs. Low-gainers. Repeated Measures ANOVA		
rat(diet)		51.1345
AR(1)	rat(diet)	0.1861
Residual		33.8754

Table 3. Analyses of variance for food intake (g and kcal) and weight gain during the maintenance period

Food intake (g) during maintenance period. Repeated Measures ANOVA			
Effect	*Df (Num, Den)*	*F*	*P*
Diet	1, 29.7	5.51	0.0257
Day	36, 937	10.68	<0.0001
Diet*Day	36, 937	4.92	<0.0001
Food intake (kcal) during maintenance period. Repeated Measures ANOVA			
Effect			
Diet	1, 29.7	19.14	0.0001
Day	36, 937	10.45	<0.0001
Diet*Day	36, 937	5.13	<0.0001
Total weight gain during maintenance period. Completely Randomized Design			
Intake (g)	1	0.12	0.7337
Diet	1	15.46	0.0005
Intake (kcal)	1	0.07	0.7926
Diet	1	12.85	0.0013

Table 4. Body weight (g) of rats (LSmean ± SEM) in control group (n = 10) and HFD group (n = 20) on each day of the 37-day maintenance period and the differences of least squares means (modified from Mavreta Vagenas' MScA project, 2013)

Day	**Control Diet** LSmeans ± SE	**High Fat Diet** LSmeans ± SE	**Difference ± SEM** HFD - CD	**Scheffé Adjusted P- values**
1	373.8 ± 8.4	366.4 ± 6.0	- 7. 4 ± 10.3	0.4768
2	379.4 ± 8.4	374.9 ± 6.0	- 4.5 ± 10.3	0.6621
3	383.5 ± 8.4	383.9 ± 6.0	0.4 ± 10.3	0.9674
4	391.1 ± 8.4	393.5 ± 6.0	2.4 ± 10.3	0.8190
5	396.8 ± 8.4	401.5 ± 6.0	4.7 ± 10.3	0.6538
6	404.7 ± 8.4	409.2 ± 6.0	4.5 ± 10.3	0.6652
7	409.2 ± 8.4	415.1 ± 6.0	5.9 ± 10.3	0.5703
8	416.5 ± 8.4	421.3 ± 6.0	4.8 ± 10.3	0.6476
9	421.0 ± 8.4	428.0 ± 6.0	7.0 ± 10.3	0.5004
10	423.3 ± 8.4	433.4 ± 6.0	10.1 ± 10.3	0.3344
11	430.1 ± 8.4	440.3 ± 6.0	10.2 ± 10.3	0.3287
12	434.1 ± 8.4	445.7 ± 6.0	11.6 ± 10.3	0.2690
13	437.1 ± 8.4	450.8 ± 6.0	13.7 ± 10.3	0.1931
14	443.0 ± 8.4	457.4 ± 6.0	14.4 ± 10.3	0.1718
15	446.1 ± 8.4	461.6 ± 6.0	15.5 ± 10.3	0.1424
16	452.0 ± 8.4	468.3 ± 6.0	16.3 ± 10.3	0.1213
17	455.9 ± 8.4	473.5 ± 6.0	17.6 ± 10.3	0.0969
18	459.7 ± 8.4	479.3 ± 6.0	19.6 ± 10.3	0.0669
19	463.2 ± 8.4	483.5 ± 6.0	20.3 ± 10.3	0.0580
20	467.6 ± 8.4	486.6 ± 6.0	19.0 ± 10.3	0.0738
21	472.3 ± 8.4	492.4 ± 6.0	20.1 ± 10.3	0.0592
22	475.9 ± 8.4	498.4 ± 6.0	22.5 ± 10.3	0.0358
23	481.1 ± 8.4	505.3 ± 6.0	24.2 ± 10.3	0.0254
24	485.7 ± 8.4	509.7 ± 6.0	24.0 ± 10.3	0.0260
25	490.6 ± 8.4	515.1 ± 6.0	24.5 ± 10.3	0.0231
26	493.9 ± 8.4	520.2 ± 6.0	26.3 ± 10.3	0.0157
27	498.1 ± 8.4	525.0 ± 6.0	26.9 ± 10.3	0.0132
28	501.4 ± 8.4	529.1 ± 6.0	27.7 ± 10.3	0.0111
29	505.6 ± 8.4	533.1 ± 6.0	27.5 ± 10.3	0.0117
30	511.0 ± 8.4	537.6 ± 6.0	26.6 ± 10.3	0.0145
31	513.5 ± 8.4	541.5 ± 6.0	28.0 ± 10.3	0.0103
32	517.5 ± 8.4	546.3 ± 6.0	28.8 ± 10.3	0.0086
33	517.5 ± 8.4	547.5 ± 6.0	30.0 ± 10.3	0.0063
34	522.3 ± 8.4	550.8 ± 6.0	28.5 ± 10.3	0.0090
35	524.4 ± 8.4	554.2 ± 6.0	29.8 ± 10.3	0.0066
36	524.8 ± 8.4	555.5 ± 6.0	30.7 ± 10.3	0.0052
37	527.4 ± 8.4	559.2 ± 6.0	31.8 ± 10.3	0.0040

Table 5. Weight variables (LSmean ± SEM) recorded over the 37-day maintenance diet period and differences between the control rats (C; n = 10) and HFD rats (HFD; n = 20) (modified from Mavreta Vagenas' MScA project, 2013)

Variable	**Control Diet**	**High Fat Diet**	**Difference ± SEM** HFD - C	**P Value**
Initial Weight (g)	373.8 ± 5.5	366.4 ± 3.9	7.4 ± 6.8	P = 0.2816
Final Weight (g)	527.4 ± 8.9	559.2 ± 7.9*	31.8 ± 12.8	P = 0.0192
Weight Gain (%)	41.2 ± 1.8	52.6 ± 1.3**	11.4 ± 2.2	P < 0.0001
Weight Gain (g/day)	4.1 ± 0.2	5.2 ± 0.1**	1.1 ± 0.2	P = 0.0001
Total Weight Gain (g)	153.6 ± 7.2	192.8 ± 5.1**	39.3 ± 8.8	P = 0.0001

* $P < 0.05$ versus control rats; ** $P < 0.01$ versus control rats

Table 6a. Within group changes in body composition variables (LSmean ± SE) between baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)

Group	Variable	Baseline	Pre-Training	Post-Training	σ_{rat}^2	σ_e^2	Differences ± SEM a. Pre-Training - Baseline b. Post-Training - Baseline c. Post-Training - Pre-Training	Bonferroni Adjusted P-Values
All Rats								
	Total Body Fat (g)	67.0 ± 5.3	147.2 ± 5.3*	178.6 ± 5.3* †	0	809.38	a. 80.2 ± 5.2	P < 0.0001
							b. 111.6 ± 6.4	P < 0.0001
							c. 31.4 ± 5.2	P < 0.0001
	Total Body Fat (%)	19.6 ± 0.8	26.7 ± 0.8*	28.9 ± 0.8* †	8.1993	10.6976	a. 7.1 ± 0.8	P < 0.0001
							b. 9.3 ± 0.9	P < 0.0001
							c. 2.2 ± 0.8	P = 0.0476
	Total Lean Mass + BMC (g)	275.1 ± 4.2	399.9 ± 4.2*	433.0 ± 4.2* †	246.88	270.53	a. 124.8 ± 4.1	P < 0.0001
							b. 157.9 ± 4.5	P < 0.0001
							c. 33.1 ± 4.1	P < 0.0001
	Total Lean Mass (g)	267.0 ± 4.2	386.4 ± 4.2*	417.5 ± 4.2* †	235.60	272.11	a. 119.4 ± 4.1	P < 0.0001
							b. 150.5 ± 4.5	P < 0.0001
							c. 31.1 ± 4.1	P < 0.0001
	Total BMC (g)	8.05 ± 0.14	13.48 ± 0.14*	15.46 ± 0.14* †	0	0.5636	a. 5.43 ± 0.11	P < 0.0001
							b. 7.41 ± 0.14	P < 0.0001
							c. 1.98 ± 0.11	P < 0.0001
	Total Mass (g)	342.1 ± 4.8	547.0 ± 4.8*	611.6 ± 4.8* †	264.23	407.54	a. 204.9 ± 4.6	P < 0.0001
							b. 269.5 ± 5.8	P < 0.0001
							c. 64.6 ± 4.6	P < 0.0001
	Abdominal Fat (g)	13.2 ± 2.1	46.1 ± 2.1*	58.5 ± 2.1* †	0	124.89	a. 32.9 ± 2.0	P < 0.0001
							b. 45.3 ± 2.5	P < 0.0001
							c. 12.4 ± 2.0	P < 0.0001
	Abdominal Fat (% of Total Abdominal Mass)	18.5 ± 0.8	30.6 ± 0.8*	32.2 ± 0.8*	5.6806	14.5689	a. 12.1 ± 0.9	P < 0.0001
							b. 13.7 ± 1.0	P < 0.0001
							c. 1.6 ± 0.9	P = 0.2681
	Abdominal Fat (% of Total Body Fat)	19.7 ± 0.4	30.9 ± 0.4*	32.1 ± 0.4*	2.3565	3.5552	a. 11.2 ± 0.5	P < 0.0001
							b. 12.4 ± 0.5	P < 0.0001
							c. 1.2 ± 0.5	P = 0.1075
	Abdominal Lean Mass + BMC (g)	58.1 ± 1.4	101.7 ± 1.4*	119.2 ± 1.4* †	23.9558	31.8740	a. 43.6 ± 1.7	P < 0.0001
							b. 61.1 ± 1.4	P < 0.0001

							c. 17.5 ± 1.7	P < 0.0001
	Abdominal Lean Mass (g)	57.6 ± 1.4	100.7 ± 1.4*	118.0 ± 1.4* †	23.5895	31.4512	a. 43.1 ± 1.7	P < 0.0001
							b. 60.4 ± 1.4	P < 0.0001
							c. 17.3 ± 1.7	P < 0.0001
	Abdominal BMC (g)	0.48 ± 0.02	0.95 ± 0.02*	1.17 ± 0.02* †	0.00262	0.00497	a. 0.47 ± 0.02	P < 0.0001
							b. 0.69 ± 0.02	P < 0.0001
							c. 0.22 ± 0.02	P < 0.0001
	Total Abdominal Mass (g)	71.4 ± 2.9	149.2 ± 2.9*	178.0 ± 2.9* †	79.3326	159.64	a. 77.8 ± 3.1	P < 0.0001
							b. 106.6 ± 3.5	P < 0.0001
							c. 28.8 ± 3.1	P < 0.0001
Control Diet								
	Total Body Fat (g)	66.7 ± 6.6	122.9 ± 6.6*	138.3 ± 6.6*	219.55	212.58	a. 56.2 ± 6.5	P < 0.0001
							b. 71.6 ± 6.5	P < 0.0001
							c. 15.4 ± 6.5	P = 0.0890
	Total Body Fat (%)	19.4 ± 1.0	23.4 ± 1.0*	23.9 ± 1.0*	6.5609	5.6144	a. 4.0 ± 1.1	P = 0.0034
							b. 4.5 ± 1.1	P = 0.0015
							c. 0.5 ± 1.1	P = 1.0000
	Total Lean Mass + BMC (g)	278.1 ± 7.4	399.8 ± 7.4*	440.3 ± 7.4* †	378.43	168.72	a. 121.7 ± 5.8	P < 0.0001
							b. 162.2 ± 5.8	P < 0.0001
							c. 40.5 ± 5.8	P < 0.0001
	Total Lean Mass (g)	269.7 ± 7.3	386.7 ± 7.3*	425.5 ± 7.3* †	357.09	170.01	a. 117.0 ± 5.8	P < 0.0001
							b. 155.8 ± 5.8	P < 0.0001
							c. 38.8 ± 5.8	P < 0.0001
	Total BMC (g)	8.30 ± 0.27	13.00 ± 0.27*	14.76 ± 0.27* †	0.5424	0.1879	a. 4.70 ± 0.19	P < 0.0001
							b. 6.46 ± 0.19	P < 0.0001
							c. 1.76 ± 0.19	P < 0.0001
	Total Mass (g)	344.7 ± 8.9	522.7 ± 8.9*	578.6 ± 8.9* †	583.45	217.23	a. 178.0 ± 6.6	P < 0.0001
							b. 233.9 ± 6.6	P < 0.0001
							c. 55.9 ± 6.6	P < 0.0001
	Abdominal Fat (g)	13.3 ± 2.4	35.1 ± 2.4*	40.6 ± 2.4*	30.1551	28.3623	a. 21.8 ± 2.4	P < 0.0001
							b. 27.3 ± 2.4	P < 0.0001
							c. 5.5 ± 2.4	P = 0.1024
	Abdominal Fat (% of Total Abdominal Mass)	18.8 ± 1.2	25.5 ± 1.2*	24.9 ± 1.2*	8.0857	7.5163	a. 6.7 ± 1.2	P = 0.0001
							b. 6.1 ± 1.2	P = 0.0003
							c. - 0.6 ± 1.2	P = 1.0000
	Abdominal Fat (% of Total Body Fat)	20.0 ± 0.8	28.4 ± 0.8*	29.0 ± 0.8*	3.2054	3.3328	a. 8.4 ± 0.8	P < 0.0001
							b. 9.0 ± 0.8	P < 0.0001
							c. 0.6 ± 0.8	P = 1.0000
	Abdominal Lean Mass + BMC (g)	57.3 ± 2.2	101.1 ± 2.2*	120.8 ± 2.2* †	21.9941	25.0378	a. 43.8 ± 2.2	P < 0.0001
							b. 63.5 ± 2.2	P < 0.0001

							c. 19.7 ± 2.2	P < 0.0001
	Abdominal Lean Mass (g)	56.8 ± 2.1	100.1 ± 2.1*	119.6 ± 2.1*†	21.6528	24.7708	a. 43.4 ± 2.2	P < 0.0001
							b. 62.8 ± 2.2	P < 0.0001
							c. 19.5 ± 2.2	P < 0.0001
	Abdominal BMC (g)	0.47 ± 0.02	0.93 ± 0.02*	1.12 ± 0.02*†	0.00264	0.00292	a. 0.46 ± 0.02	P < 0.0001
							b. 0.65 ± 0.02	P < 0.0001
							c. 0.19 ± 0.02	P < 0.0001
	Total Abdominal Mass (g)	70.7 ± 4.2	140.1 ± 4.2*	162.3 ± 4.2*†	95.5187	80.9497	a. 69.4 ± 4.0	P < 0.0001
							b. 91.6 ± 4.0	P < 0.0001
							c. 22.2 ± 4.0	P < 0.0001
High Fat Diet								
	Total Body Fat (g)	67.1 ± 7.3	157.9 ± 7.3*	195.7 ± 7.3*†	498.76	574.85	a. 90.8 ± 7.6	P < 0.0001
							b. 128.6 ± 7.6	P < 0.0001
							c. 37.8 ± 7.6	P < 0.0001
	Total Body Fat (%)	19.7 ± 1.0	28.4 ± 1.0*	31.3 ± 1.0*†	9.5113	11.9034	a. 8.7 ± 1.1	P < 0.0001
							b. 11.6 ± 1.1	P < 0.0001
							c. 2.9 ± 1.1	P = 0.0320
	Total Lean Mass + BMC (g)	273.5 ± 6.1	395.5 ± 6.1*	425.9 ± 6.1*†	354.76	382.12	a. 122.0 ± 6.2	P < 0.0001
							b. 152.4 ± 6.2	P < 0.0001
							c. 30.4 ± 6.2	P < 0.0001
	Total Lean Mass (g)	265.5 ± 6.0	381.9 ± 6.0*	410.2 ± 6.0*†	339.88	374.98	a. 116.4 ± 6.1	P < 0.0001
							b. 144.7 ± 6.1	P < 0.0001
							c. 28.3 ± 6.1	P = 0.0001
	Total BMC (g)	7.91 ± 0.20	13.59 ± 0.20*	15.67 ± 0.20*†	0.5069	0.3102	a. 5.68 ± 0.18	P < 0.0001
							b. 7.76 ± 0.18	P < 0.0001
							c. 2.08 ± 0.18	P < 0.0001
	Total Mass (g)	340.5 ± 7.9	553.4 ± 7.9*	621.6 ± 7.9*†	785.34	459.85	a. 212.9 ± 6.8	P < 0.0001
							b. 281.1 ± 6.8	P < 0.0001
							c. 68.2 ± 6.8	P < 0.0001
	Abdominal Fat (g)	13.2 ± 2.9	51.0 ± 2.9*	66.3 ± 2.9*†	74.2990	94.8965	a. 37.8 ± 3.1	P < 0.0001
							b. 53.1 ± 3.1	P < 0.0001
							c. 15.3 ± 3.1	P < 0.0001
	Abdominal Fat (%of Total Abdominal Mass)	18.4 ± 1.1	33.2 ± 1.1*	35.6 ± 1.1*	8.9068	13.1983	a. 14.8 ± 1.1	P < 0.0001
							b. 17.2 ± 1.1	P < 0.0001
							c. 2.4 ± 1.1	P = 0.1207
	Abdominal Fat (% of Total Body Fat)	19.6 ± 0.5	32.1 ± 0.5*	33.6 ± 0.5*	1.0349	4.4686	a. 12.5 ± 0.7	P < 0.0001
							b. 14.0 ± 0.7	P < 0.0001
							c. 1.5 ± 0.7	P = 0.0777
	Abdominal Lean Mass + BMC (g)	58.9 ± 2.0	100.8 ± 2.0*	117.6 ± 2.0*†	16.2819	60.8313	a. 41.9 ± 2.5	P < 0.0001
							b. 58.7 ± 2.5	P < 0.0001

							c. 16.8 ± 2.5	P < 0.0001
	Abdominal Lean Mass (g)	58.4 ± 1.9	99.8 ± 1.9*	116.5 ± 1.9*†	15.9891	59.8931	a. 41.4 ± 2.4	P < 0.0001
							b. 58.1 ± 2.4	P < 0.0001
							c. 16.7 ± 2.4	P < 0.0001
	Abdominal BMC (g)	0.48 ± 0.02	0.95 ± 0.02*	1.17 ± 0.02*†	0.00338	0.00764	a. 0.47 ± 0.03	P < 0.0001
							b. 0.69 ± 0.03	P < 0.0001
							c. 0.22 ± 0.03	P < 0.0001
	Total Abdominal Mass (g)	72.1 ± 4.0	151.7 ± 4.0*	183.9 ± 4.0*†	141.52	187.96	a. 79.6 ± 4.3	P < 0.0001
							b. 111.8 ± 4.3	P < 0.0001
							c. 32.2 ± 4.3	P < 0.0001
Low Gainers								
	Total Body Fat (g)	66.3 ± 7.5	152.2 ± 7.5*	184.1 ± 7.5*†	225.11	496.97	a. 85.9 ± 8.7	P < 0.0001
							b. 117.8 ± 8.7	P < 0.0001
							c. 31.9 ± 8.7	P = 0.0038
	Total Body Fat (%)	19.7 ± 1.3	28.6 ± 1.3*	30.7 ± 1.3*	6.0349	14.2235	a. 8.9 ± 1.5	P < 0.0001
							b. 11.0 ± 1.5	P < 0.0001
							c. 2.1 ± 1.5	P = 0.5296
	Total Lean Mass + BMC (g)	272.9 ± 6.5	378.0 ± 6.5*	412.3 ± 6.5*†	221.36	288.80	a. 105.1 ± 6.9	P < 0.0001
							b. 139.4 ± 6.9	P < 0.0001
							c. 34.3 ± 6.9	P = 0.0002
	Total Lean Mass (g)	264.9 ± 6.5	364.9 ± 6.5*	397.1 ± 6.5*†	217.25	290.96	a. 100.0 ± 7.0	P < 0.0001
							b. 132.2 ± 7.0	P < 0.0001
							c. 32.2 ± 7.0	P = 0.0004
	Total BMC (g)	7.90 ± 0.15	13.07 ± 0.15*	15.12 ± 0.15*†	0.1560	0.1146	a. 5.17 ± 0.14	P < 0.0001
							b. 7.22 ± 0.14	P < 0.0001
							c. 2.05 ± 0.14	P < 0.0001
	Total Mass (g)	339.6 ± 6.1	530.1 ± 6.1*	595.8 ± 6.1*†	267.51	179.20	a. 190.5 ± 5.5	P < 0.0001
							b. 256.2 ± 5.5	P < 0.0001
							c. 65.7 ± 5.5	P < 0.0001
	Abdominal Fat (g)	13.4 ± 3.1	48.3 ± 3.1*	61.6 ± 3.1*†	46.4971	71.6860	a. 34.9 ± 3.5	P < 0.0001
							b. 48.2 ± 3.5	P < 0.0001
							c. 13.3 ± 3.5	P = 0.0026
	Abdominal Fat (%of Total Abdominal Mass)	18.2 ± 1.3	33.1 ± 1.3*	34.8 ± 1.3*	6.0421	14.7732	a. 14.9 ± 1.6	P < 0.0001
							b. 16.6 ± 1.6	P < 0.0001
							c. 1.7 ± 1.6	P = 0.8711
	Abdominal Fat (% of Total Body Fat)	20.0 ± 0.6	31.5 ± 0.6*	33.5 ± 0.6*	1.8800	2.6080	a. 11.5 ± 0.6	P < 0.0001
							b. 13.5 ± 0.6	P < 0.0001
							c. 2.0 ± 0.6	P = 0.0183
	Abdominal Lean Mass + BMC (g)	60.5 ± 2.3	95.7 ± 2.3*	114.3 ± 2.3*†	26.2012	39.6714	a. 35.2 ± 2.6	P < 0.0001
							b. 53.8 ± 2.6	P < 0.0001

							c. 18.6 ± 2.6	P < 0.0001
	Abdominal Lean Mass (g)	60.0 ± 2.3	94.8 ± 2.3*	113.2 ± 2.3*†	25.9175	39.1702	a. 34.8 ± 2.5	P < 0.0001
							b. 53.2 ± 2.5	P < 0.0001
							c. 18.4 ± 2.5	P < 0.0001
	Abdominal BMC (g)	0.49 ± 0.02	0.92 ± 0.02*	1.11 ± 0.02*†	0.00235	0.00415	a. 0.43 ± 0.03	P < 0.0001
							b. 0.62 ± 0.03	P < 0.0001
							c. 0.19 ± 0.03	P < 0.0001
	Total Abdominal Mass (g)	73.9 ± 4.3	144.0 ± 4.3*	175.9 ± 4.3*†	111.84	112.54	a. 70.1 ± 4.3	P < 0.0001
							b. 102.0 ± 4.3	P < 0.0001
							c. 31.9 ± 4.3	P < 0.0001
High Gainers								
	Total Body Fat (g)	67.7 ± 13.3	166.5 ± 13.3*	214.0 ± 13.3*†	856.45	564.37	a. 98.8 ± 11.9	P < 0.0001
							b. 146.3 ± 11.9	P < 0.0001
							c. 47.5 ± 11.9	P = 0.0040
	Total Body Fat (%)	19.8 ± 1.8	28.1 ± 1.8*	32.2 ± 1.8*	16.4767	9.2710	a. 8.3 ± 1.5	P = 0.0002
							b. 12.4 ± 1.5	P < 0.0001
							c. 4.1 ± 1.5	P = 0.0525
	Total Lean Mass + BMC (g)	274.4 ± 7.8	421.8 ± 7.8*	446.4 ± 7.8*	245.20	246.22	a. 147.4 ± 7.8	P < 0.0001
							b. 172.0 ± 7.8	P < 0.0001
							c. 24.6 ± 7.8	P = 0.0219
	Total Lean Mass (g)	266.4 ± 7.8	407.4 ± 7.8*	429.9 ± 7.8*	236.98	245.19	a. 141.0 ± 7.8	P < 0.0001
							b. 163.5 ± 7.8	P < 0.0001
							c. 22.5 ± 7.8	P = 0.0370
	Total BMC (g)	7.94 ± 0.35	14.37 ± 0.35*	16.49 ± 0.35*†	0.6975	0.2799	a. 6.43 ± 0.26	P < 0.0001
							b. 8.55 ± 0.26	P < 0.0001
							c. 2.12 ± 0.26	P < 0.0001
	Total Mass (g)	342.0 ± 10.9	588.3 ± 10.9*	660.4 ± 10.9*†	782.06	165.37	a. 246.3 ± 6.4	P < 0.0001
							b. 318.4 ± 6.4	P < 0.0001
							c. 72.1 ± 6.4	P < 0.0001
	Abdominal Fat (g)	13.0 ± 5.4	55.0 ± 5.4*	73.3 ± 5.4*†	112.09	119.91	a. 42.0 ± 5.5	P < 0.0001
							b. 60.3 ± 5.5	P < 0.0001
							c. 18.3 ± 5.5	P = 0.0147
	Abdominal Fat (% of Total Abdominal Mass)	18.6 ± 1.8	33.3 ± 1.8*	36.8 ± 1.8*	14.4148	11.9155	a. 14.7 ± 1.7	P < 0.0001
							b. 18.2 ± 1.7	P < 0.0001
							c. 3.5 ± 1.7	P = 0.1765
	Abdominal Fat (% of Total Body Fat)	19.1 ± 0.9	33.0 ± 0.9*	33.9 ± 0.9*	0.1201	6.9886	a. 13.9 ± 1.3	P < 0.0001
							b. 14.8 ± 1.3	P < 0.0001
							c. 0.9 ± 1.3	P = 1.0000
	Abdominal Lean Mass + BMC (g)	56.5 ± 2.5	108.3 ± 2.5*	122.6 ± 2.5*†	0	50.1239	a. 51.8 ± 3.5	P < 0.0001
							b. 66.1 ± 3.5	P < 0.0001

							c. 14.3 ± 3.5	P = 0.0018
	Abdominal Lean Mass (g)	55.9 ± 2.5	107.3 ± 2.5*	121.3 ± 2.5* †	0	49.1994	a. 51.3 ± 3.5	P < 0.0001
							b. 65.4 ± 3.5	P < 0.0001
							c. 14.0 ± 3.5	P = 0.0019
	Abdominal BMC (g)	0.48 ± 0.04	1.00 ± 0.04*	1.27 ± 0.04* †	0.00310	0.008869	a. 0.52 ± 0.05	P < 0.0001
							b. 0.79 ± 0.05	P < 0.0001
							c. 0.27 ± 0.05	P = 0.0001
	Total Abdominal Mass (g)	69.5 ± 6.7	163.3 ± 6.7*	195.9 ± 6.7* †	159.73	201.15	a. 93.8 ± 7.1	P < 0.0001
							b. 126.4 ± 7.1	P < 0.0001
							c. 32.6 ± 7.1	P < 0.0013

* Significantly different from Baseline; † Significantly different from Pre-Training

Table 6b. Repeated measures ANOVA for each body composition variable for the total duration of the experiment.

Variable	NDF	DDF	F	P
Toatl body fat (g)	4	56.3	5.50	0.0008
Total body fat (%)	4	37.8	3.81	0.0106
Total lean mass + BMC (g)	4	40.5	4.79	0.0029
Total lean mass (g)	4	40.6	4.56	0.0039
Total BMC (g)	4	56.2	11.83	<0.0001
Total mass (g)	4	38.2	10.48	<0.0001
Abdominal fat (g)	4	55.7	7.43	<0.0001
Abdominal fat (% of total abdominal mass)	4	38.6	7.01	0.0002
Abdominal fat (% of total body fat)	4	37.7	7.06	0.0002
Abdominal lean mass + BMC (g)	4	39.5	5.47	0.0013
Abdominal lean mass (g)	4	39.6	5.45	0.0014
Abdominal BMC (g)	4	40.3	3.69	0.0120
Total abdominal mass (g)	4	36.3	4.50	0.0047

Table 7. Coefficients of variation (%) reported as mean ± SEM, in Sprague-Dawley rats determined at 2 time points: pre-training (n=4) and post-training (n =5) using triplicate dual-energy x-ray absorptiometry scans (modified from Mavreta Vagenas' MScA project, 2013)

	Total Body Fat (g)	**Total Body Fat (%)**	**Total Lean Mass + BMC (g)**	**Total Lean Mass (g)**	**Total BMC (g)**	**Total Mass (g)**	**Abdominal Fat Mass (g)**	**Abdominal Fat (% of Total Abdominal Mass)**	**Abdominal Lean Mass + BMC**	**Abdominal Lean Mass (g)**	**Abdominal BMC (g)**	**Total Abdominal Mass (g)**
Pre-training	2.5 ± 1.0	2.2 ± 0.9	0.9 ± 0.5	0.9 ± 0.5	1.3 ± 0.3	0.2 ± 0.1	1.8 ± 0.3	2.8 ± 0.8	5.8 ± 1.7	5.8 ± 1.7	4.3 ± 1.5	4.2 ± 1.0
Post-training	3.1 ± 1.0	2.8 ± 1.0	1.1 ± 0.3	1.2 ± 0.3	1.0 ± 0.3	0.4 ± 0.1	6.4 ± 1.7	4.3 ± 0.8	5.0 ± 1.6	5.0 ± 1.6	6.1 ± 2.0	4.5 ± 1.5

Table 8. Lee Obesity Index (LSmean ± SE) determined at baseline, pre-training and post-training of rats on a control diet (CD), HFD, and low gainers (LG) and high gainers (HG) of the HFD group (modified from Mavreta Vagenas' MScA project, 2013)

	Control Diet (n=10)	**High Fat Diet** (n=20)	**High Fat Diet**		**Differences ± SE** a. HFD - CD b. HG - CD c. LG - CD d. HG - LG	**P-values**
			Low Gainers (n=12)	**High Gainers** (n=8)		
Lee Obesity Index[1] Baseline	304.9 ± 2.7	303.6 ± 1.9	300.8 ± 2.4	307.7 ± 2.9	a. -1.3 ± 3.3	P = 0.6962
					b. 2.8 ± 3.9	P = 0.4744
					c. - 4.1 ± 3.5	P = 0.2581
					d. 6.9 ± 3.7	P = 0.0771
Lee Obesity Index Pre-Training	306.5 ± 2.5	309.0 ± 1.7	307.7 ± 2.3	310.8 ± 2.8	a. 2.5 ± 3.0	P = 0.4170
					b. -1.3 ± 3.6	P = 0.7049
					c. -4.3 ± 3.7	P = 0.2584
					d. 3.0 ± 3.6	P = 0.4079
Lee Obesity Index Post-Training	305.0 ± 2.1	307.7 ± 1.5	306.6 ± 1.9	309.4 ± 2.4	a. 2.7 ± 2.6	P = 0.3121
					b. -1.6 ± 2.9	P = 0.5875
					c. -4.3 ± 3.2	P = 0.1883
					d. 2.7 ± 3.1	P = 0.3825

[1] Lee Obesity Index=$3\sqrt{}$[wt(g)]/[naso-anal length (mm)] x 10^4

Table 9. Abdominal area fat pad mass (LSmean ± SEM) in control rats (CD), HFD rats, low gainers (LG) and high gainers (HG) (modified from Mavreta Vagenas' MScA project, 2013)

	Control (n = 10)	**High Fat Diet** (n = 20)	**High Fat Diet**		**Differences ± SE** a. HFD - CD b. HG - CD c. LG - CD d. HG - LG	**P-Value**
			Low Gainers (n = 12)	**High Gainers** (n = 8)		
Fat Pad Mass (g)	37.8 ± 3.3	55.3 ± 2.3***	52.5 ± 2.9**	59.6 ± 3.6***	a. 17.5 ± 4.0	P = 0.0002
					b. 21.8 ± 4.8	P = 0.0001
					c. 14.7 ± 4.4	P = 0.0024
					d. 7.1 ± 4.7	P = 0.1391

** P < 0.01 versus control rats; *** P < 0.001 versus control rat

Table 10. Repeated measures analyses of variance for test food intake before 8 h fast and maintenance food intake 1 h after fast

Effect	*Df* (num, denom)	*F*	*P*
Test food intake (g) prior to 8h fast.			
Diet	1, 28	1.57	0.2201
Cycle	9, 199	27.85	<0.0001
Diet*Cycle	9, 199	1.04	0.4124
Trend			
Linear	1, 54.6	165.96	<0.0001
Quadratic	1, 89	48.49	<0.0001
Cubic	1, 151	6.10	0.0146
Quartic	1, 229	1.10	0.2946
Test food intake (g) prior to 8h fast- Control, High-gainers vs. Low-gainers			
Group	2, 27	2.17	0.1341
Cycle	9, 192	27.99	<0.0001
Group*Cycle	18, 205	0.68	0.8242
Trend			
Linear	1, 53	165.96	<0.0001
Quadratic	1, 85.9	48.49	<0.0001
Cubic	1, 145	6.10	0.0067
Quartic	1, 220	1.10	0.4018
Maintenance food intake (g) during 1 h access following 8 h fast.			
Diet	1, 28	0.04	0.8446
Cycle	9, 205	8.66	<0.0001
Diet*Cycle	9, 205	1.14	0.3363
Trend			
Linear	1, 64.4	36.26	<0.0001
Quadratic	1, 99.7	1.39	0.2418
Cubic	1, 159	0.01	0.9040
Quartic	1, 228	5.23	0.0231
Maintenance food intake (kcal) during 1 h access following 8 h fast.			
Diet	1, 28	5.95	0.0213
Cycle	9, 205	7.79	<0.0001
Diet*Cycle	9, 205	1.10	0.3632
Trend			
Linear	1, 64.6	33.70	<0.0001
Quadratic	1, 100	1.13	0.2902
Cubic	1, 159	0.00	0.9918
Quartic	1, 228	4.36	0.0374
Maintenance food intake (g) during 1 h access following 8 h fast- Control, High-gainers vs. Low-gainers.			
Group	2, 27	0.38	0.6858
Cycle	9, 198	9.60	<0.0001
Group*Cycle	18, 209	0.92	0.5584
Trend			
Linear	1, 63.5	43.93	<0.0001
Quadratic	1, 97.7	0.69	0.4089
Cubic	1, 154	0.22	0.6361
Quartic	1, 220	5.33	0.0218
Maintenance food intake (kcal) during 1 h access following 8 h fast- Control, High-gainers vs. Low-gainers.			
Group	2, 27	3.35	0.0500
Cycle	9, 198	9.37	<0.0001
Group*Cycle	18, 210	0.94	0.5340
Trend			
Linear	1, 63.9	43.66	<0.0001
Quadratic	1, 98.1	0.56	0.4541
Cubic	1, 154	0.34	0.5634
Quartic	1, 220	4.90	0.0279

Table 11. Chi-Square Test between both diets for each cycle

Control; FREQ procedure			
Peaks/Trough	**DF**	**Value**	**Prob**
Peaks	7	14.07	0.0500
Troughs	7	22.91	0.0018
HFD; FREQ procedure			
Peaks	7	16.60	0.0202
Troughs	7	29.94	<0.0001
GENMOD procedure comparing both groups			
Effect	**DF**	**Chi-Square**	**Pr>ChiSq**
Peaks			
Diet	1	0.86	0.3529
Cycle	7	25.40	0.0006
Troughs			
Diet	1	0.25	0.6142
Cycle	7	37.86	<0.0001

Table 12. First peak and next trough using criterion of greater than one gram. Peaks are in bold and troughs in italics. Classification within the HFD group is labeled as HG for high-weight gainers and LG for low-weight gainers; H for high-global fat gainers and L for low-global fat gainers. Percentages (%) are the proportions of rats in their respective group experiencing a peak or a trough.

Control Group									
				Cycle					
Rat		3	4	5	6	7	8	9	10
1		1.38	2.13	**2.48**	*-4.37*	0.12	0.00	-2.15	0.24
2		**1.32**	0.73	4.83	1.73	-1.36	*-2.20*	3.80	-0.29
6		**7.14**	0.23	-0.79	2.72	*-1.29*	3.32	-2.77	1.43
10		**3.01**	1.42	2.28	4.51	1.63	*-2.29*	0.50	2.50
11		1.51	**5.60**	-0.44	2.52	3.20	*-4.12*	-1.44	1.10
18		-1.00	3.96	**6.64**	-0.23	0.53	*-1.63*	4.21	-0.13
19		2.40	2.51	4.00	**5.57**	1.48	*-2.24*	-0.13	0.95
22		**4.37**	0.67	1.09	3.03	-0.03	*-4.67*	2.76	-0.34
25		**2.36**	0.90	1.75	1.06	1.99	-0.42	-0.95	*-1.94*
28		0.05	**6.71**	3.55	*-2.35*	0.50	-1.21	2.81	-0.75
# of peaks (N, %)		**5, 50%**	**2, 20%**	**2, 20%**	**1, 10%**	**0**	**0**	**0**	**0**
# of troughs		*0*	*0*	*0*	*2*	*1*	*6*	*2*	*1*
HFD Group									
				Cycle					
Rat	Weight/Global Fat Category	3	4	5	6	7	8	9	10
3	HG/L	**3.50**	1.55	*-1.69*	3.37	1.78	-1.81	0.18	-2.06
4	HG/L	**5.60**	1.67	0.58	4.11	*-3.09*	-1.13	2.12	0.91
5	LG/L	**2.66**	-1.97	*-2.83*	3.02	1.23	-3.44	1.23	2.97
7	LG/H	0.06	0.13	**3.87**	2.22	0.13	-0.40	1.68	3.10
8	LG/H	**1.54**	0.14	*-2.84*	2.56	1.70	1.24	0.23	-0.45
9	LG/H	-1.53	1.38	**1.81**	*-2.53*	2.85	-3.09	-1.55	0.12
12	HG/H	-0.32	**6.29**	*-2.79*	2.59	5.66	-2.10	2.09	1.08
13	HG/H	**1.85**	1.65	2.56	-0.82	0.57	-0.79	0.75	1.58
14	HG/H	0.69	-0.98	**3.21**	2.39	0.41	-0.17	-0.25	*-1.78*
15	HG/H	**1.19**	0.05	-0.65	-0.71	-0.04	3.51	0.88	*-1.36*
16	LG/L	**1.72**	0.58	0.54	2.50	0.04	-1.47	*-1.93*	-1.18
17	LG/L	**5.53**	1.36	2.70	*-1.09*	1.66	-5.43	4.54	2.68
20	LG/H	**4.12**	1.77	2.27	1.71	*-2.89*	-2.63	3.48	-1.02
21	LG/L	**4.47**	2.10	*-1.51*	-0.09	0.76	-1.57	-0.10	-0.69
23	HG/L	1.41	**2.92**	0.31	1.83	*-1.78*	-0.78	1.85	3.36
24	LG/L	**1.20**	0.43	0.89	3.64	-0.35	*-2.56*	0.28	4.28
26	LG/H	2.00	**3.15**	0.43	3.22	*-2.12*	-1.23	1.22	0.44
27	HG/L	**4.42**	1.93	1.09	2.62	3.01	*-4.67*	0.75	4.79
29	LG/L	-0.15	**3.40**	0.25	1.50	*-1.23*	0.29	-0.05	5.65
30	LG/H	**9.72**	1.11	3.96	1.73	2.33	*-2.63*	1.24	1.99
# of peaks (N, %)		**13,65%**	**4, 20%**	**3, 15%**	**0**	**0**	**0**	**0**	**0**
	HG 8	**5, 61%**	**2, 25%**	**1, 13%**					
	LG 12	**8, 67%**	**2, 17%**	**2, 17%**					
	H 10	**5, 50%**	**2, 20%**	**3, 30%**					
	L 10	**8, 80%**	**2, 20%**	**3, 30%**					
HG/L 4		**3, 75**	**1, 25**	**0, 0**					
LG/L 6		**5, 83**	1, 17	**0, 0**					
LG/H 6		**3, 50**	1, 17	**2, 33**					
HG/H 4		**2, 33**	1, 25	**1, 25**					
# of troughs		*0*	*0*	*5*	*2*	*5*	*3*	*1*	*2*
Total peaks		**18**	**6**	**5**	**1**	**0**	**0**	**0**	**0**
Total troughs		*0*	*0*	*5*	*4*	*6*	*9*	*3*	*2*

Table 13. First peak and next trough using criterion of greater than two grams. Peaks are in bold and troughs in italics. Classification within the HFD group is labelled as HG for high-weight gainers and LG for low-weight gainers; H for high-global fat gainers and L for low-global fat gainers. Percentages (%) are the proportions of rats in their respective group experiencing a peak or a trough.

Control Group									
		Cycle							
Rat		3	4	5	6	7	8	9	10
1		1.38	2.13	**2.48**	*-4.37*	0.12	0.00	-2.15	0.24
2		1.32	0.73	**4.83**	1.73	-1.36	*-2.20*	3.80	-0.29
6		**7.14**	0.23	-0.79	2.72	-1.29	3.32	*-2.77*	1.43
10		**3.01**	1.42	2.28	4.51	1.63	*-2.29*	0.50	2.50
11		1.51	**5.60**	-0.44	2.52	3.20	*-4.12*	-1.44	1.10
18		-1.00	3.96	**6.64**	-0.23	0.53	-1.63	4.21	-0.13
19		2.40	2.51	4.00	**5.57**	1.48	*-2.24*	-0.13	0.95
22		**4.37**	0.67	1.09	3.03	-0.03	*-4.67*	2.76	-0.34
25		**2.36**	0.90	1.75	1.06	1.99	-0.42	-0.95	-1.94
28		0.05	**6.71**	3.55	*-2.35*	0.50	-1.21	2.81	-0.75
# of peaks (N, %)		**4,** 40%	**2,** 20%	**3,** 30%	**1,** 10%	**0**	**0**	**0**	**0**
# of troughs		*0*	*0*	*0*	*2*	*0*	*5*	*1*	*0*
HFD Group									
		Cycle							
Rat	Weight/Global Fat Category	3	4	5	6	7	8	9	10
3	HG/L	**3.50**	1.55	*-1.69*	3.37	1.78	-1.81	0.18	-2.06
4	HG/L	**5.60**	1.67	0.58	4.11	*-3.09*	-1.13	2.12	0.91
5	LG/L	**2.66**	-1.97	*-2.83*	3.02	1.23	-3.44	1.23	2.97
7	LG/H	0.06	0.13	**3.87**	2.22	0.13	-0.40	1.68	3.10
8	LG/H	1.54	0.14	*-2.84*	**2.56**	1.70	1.24	0.23	-0.45
9	LG/H	-1.53	1.38	1.81	*-2.53*	**2.85**	-3.09	-1.55	0.12
12	HG/H	-0.32	**6.29**	*-2.79*	2.59	5.66	-2.10	2.09	1.08
13	HG/H	1.85	1.65	**2.56**	-0.82	0.57	-0.79	0.75	1.58
14	HG/H	0.69	-0.98	**3.21**	2.39	0.41	-0.17	-0.25	*-1.78*
15	HG/H	1.19	0.05	-0.65	-0.71	-0.04	**3.51**	0.88	*-1.36*
16	LG/L	1.72	0.58	0.54	**2.50**	0.04	-1.47	*-1.93*	-1.18
17	LG/L	**5.53**	1.36	2.70	*-1.09*	1.66	-5.43	4.54	2.68
20	LG/H	**4.12**	1.77	2.27	1.71	*-2.89*	-2.63	3.48	-1.02
21	LG/L	**4.47**	2.10	*-1.51*	-0.09	0.76	-1.57	-0.10	-0.69
23	HG/L	1.41	**2.92**	0.31	1.83	*-1.78*	-0.78	1.85	3.36
24	LG/L	1.20	0.43	0.89	**3.64**	-0.35	*-2.56*	0.28	4.28
26	LG/H	2.00	**3.15**	0.43	3.22	*-2.12*	-1.23	1.22	0.44
27	HG/L	**4.42**	1.93	1.09	2.62	3.01	*-4.67*	0.75	4.79
29	LG/L	-0.15	**3.40**	0.25	1.50	*-1.23*	0.29	-0.05	5.65
30	LG/H	**9.72**	1.11	3.96	1.73	2.33	*-2.63*	1.24	1.99
# of peaks (N, %)		**8, 40%**	**4, 40%**	**4, 15%**	**2**	**1**	**1**	**0**	**0**
	HG 8	**3, 38%**	**2, 25%**	**2, 25%**	**0**	**0**	**1, 5%**		
	LG 12	**3, 25%**	**2, 17%**	**2, 17%**	**2, 17%**	**1, 5%**	**0**		
	H 10	**3, 30%**	**2, 20%**	**4, 40%**	**1, 5%**	**1, 5%**	**1, 5%**		
	L 10	**5, 50%**	**2, 20%**	**3, 30%**	**1, 5%**	**0**	**0**		
# of troughs		*0*	*0*	*5*	*2*	*5*	*3*	*1*	*2*
Total peaks		**12**	**6**	**7**	**3**	**1**	**1**	**0**	**0**
Total troughs		*0*	*0*	*5*	*4*	*6*	*9*	*3*	*2*

Table 14. Survival analysis testing occurrence of first peaks using criterion of greater than two grams

Control vs. HFD			
Test	**Chi-Square**	**DF**	**Pr>Chi-Square**
Log-Rank	0.5014	1	0.4789
Wilcoxon	0.1028	1	0.7485
-2Log(LR)	0.0330	1	0.8559
Control vs. High-weight gainers vs. Low-weight gainers			
Test	**Chi-Square**	**DF**	**Pr>Chi-Square**
Log-Rank	0.5596	2	0.7559
Wilcoxon	0.7564	2	0.6851
-2Log(LR)	0.0534	2	0.9736

Table 15. Survival analysis testing occurrence of first peaks using criterion of greater than one gram

Control vs. HFD			
Test	**Chi-Square**	**DF**	**Pr>Chi-Square**
Log-Rank	0.3225	1	0.5701
Wilcoxon	0.7564	1	0.3845
-2Log(LR)	0.0431	1	0.8356
Control vs. High-weight gainers vs. Low-weight gainers			
Test	**Chi-Square**	**DF**	**Pr>Chi-Square**
Log-Rank	0.5596	2	0.7559
Wilcoxon	0.7564	2	0.6851
-2Log(LR)	0.0534	2	0.9736

Table 16. Analyses of variance for ‘reward’ and ‘satiety’ hypotheses

‘Reward’					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Cycle	8	3.65	0.456	3.58	0.0045
Group	4	2.75	0.688	5.41	0.0019
‘Satiety’					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Cycle	8	2.207	0.276	1.98	0.0818
Group	4	3.012	0.753	5.40	0.0019

Table 17. Least square means for cycle correlations for 'reward' and 'satiety' hypotheses

'Reward'			
Cycle	LS mean	Stand. Error	Pr>t
1	0.618	0.160	0.0005
2	0.365	0.160	0.0290
3	0.584	0.160	0.0009
4	0.714	0.160	<0.0001
5	0.111	0.160	0.4917
6	0.262	0.160	0.1102
7	-0.160	0.160	0.3230
8	0.129	0.160	0.4248
9	-0.008	0.160	0.9583
'Satiety'			
Cycle	LS mean	Stand. Error	Pr>t
1	0.331	0.167	0.0559
2	0.503	0.167	0.0050
3	0.432	0.167	0.0144
4	0.583	0.167	0.0014
5	0.216	0.167	0.2042
6	0.208	0.167	0.2219
7	-0.121	0.167	0.4724
8	0.028	0.167	0.8660
9	0.060	0.167	0.7226

Table 18. Least squares means for cycle effect for 'reward' and 'satiety' hypotheses, Pr>|t| for H0: LSMean(i)=LSMean(j). Dependent variable: correlation

'Reward'									
i/j	1	2	3	4	5	6	7	8	9
1		1.0000	1.0000	1.0000	1.0000	1.0000	0.0578	1.0000	0.3293
2	1.0000		1.0000	1.0000	1.0000	1.0000	0.9538	1.0000	1.0000
3	1.0000	1.0000		1.0000	1.0000	1.0000	0.0862	1.0000	0.4744
4	1.0000	1.0000	1.0000		0.4235	1.0000	0.0180	0.5132	0.1112
5	1.0000	1.0000	1.0000	0.4235		1.0000	1.0000	1.0000	1.0000
6	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000
7	0.0578	0.9538	0.0862	0.0180	1.0000	1.0000		1.0000	1.0000
8	1.0000	1.0000	1.0000	0.5132	1.0000	1.0000	1.0000		1.0000
9	0.3293	1.0000	0.4744	0.1112	1.0000	1.0000	1.0000	1.0000	
'Satiety'									
i/j	1	2	3	4	5	6	7	8	9
1		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
2	1.0000		1.0000	1.0000	1.0000	1.0000	0.4526	1.0000	1.0000
3	1.0000	1.0000		1.0000	1.0000	1.0000	0.9150	1.0000	1.0000
4	1.0000	1.0000	1.0000		1.0000	1.0000	0.1935	0.8993	1.0000
5	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000
6	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000
7	1.0000	0.4526	0.9150	0.1935	1.0000	1.0000		1.0000	1.0000
8	1.0000	1.0000	1.0000	0.8993	1.0000	1.0000	1.0000		1.0000
9	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	

Table 19. Least square means for group correlations for 'reward' and 'satiety' hypotheses. 1 is control, 2 is high weight gainers/low fat gainers, 3 is low weight gainers/low fat gainers, 4 is low weight gainers/high fat gainers, and 5 is high weight gainers/high fat gainers

'Reward'			
Group	LS mean	Stand. Error	Pr>t
1	0.1236	0.1189	0.3067
2	0.3394	0.1189	0.0075
3	0.6643	0.1189	<0.0001
4	-0.0647	0.1189	0.5904
5	0.3897	0.1189	0.0025
'Satiety'			
Group	LS mean	Stand. Error	Pr>t
1	0.0498	0.1244	0.6918
2	0.2526	0.1244	0.0508
3	0.6161	0.1244	<0.0001
4	-0.1063	0.1244	0.3992
5	0.4331	0.1244	0.0015

Table 20. Least squares means for group effect for 'reward' and 'satiety' hypotheses, Pr>|t| for H0: LSMean(i)=LSMean(j). Dependent variable: correlation. 1 is control, 2 is high weight gainers/low fat gainers, 3 is low weight gainers/low fat gainers, 4 is low weight gainers/high fat gainers, and 5 is high weight gainers/high fat gainers

'Reward'					
i/j	1	2	3	4	5
1		1.0000	0.0298	1.0000	1.0000
2	1.0000		0.6233	0.2226	1.0000
3	0.0298	0.6233		0.0014	1.0000
4	1.0000	0.2226	0.0014		0.1096
5	1.0000	1.0000	1.0000	0.1096	
'Satiety'					
i/j	1	2	3	4	5
1		1.0000	0.0295	1.0000	0.3688
2	1.0000		0.4703	0.4976	1.0000
3	0.0295	0.4703		0.0026	1.0000
4	1.0000	0.4976	0.0026		0.0440
5	0.3688	1.0000	1.0000	0.0440	

Appendix 2. Supplementary Figures

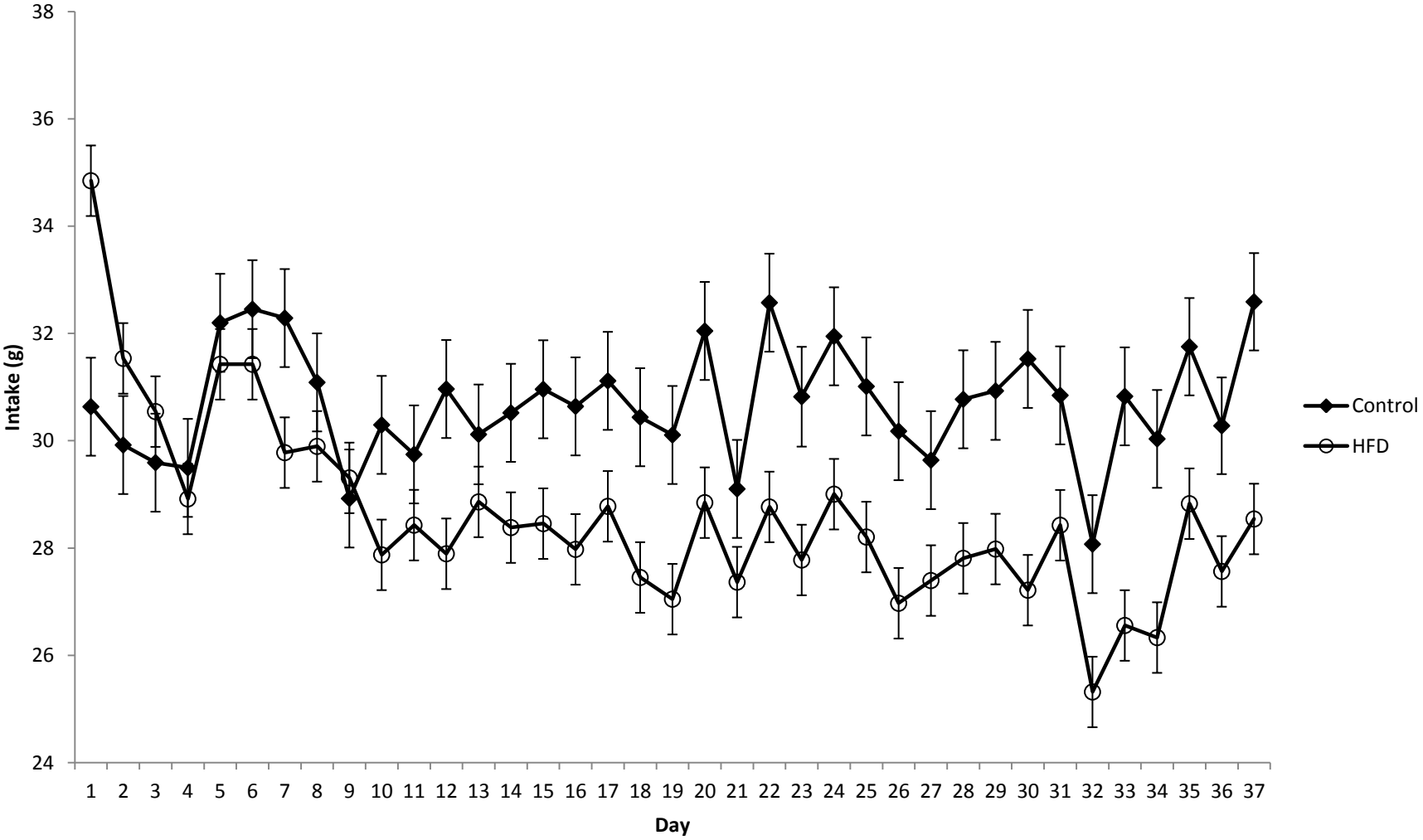


Figure 1. Intake of respective maintenance food (g, LSmean ± SEM) over 37 days for control group and HFD group prior to training.

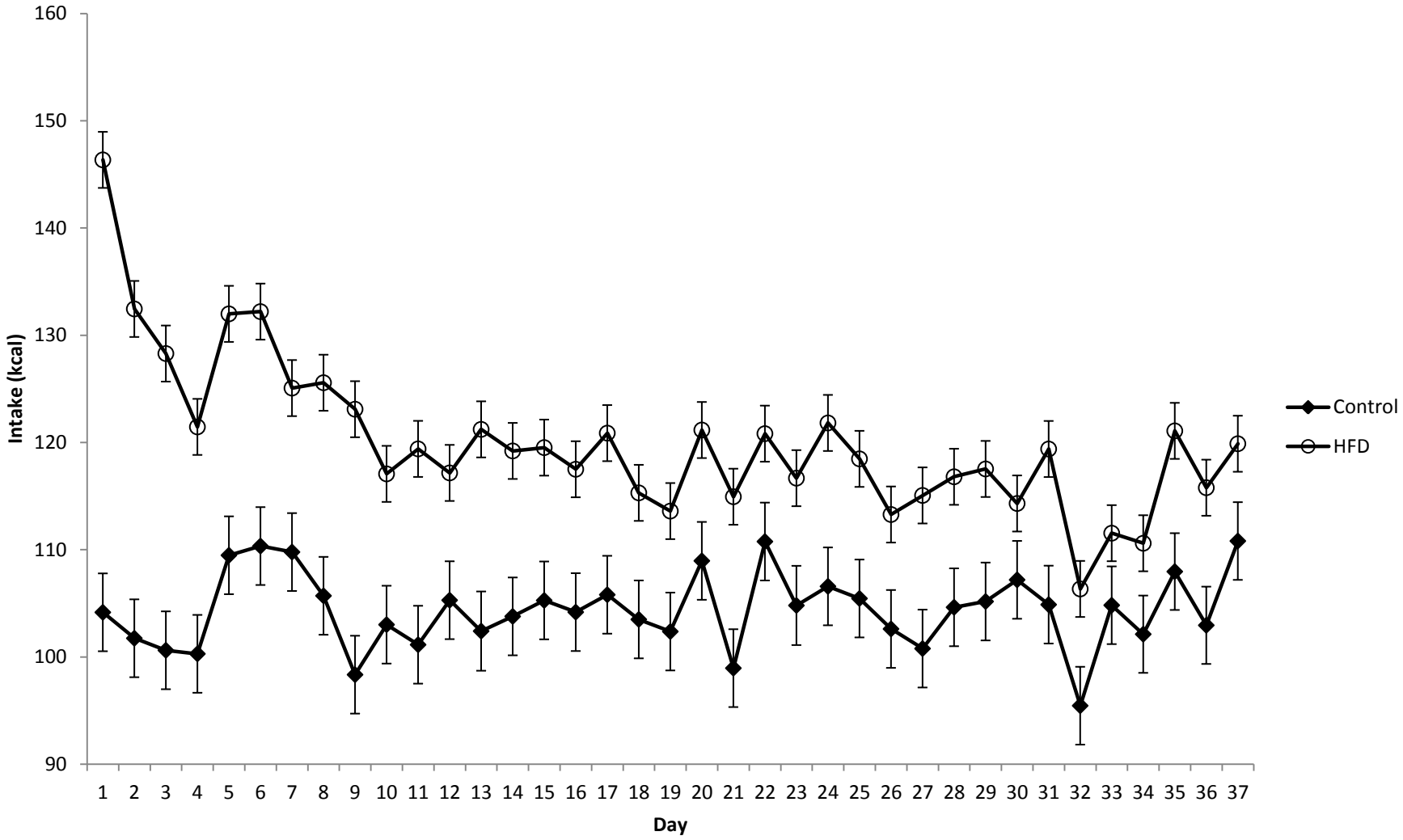


Figure 2. Intake of respective maintenance food (kcal, LSmean ± SEM) 37 days for control group and HFD group prior to training.

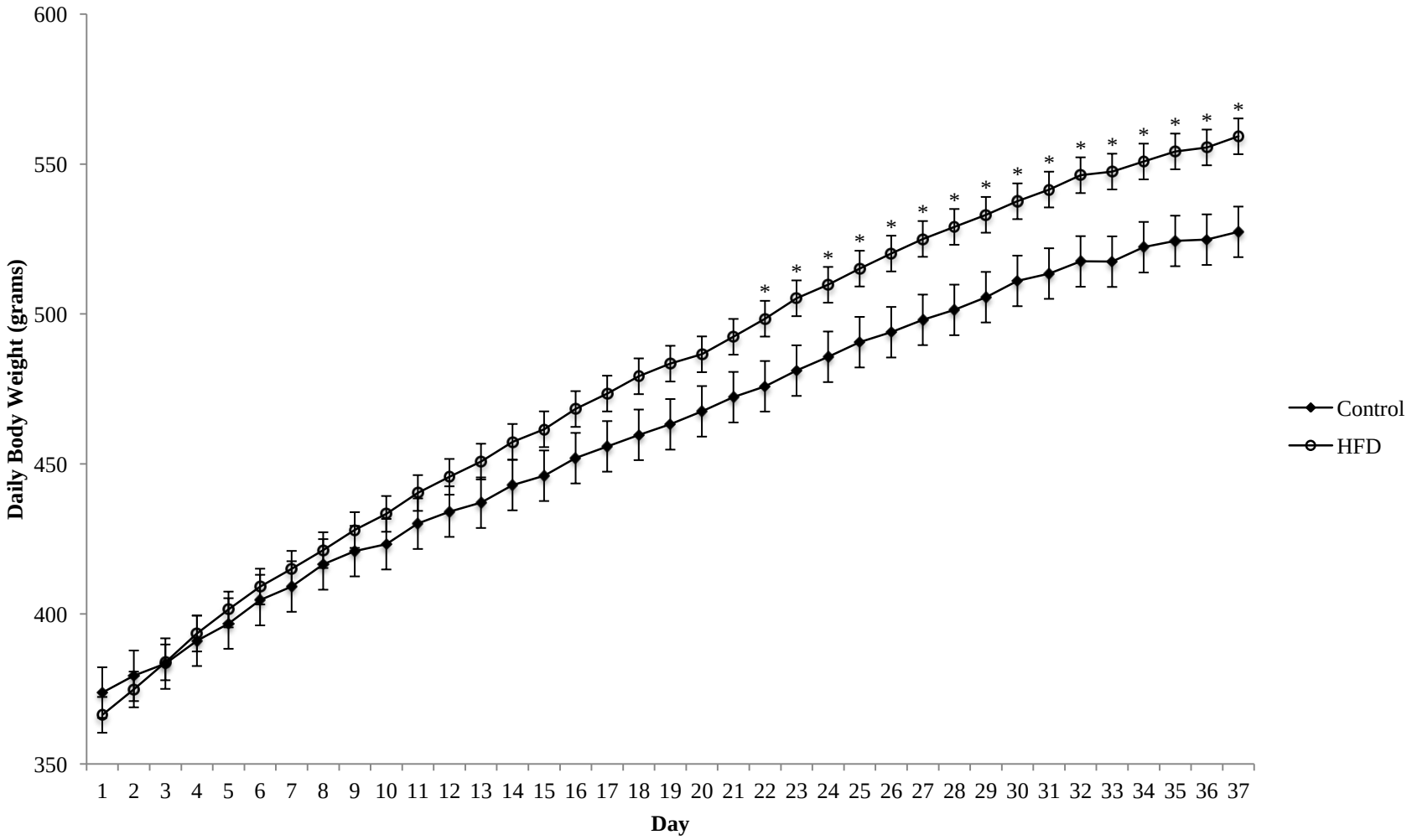


Figure 3. Daily body weight (LSmeans ± SEM) of rats fed a control diet or a HFD during the maintenance period (modified from Mavreta Vagenas' MScA project, 2013)
* Significant difference between control diet and high fat diet within day

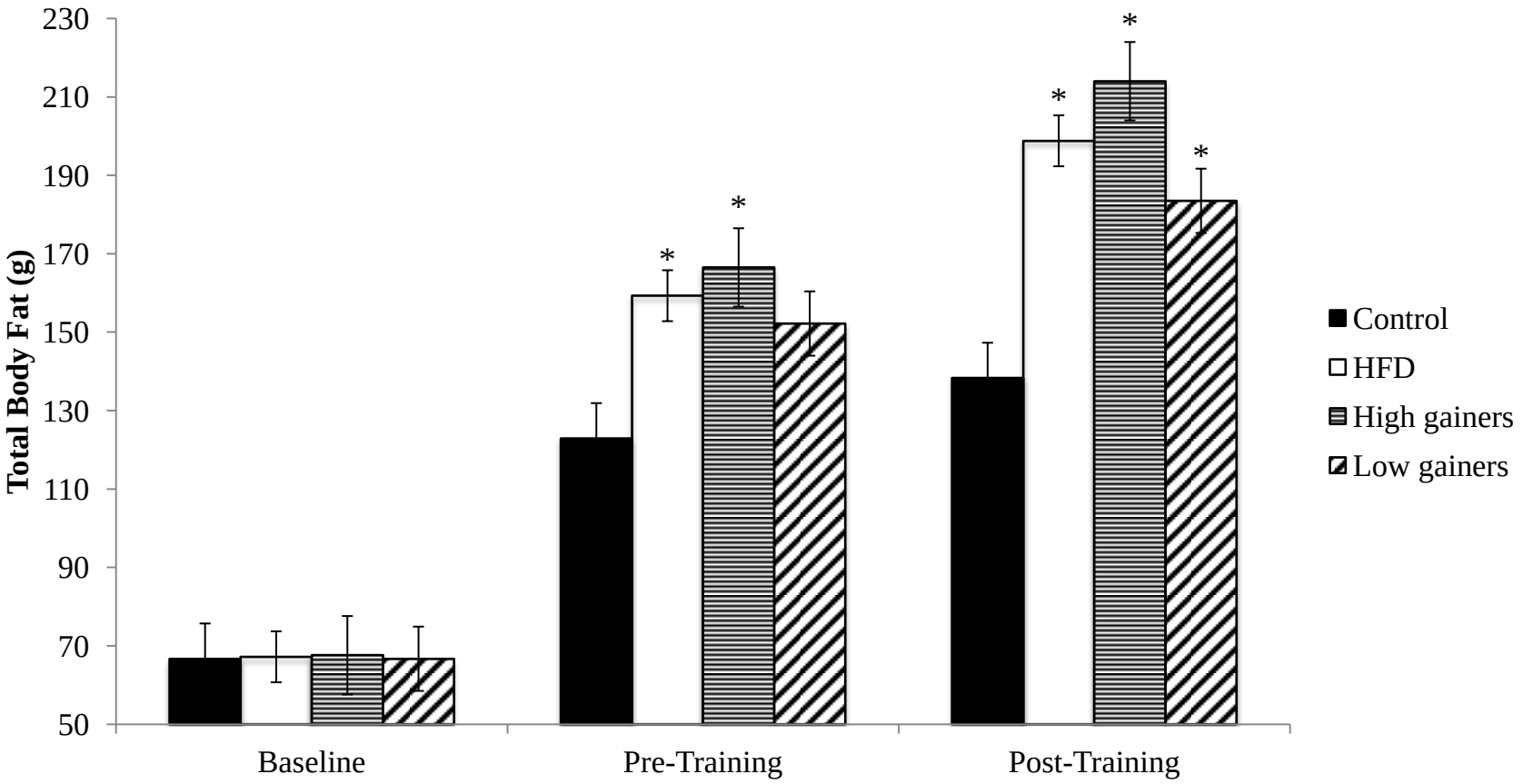


Figure 4. Total body fat measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8), and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point (i.e. baseline, pre-training, or post-training); † Significantly different from low gainers at a given time point

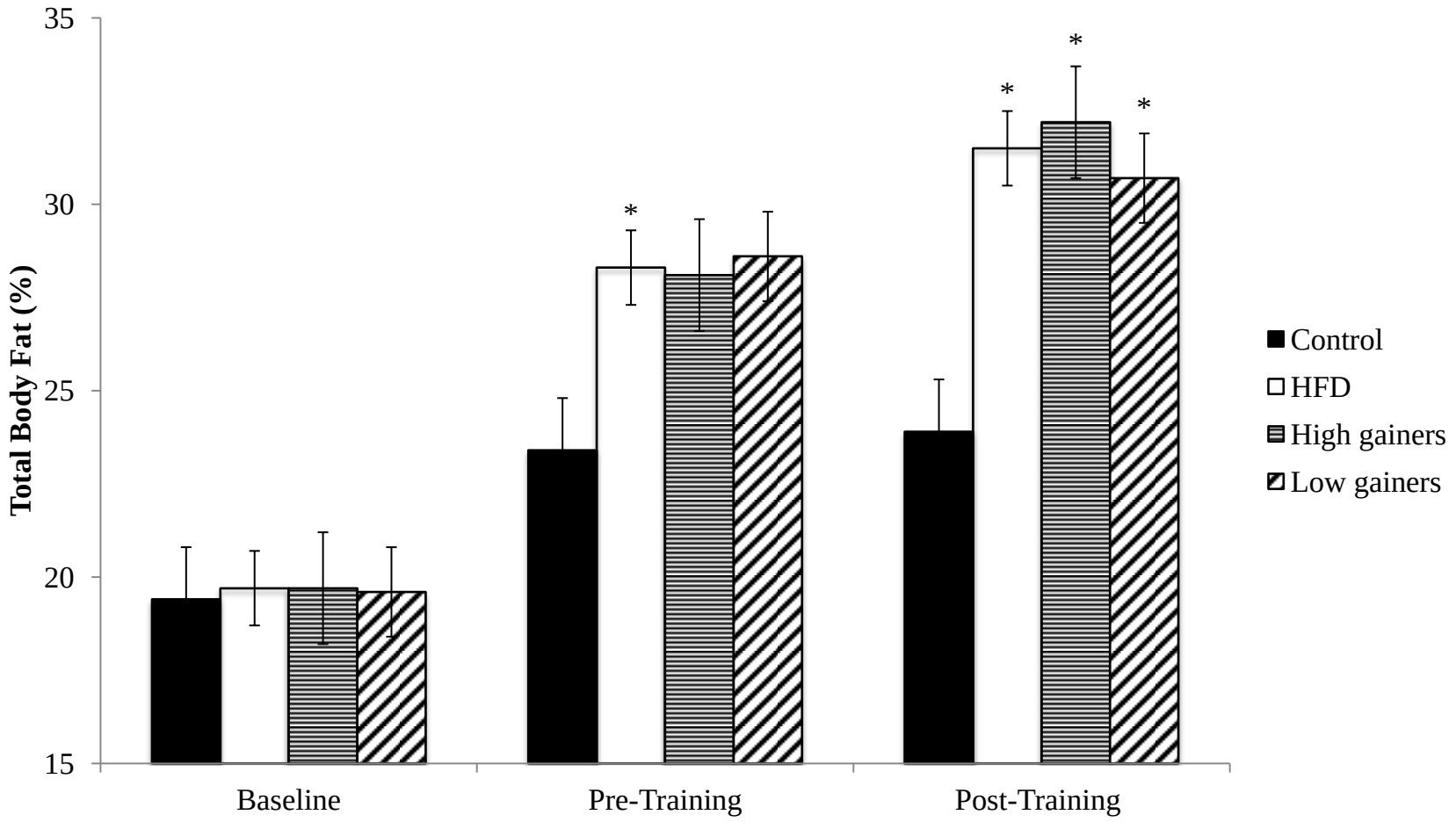


Figure 5. Total body fat (%) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8), and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

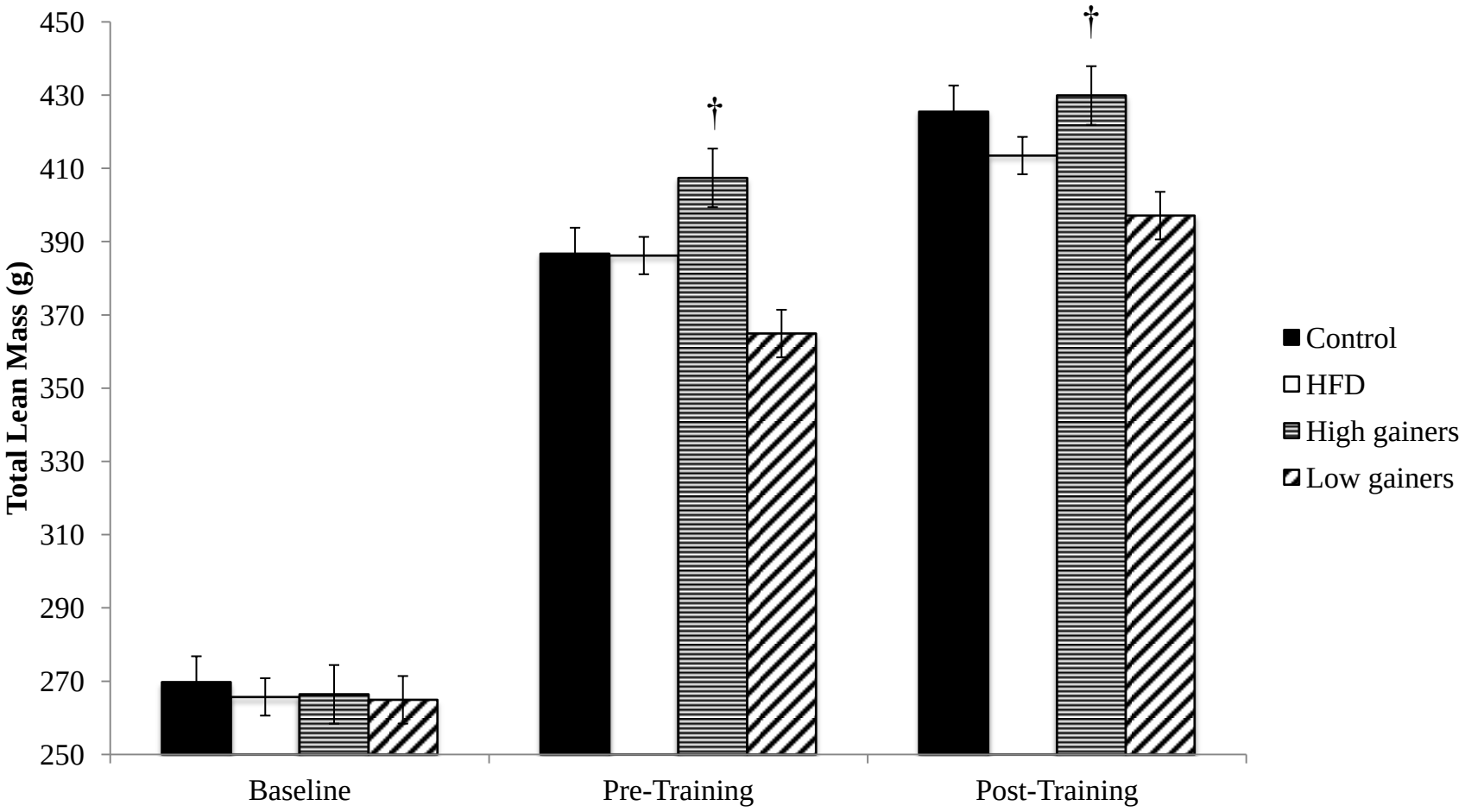


Figure 6. Total lean mass (g) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8), and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

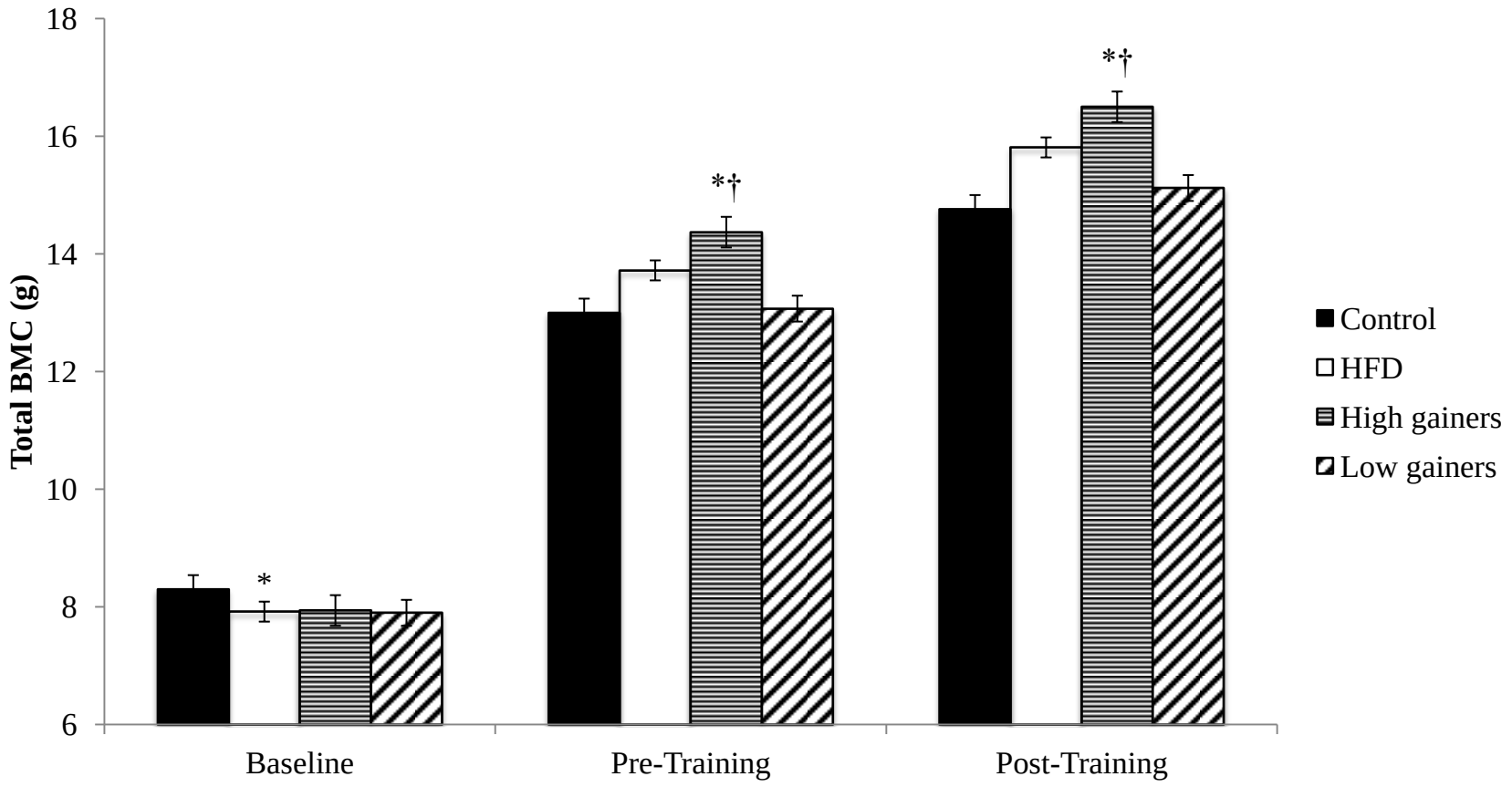


Figure 7. Total BMC (g) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8) and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

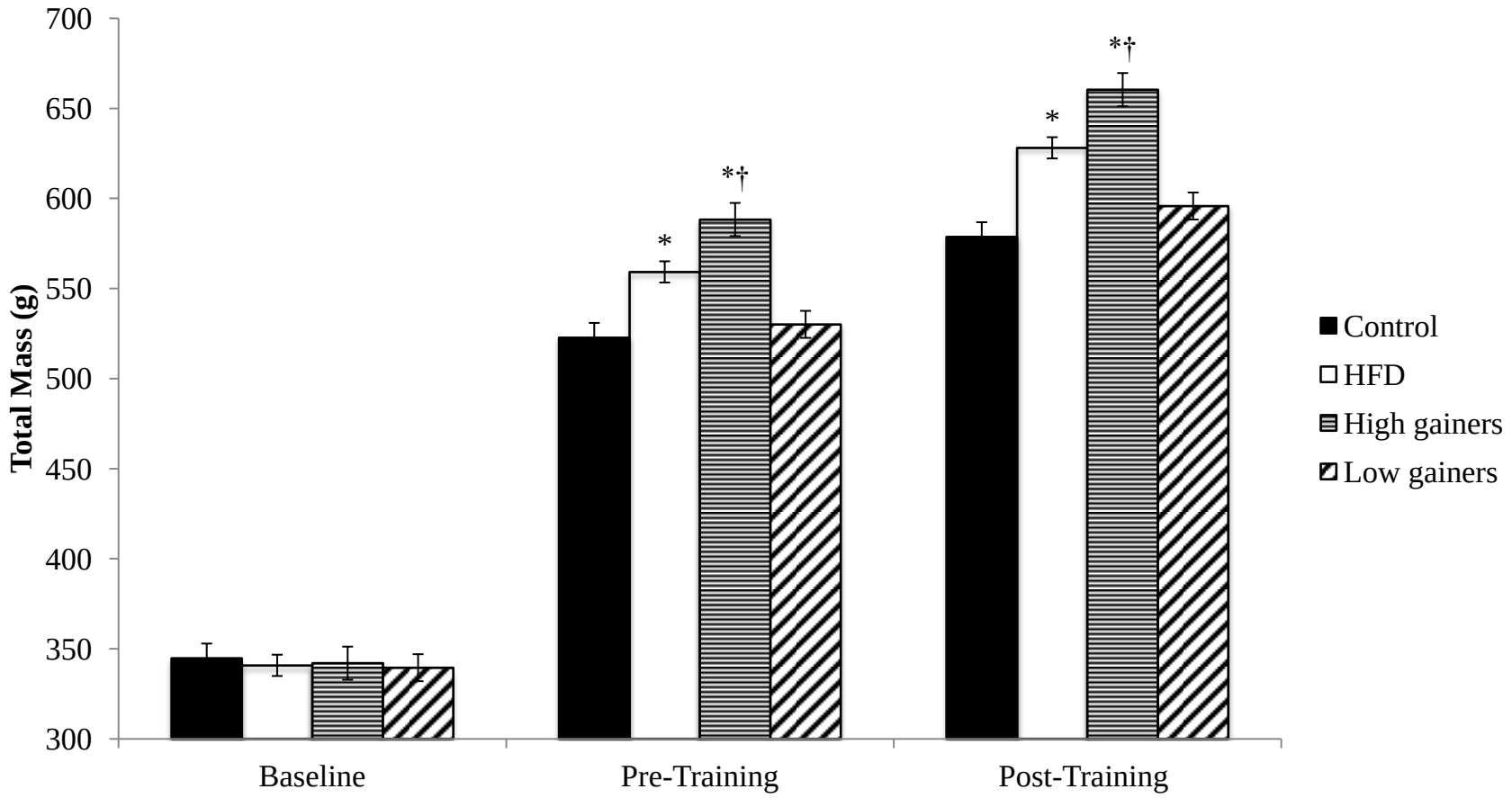


Figure 8. Total mass (g) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8) and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

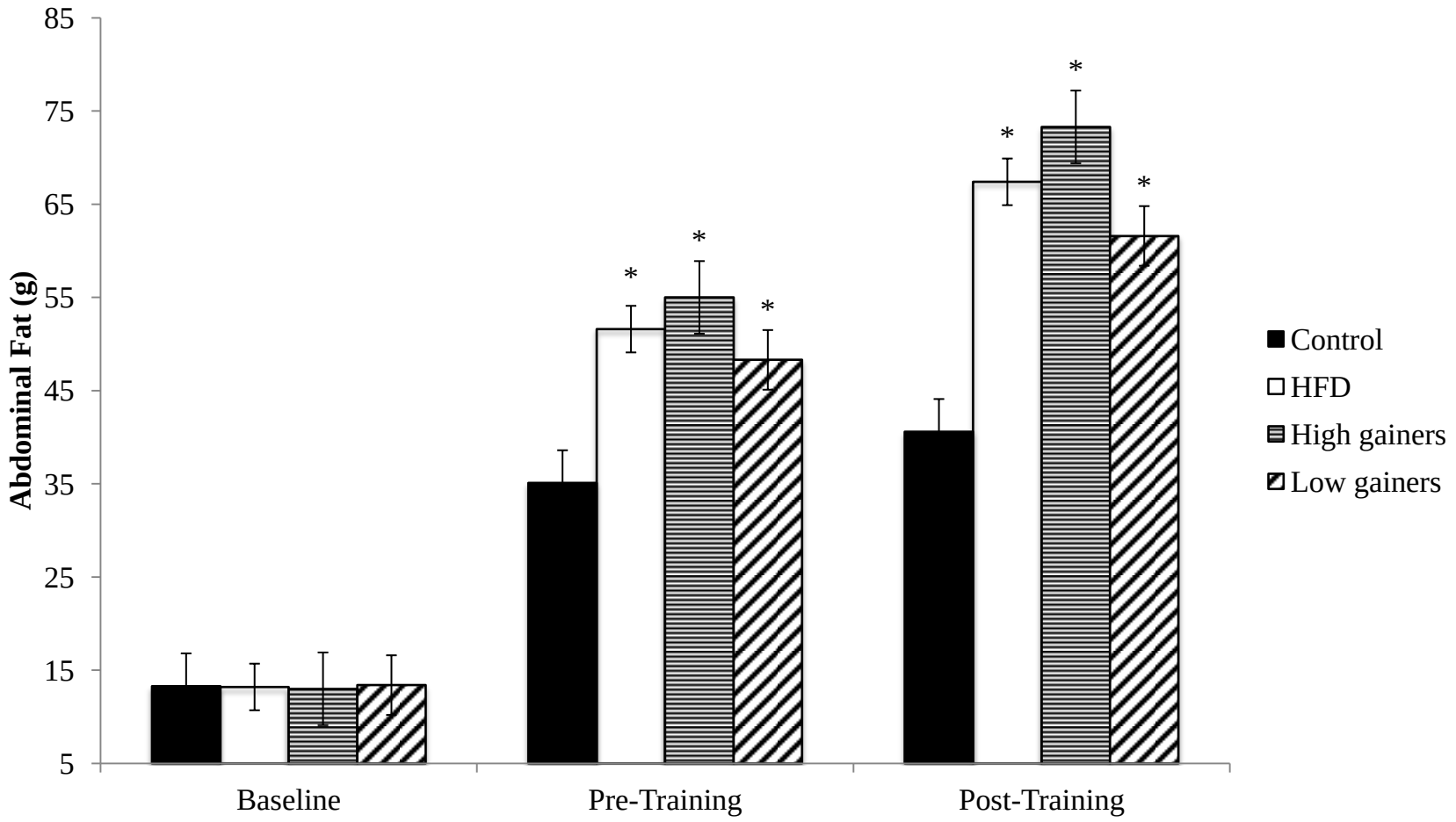


Figure 9. Abdominal fat (g) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8) and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

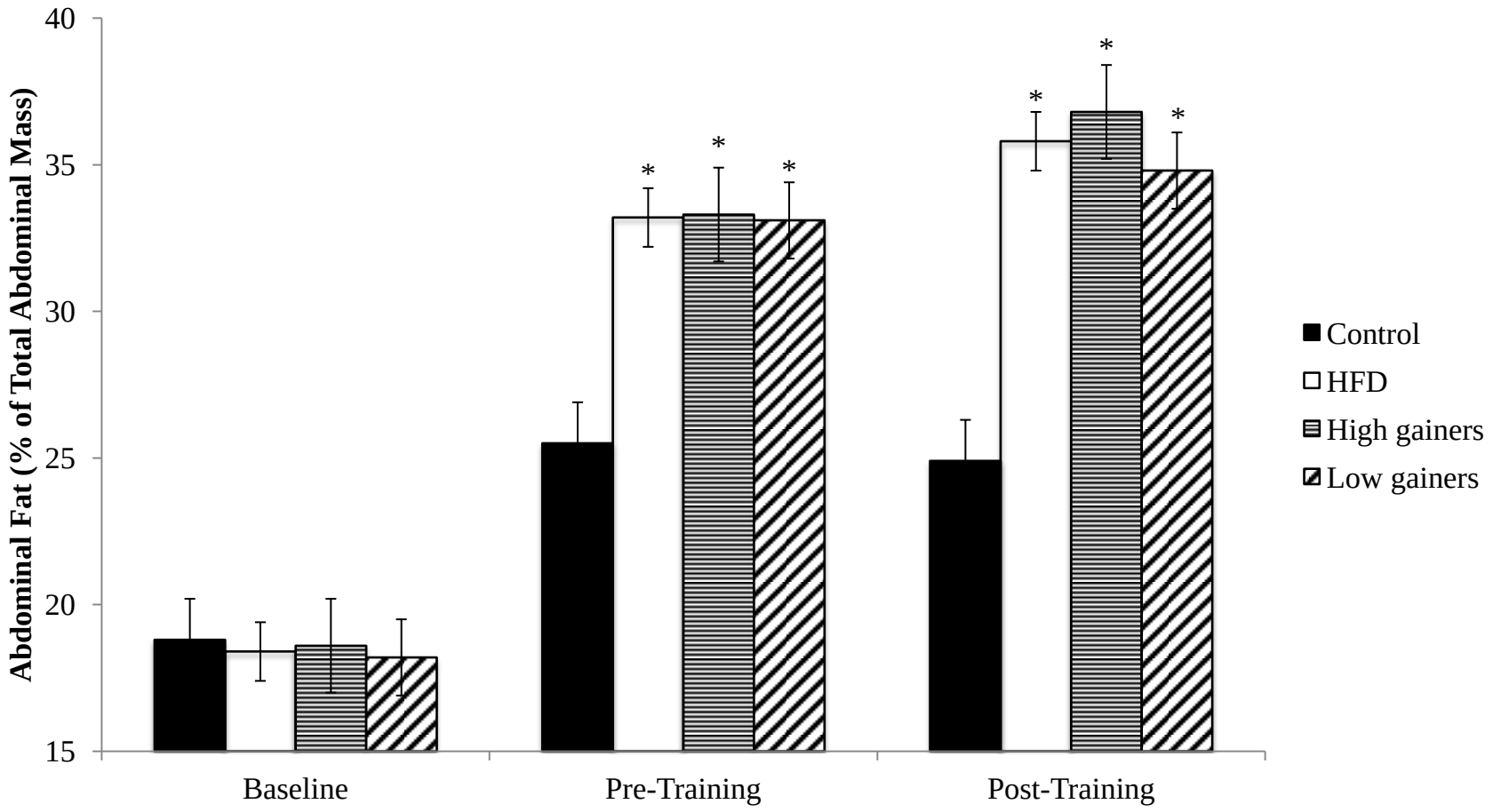


Figure 10. Abdominal fat (% of total abdominal mass) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8) and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

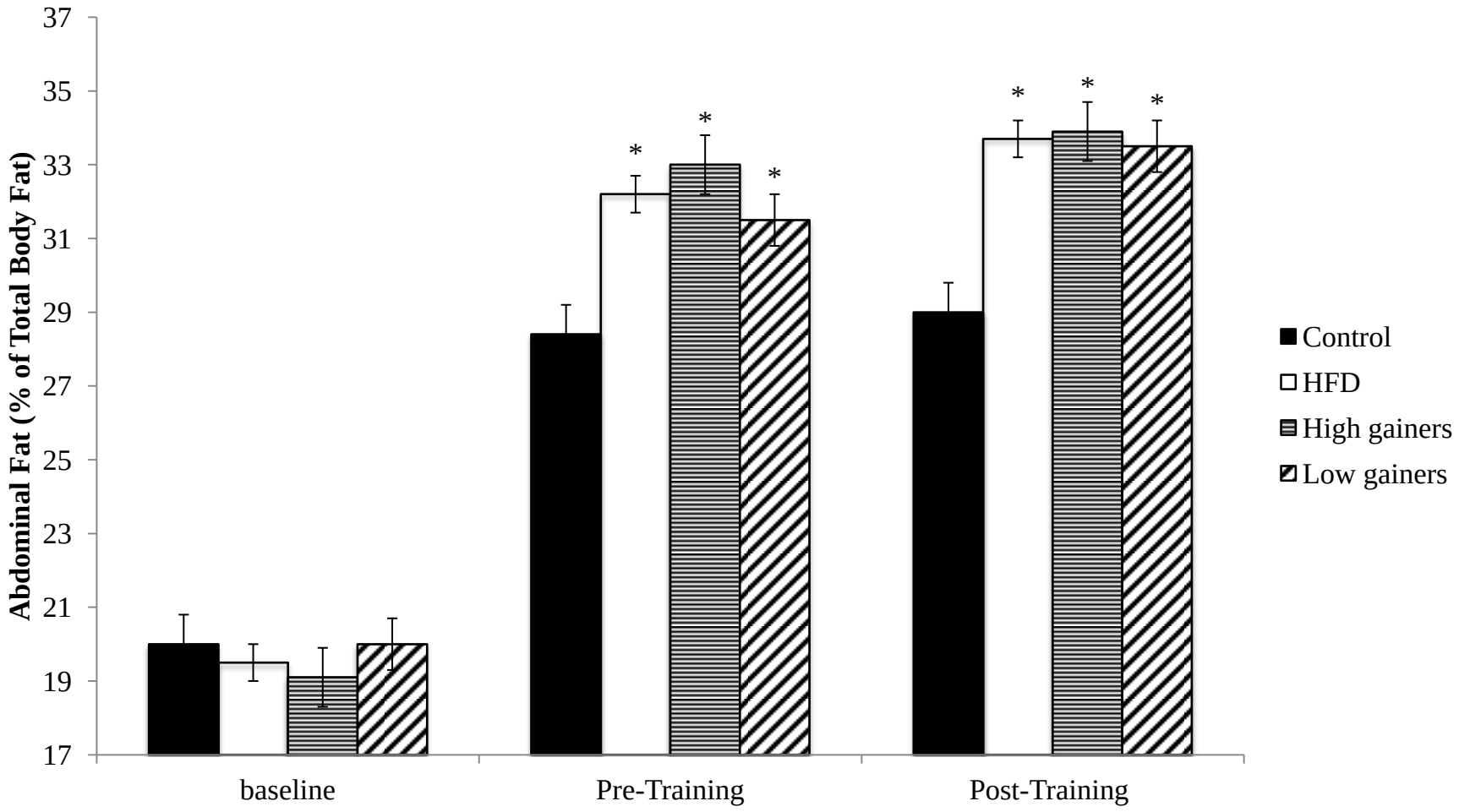


Figure 11. Abdominal fat (% of total body fat) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8) and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

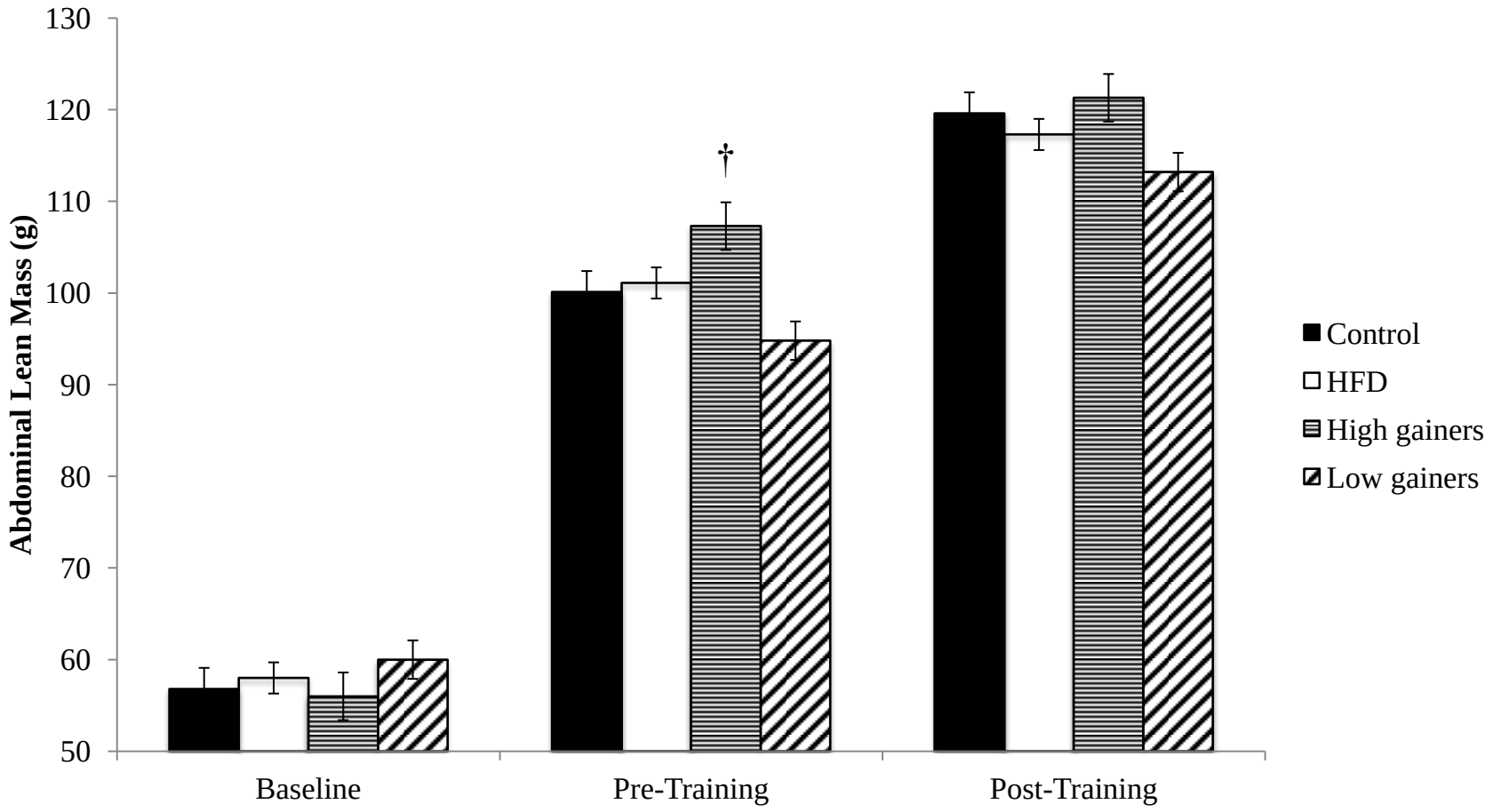


Figure 12. Abdominal lean mass (g) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers rats (n =8) and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

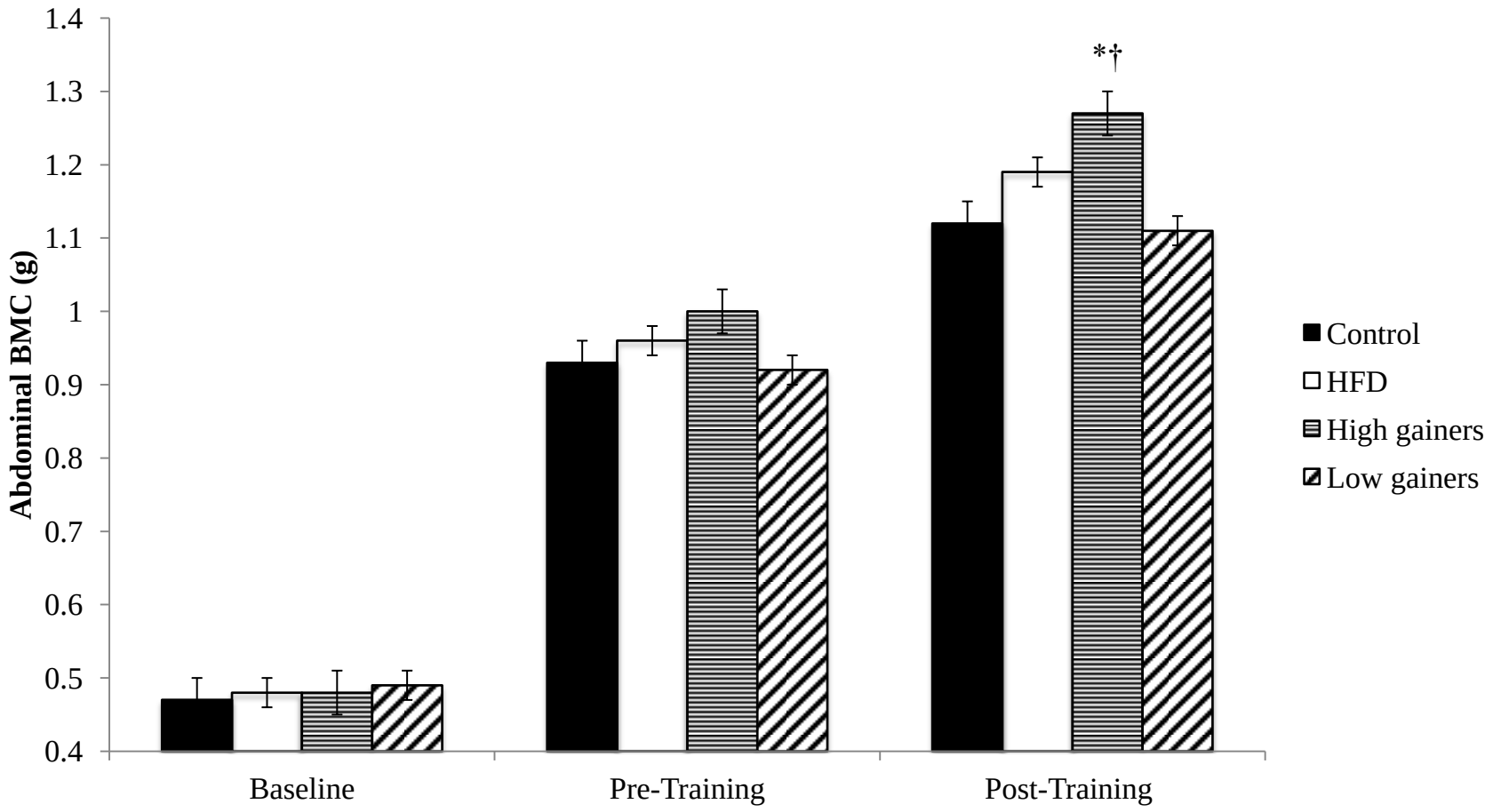


Figure 13. Abdominal BMC (g) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8) and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

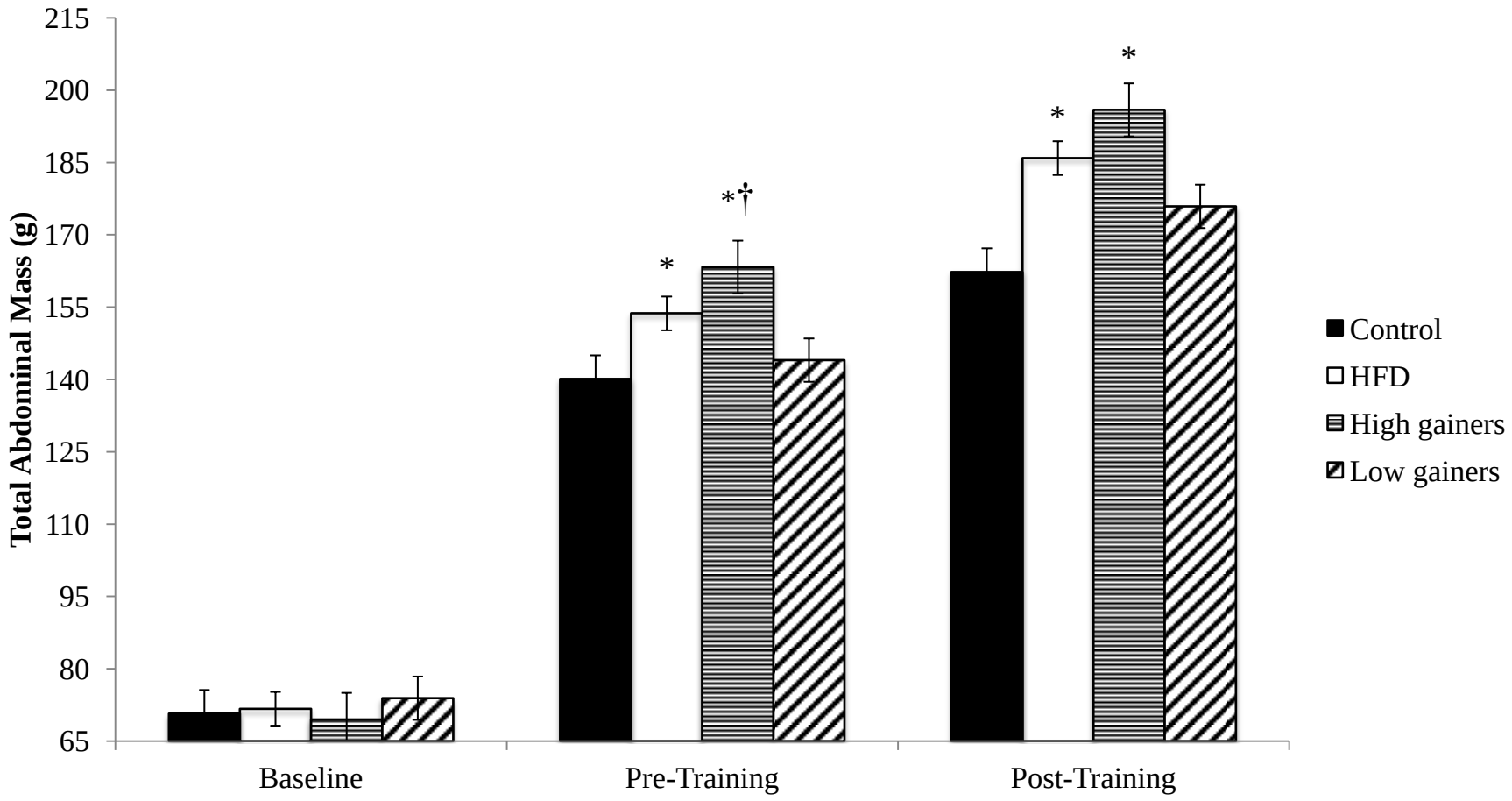


Figure 14. Total abdominal mass (g) measured by DXA in control rats (n =10), HFD rats (n =20), high weight gainers (n =8) and low weight gainers (n =12) at baseline, pre-training, and post-training (modified from Mavreta Vagenas' MScA project, 2013)
* Significantly different from control at a given time point; † Significantly different from low gainers at a given time point

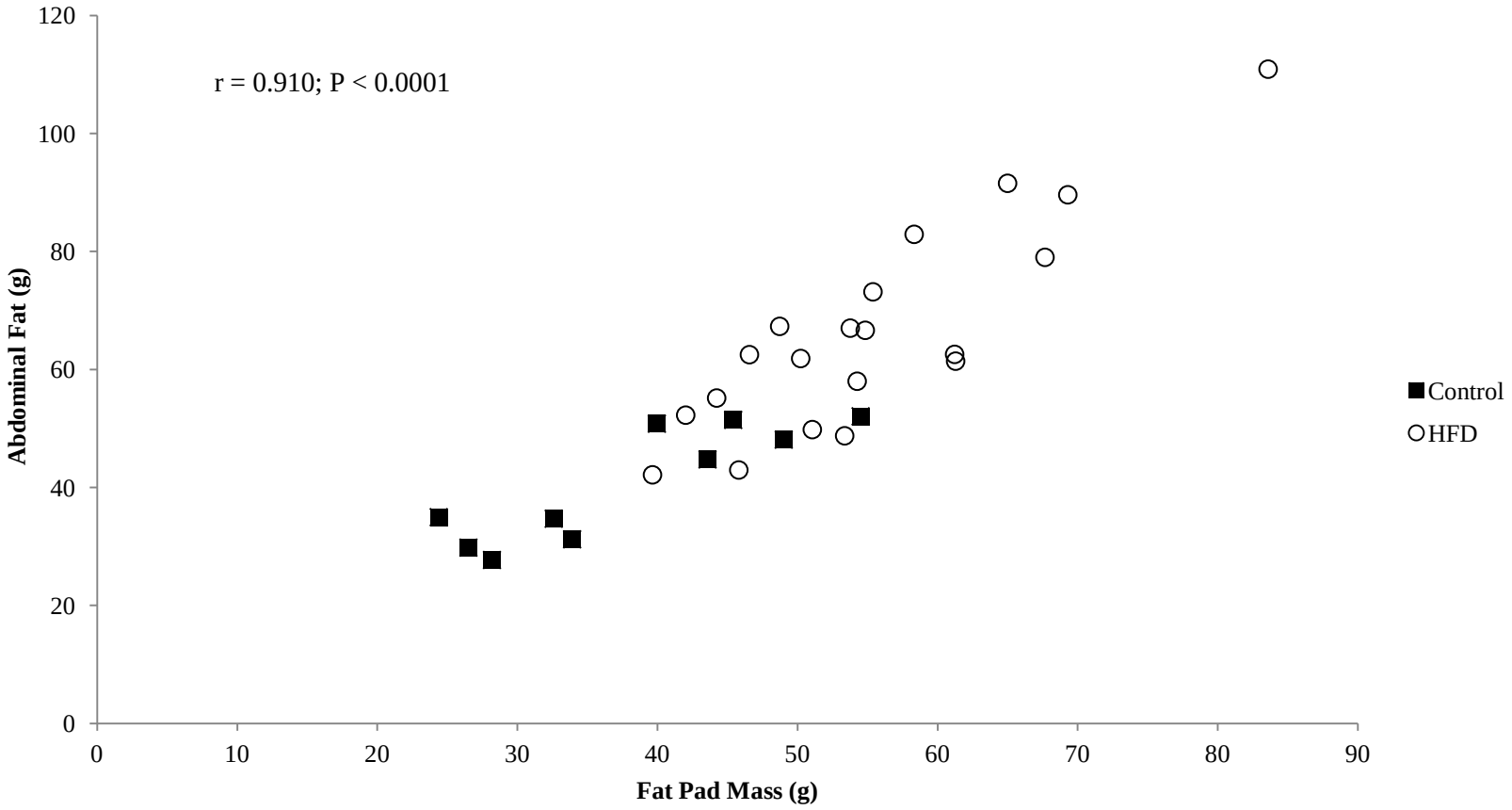


Figure 15. Correlation between abdominal fat (g) measured post-training by DXA and abdominal area fat pad mass (g) measured at sacrifice, in 30 male, Sprague-Dawley rats (modified from Mavreta Vagenas' MScA project, 2013)

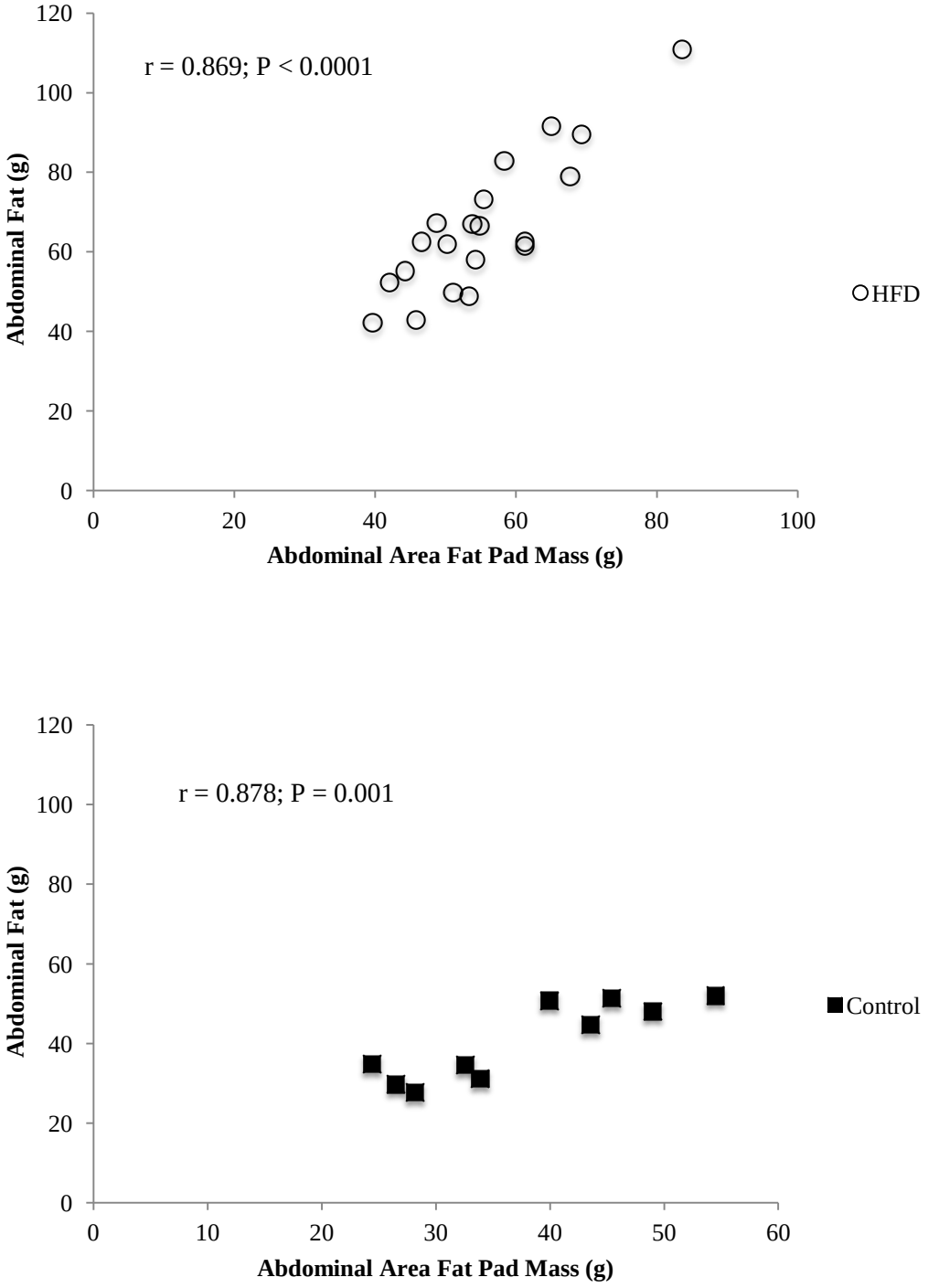
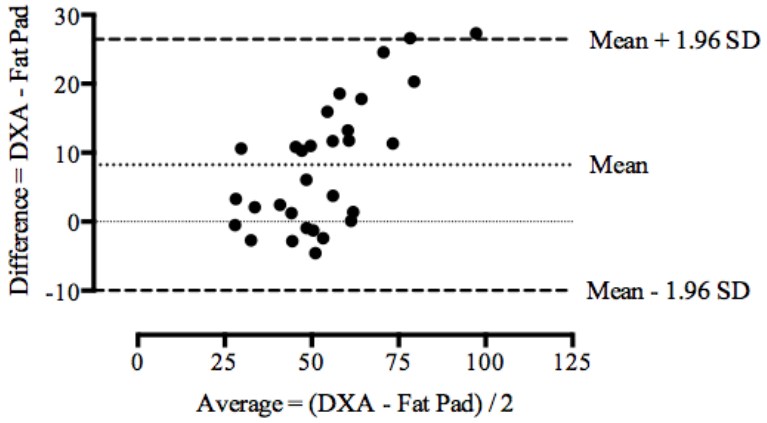
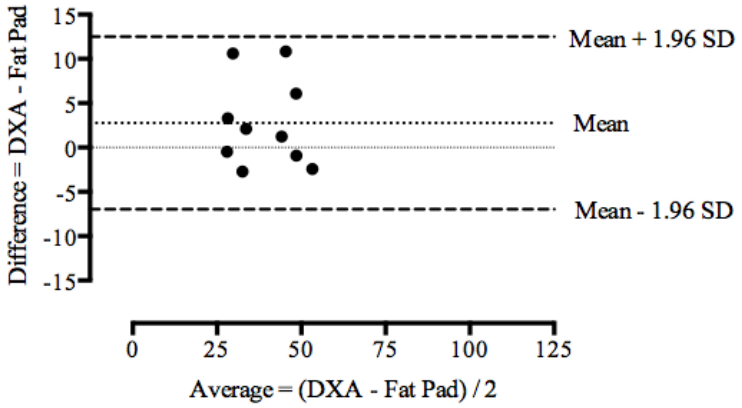


Figure 16. Correlation between abdominal fat (g) measured post-training by DXA and abdominal area fat pad mass (g) measured at sacrifice in HFD fed rats (n = 20; Panel A) and rats fed the control diet (n=10; Panel B) (modified from Mavreta Vagenas' MScA project, 2013)

A



B



C

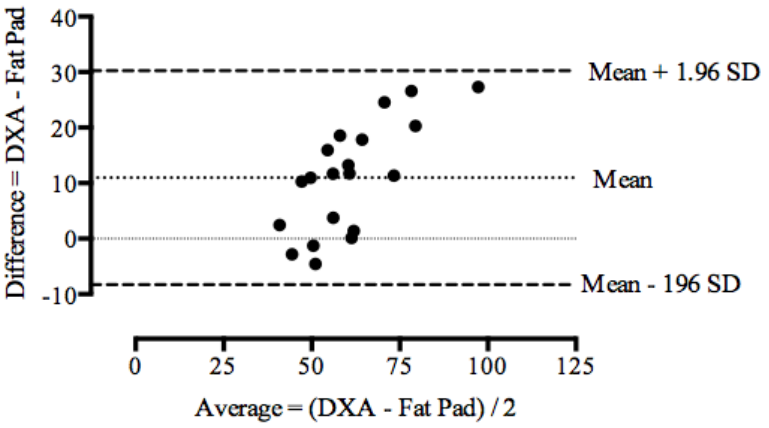
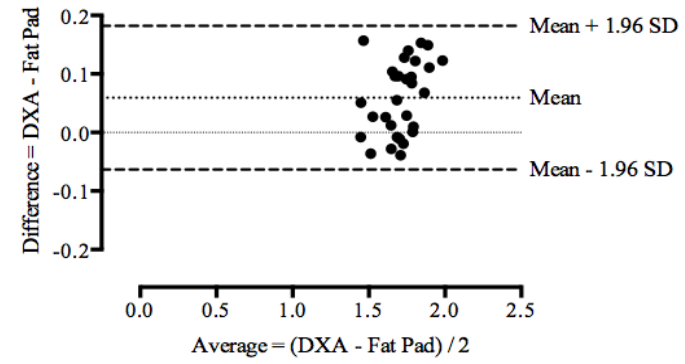
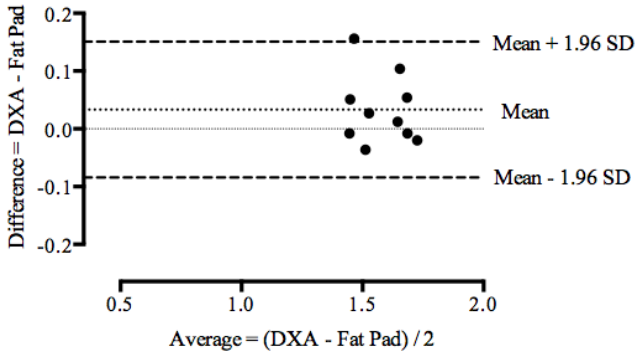


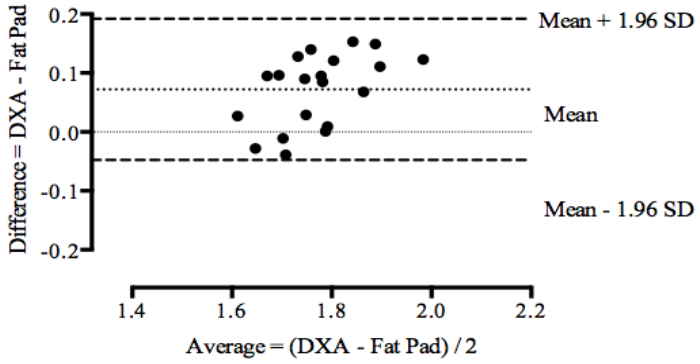
Figure 17. Bland-Altman plots comparing two methods of assessing abdominal fat in rats; in all Sprague-Dawley rats (n=30; Panel A), in control rats (n=10; Panel B), and in HFD fed rats (n=20; Panel C) (modified from Mavreta Vagenas' MScA project, 2013)



A



B



C

Figure 18. Bland-Altman plots comparing two methods of assessing abdominal fat in rats following log transformation of data; in all Sprague-Dawley rat (n=30; Panel A), in control rats (n=10; Panel B), in HFD fed rats (n=20; Panel C) (modified from Mavreta Vagenas' MScA project, 2013)

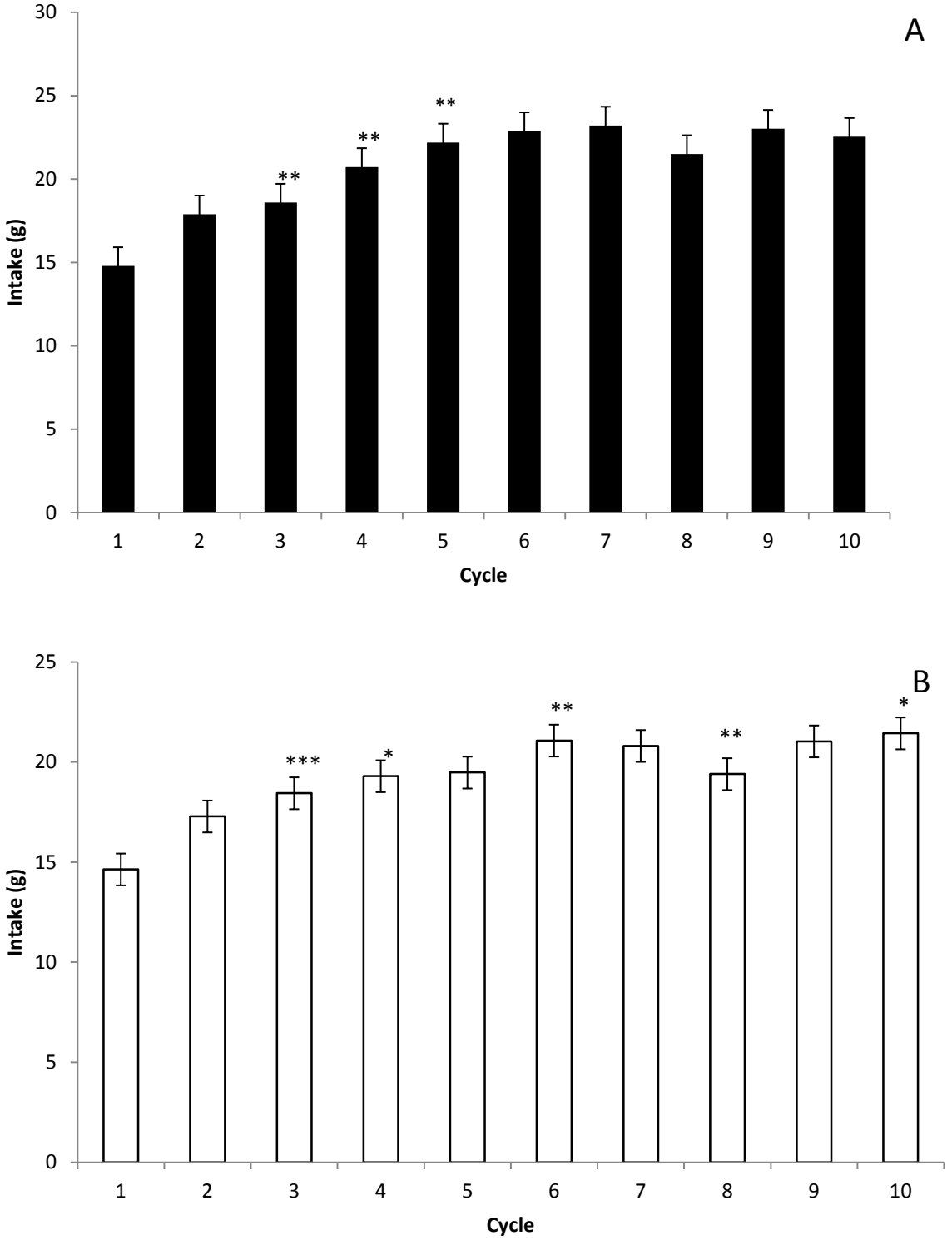


Figure 19. Intake of test food (g, LSmean ± SEM) over ten successive cycles for control group (Panel A) and HFD group (Panel B) before 8 h fast.
Asterisks indicate a significant peak of intake, where the intake at that trial is significantly greater (or less) than the average of the 2 previous days' intakes. *P<0.05, **P<0.01, ***P<0.001

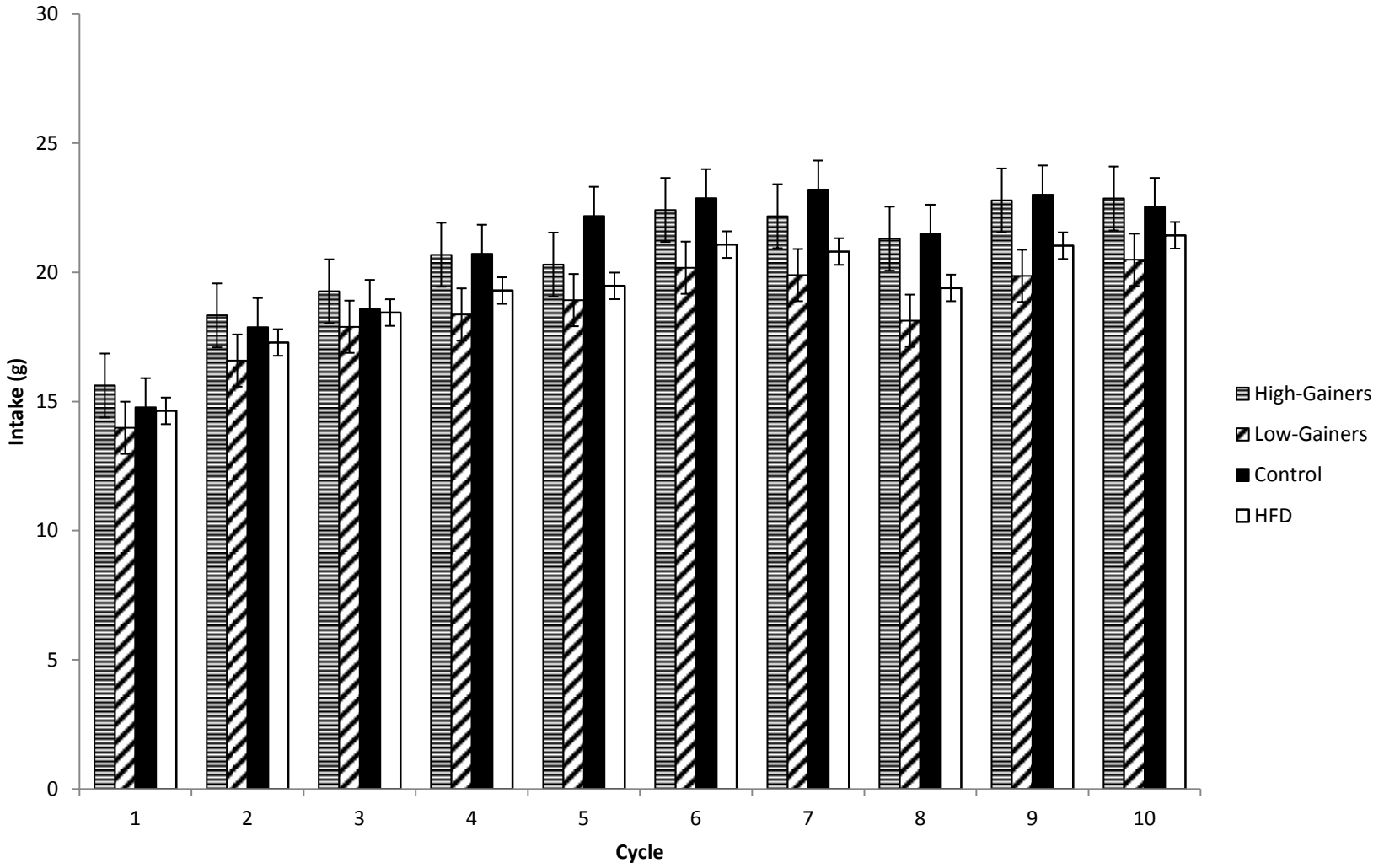


Figure 20. Intake of test food (g, LSmean ± SEM) over ten successive cycles for control group, HFD group, high gainers and low gainers (within HFD group) before 8 h fast.

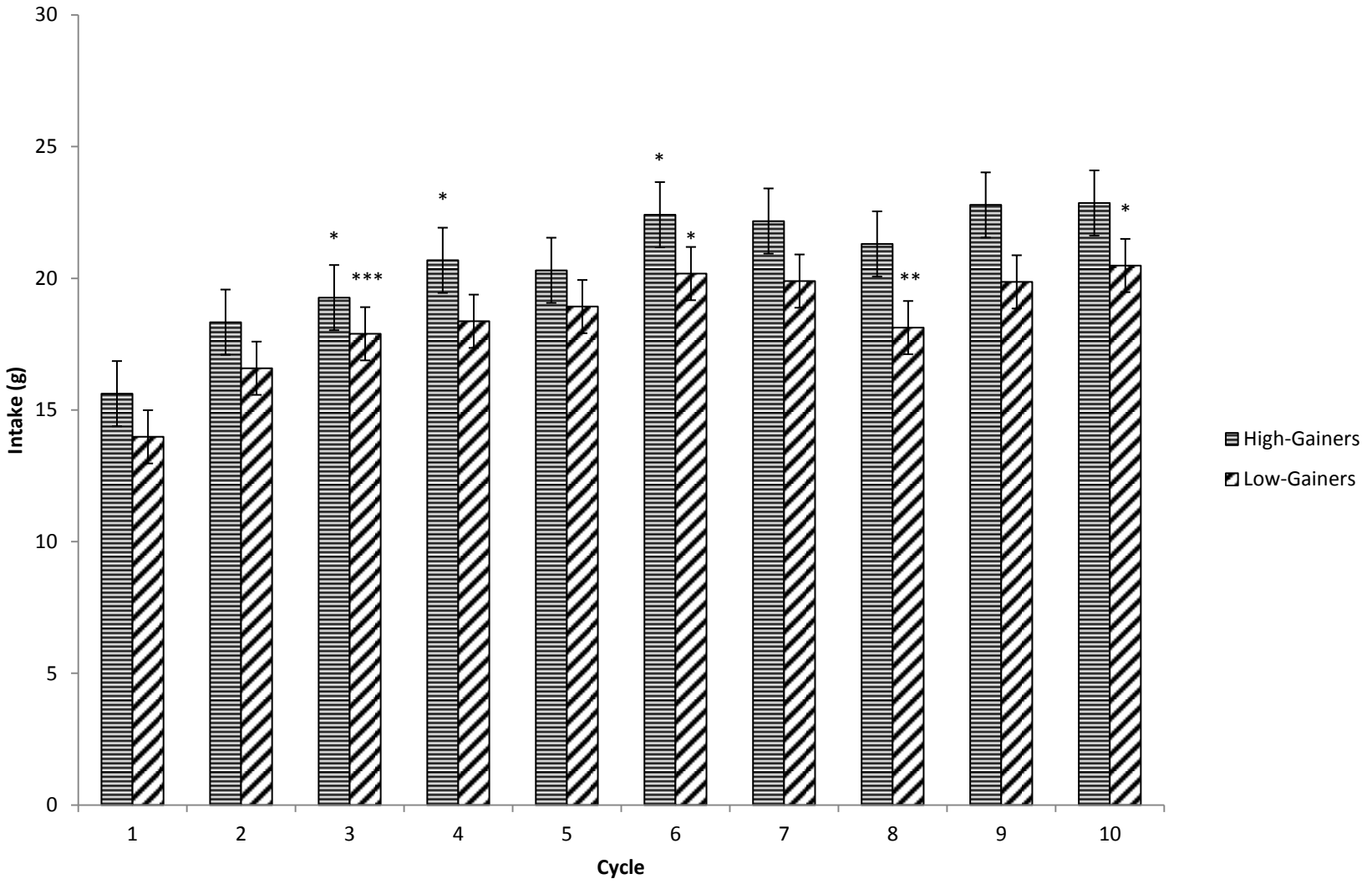


Figure 21. Intake of test food (g, LSmean ± SEM) over ten successive cycles for high-gainers and low-gainers (within HFD group) before 8 h fast. Asterisks indicate a within-group significant peak of intake, where the intake at that trial is significantly greater (or less) than the average of the 2 previous days' intakes. *P<0.05, **P<0.01, ***P<0.001

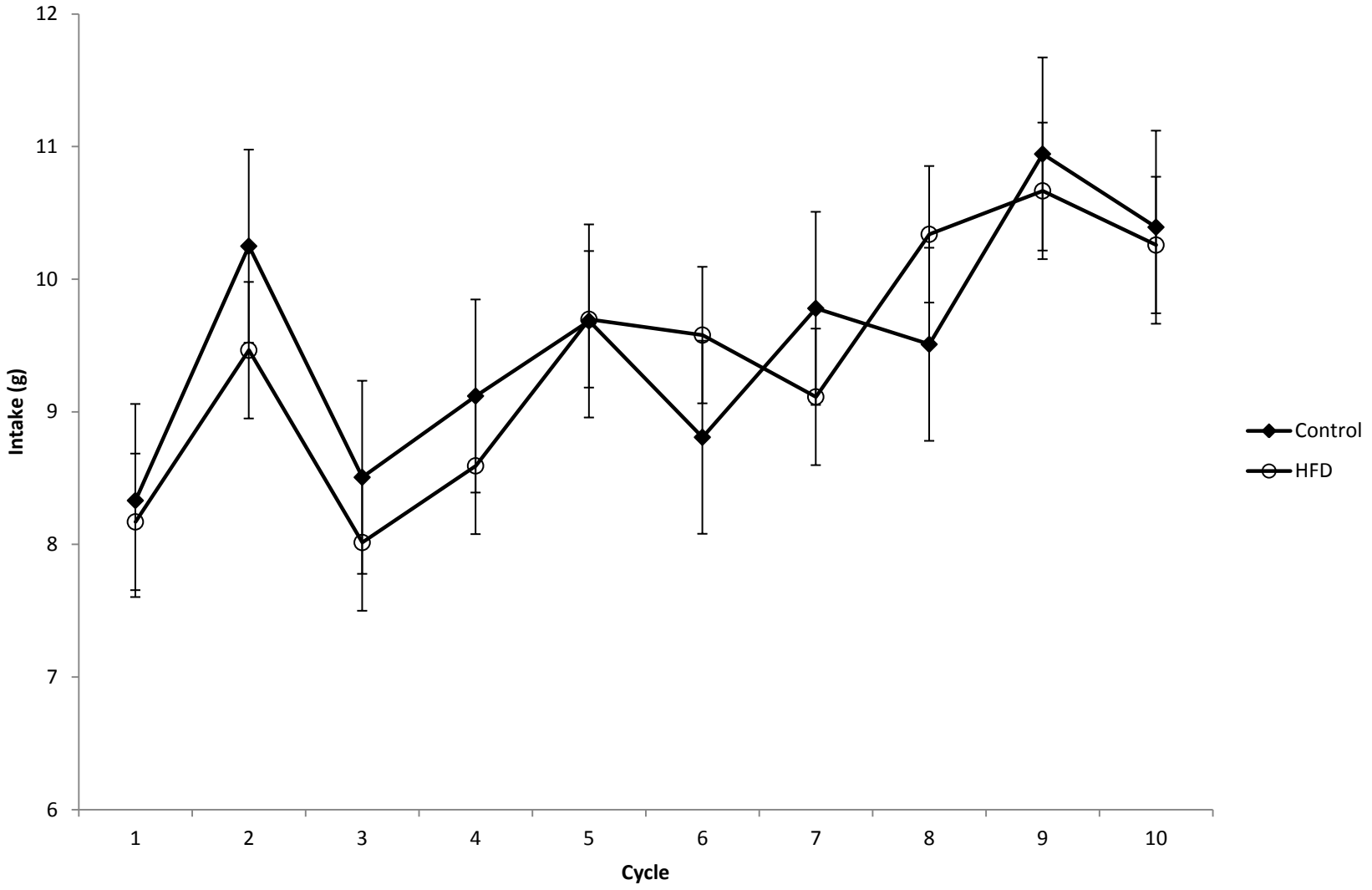


Figure 22. Intake of respective maintenance food (g, LSmean ± SEM) over ten successive cycles for control group and HFD group 1 h after fast.

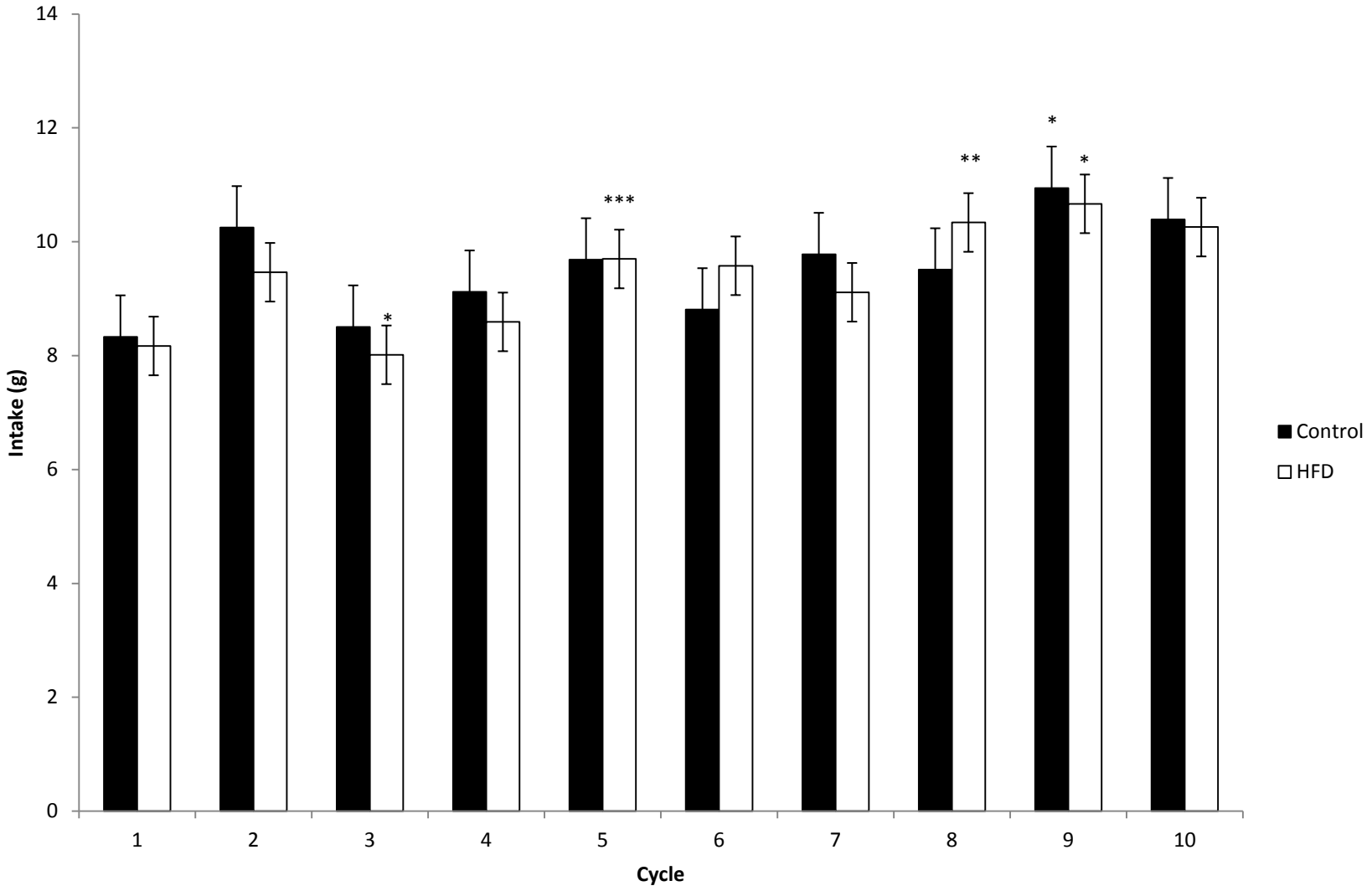


Figure 23. Intake of maintenance food (g, LSmean ± SEM) for over ten successive cycles for control group and HFD group 1 h after fast. Asterisks indicate a significant peak of intake, where the intake at that trial is significantly greater (or less) than the average of the 2 previous days' intakes. *P<0.05, **P<0.01, ***P<0.001

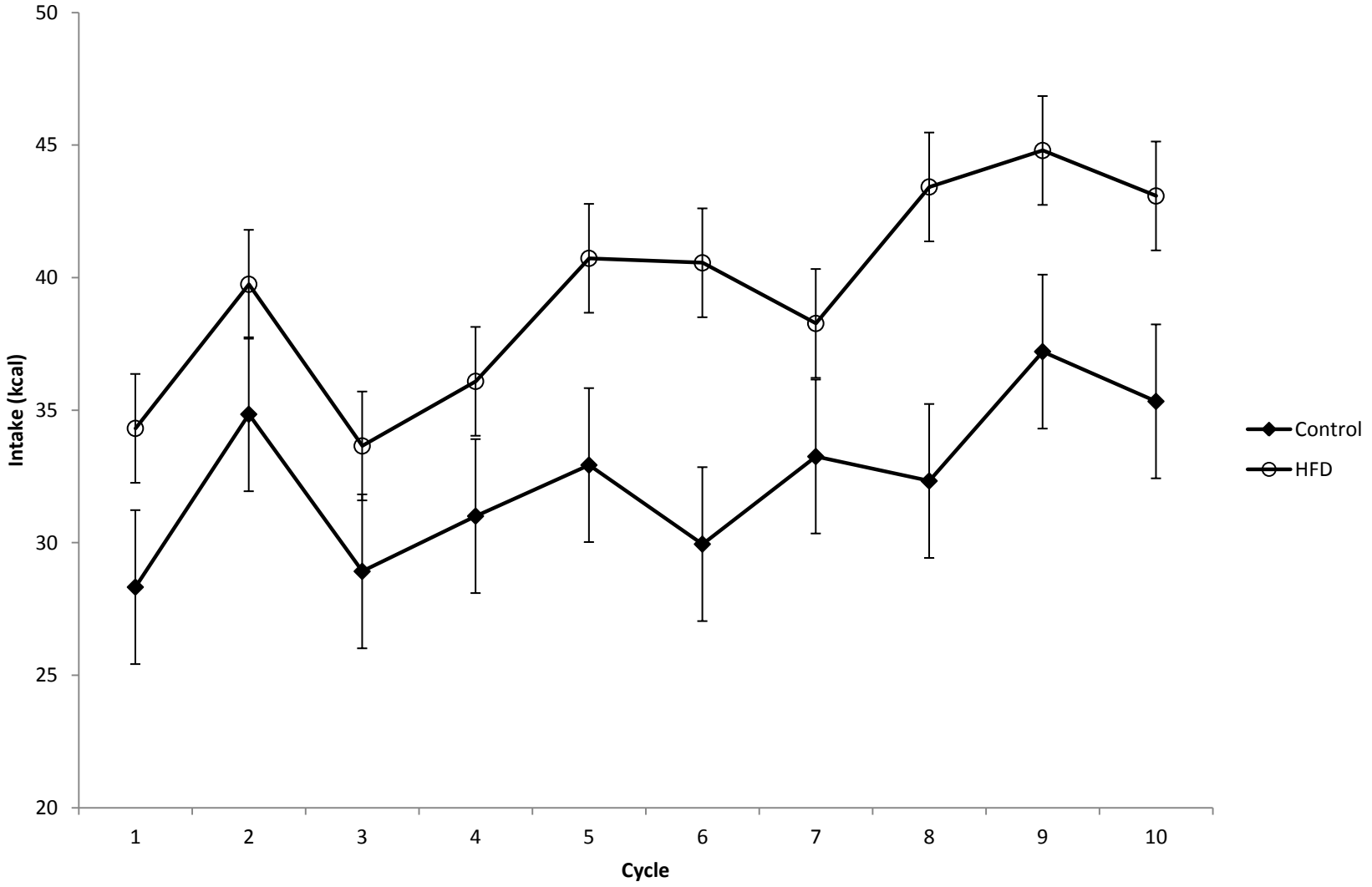


Figure 24a. Intake of respective maintenance food (kcal, LSmean ± SEM) over ten successive cycles for control group and HFD group 1 h after fast.

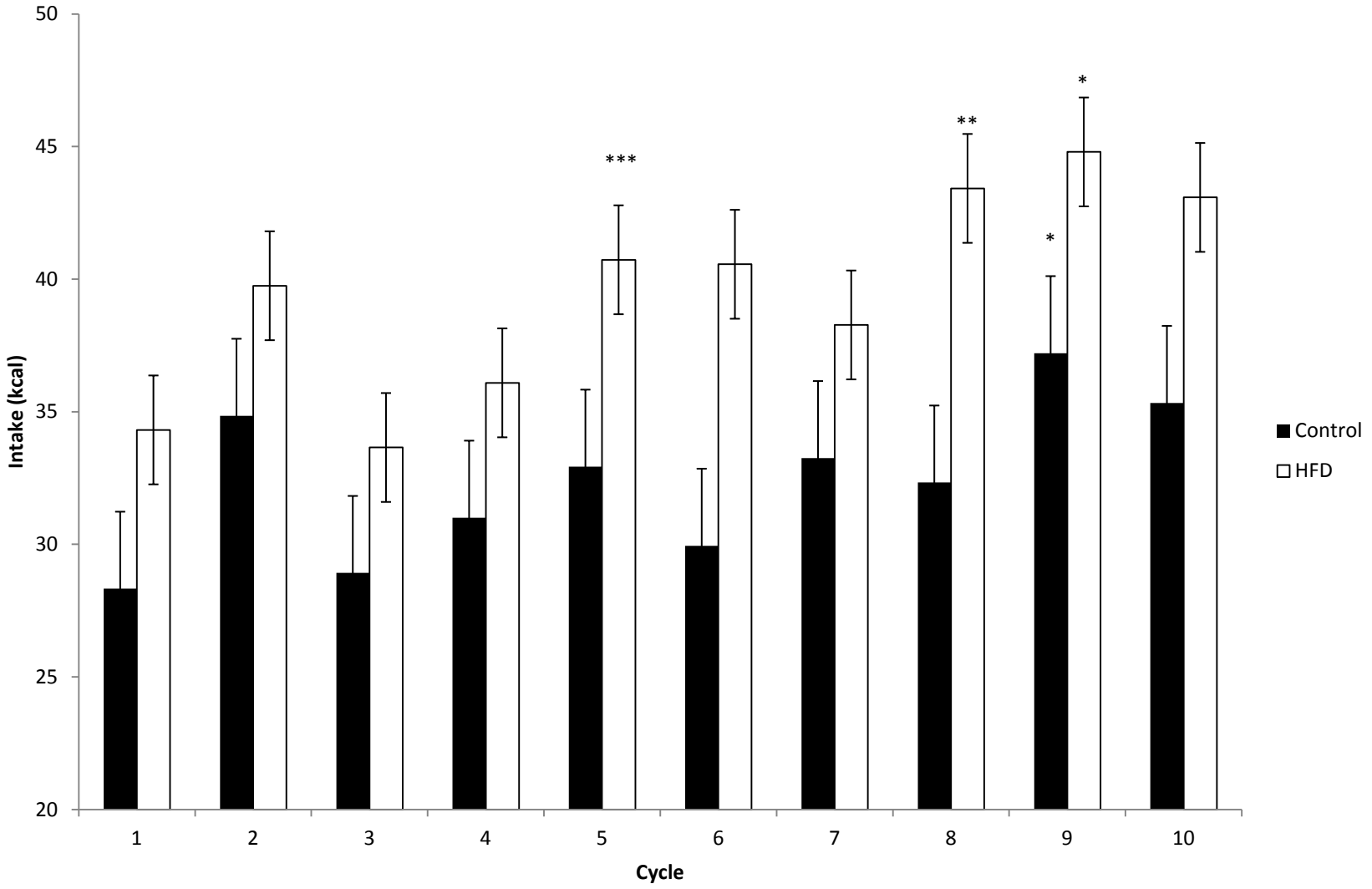


Figure 24b. Intake of maintenance food (kcal, LSmean ± SEM) over ten successive cycles for control and HFD group 1 h after fast.
Asterisks indicate a significant peak of intake, where the intake at that trial is significantly greater (or less) than the average of the 2 previous days' intakes. *P<0.05, **P<0.01, ***P<0.001

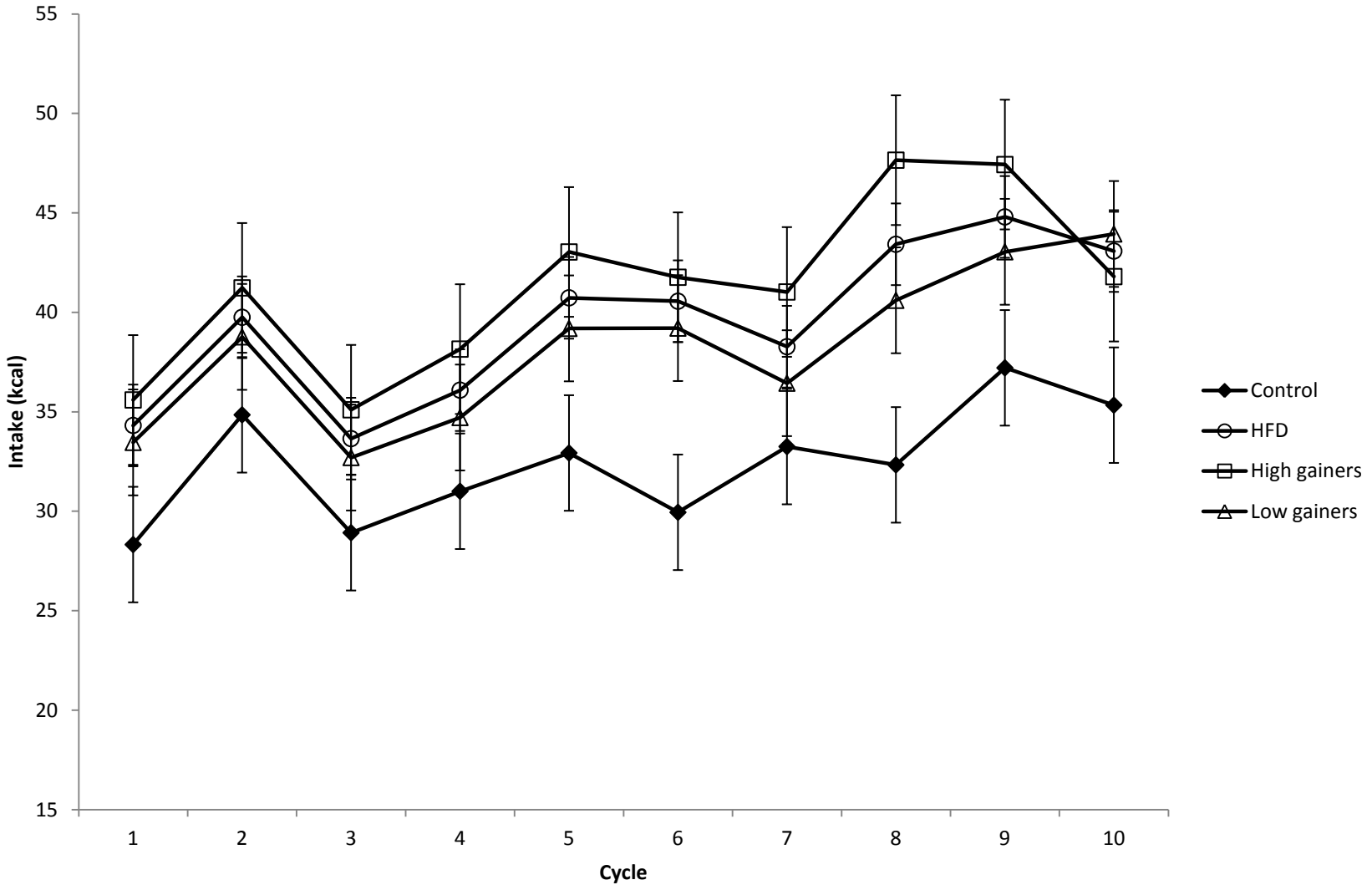


Figure 25a. Intake of respective maintenance food (kcal, LSmean ± SEM) over ten successive cycles for control group and HFD group, and high gainers and low gainers (within HFD group) 1 h after fast.

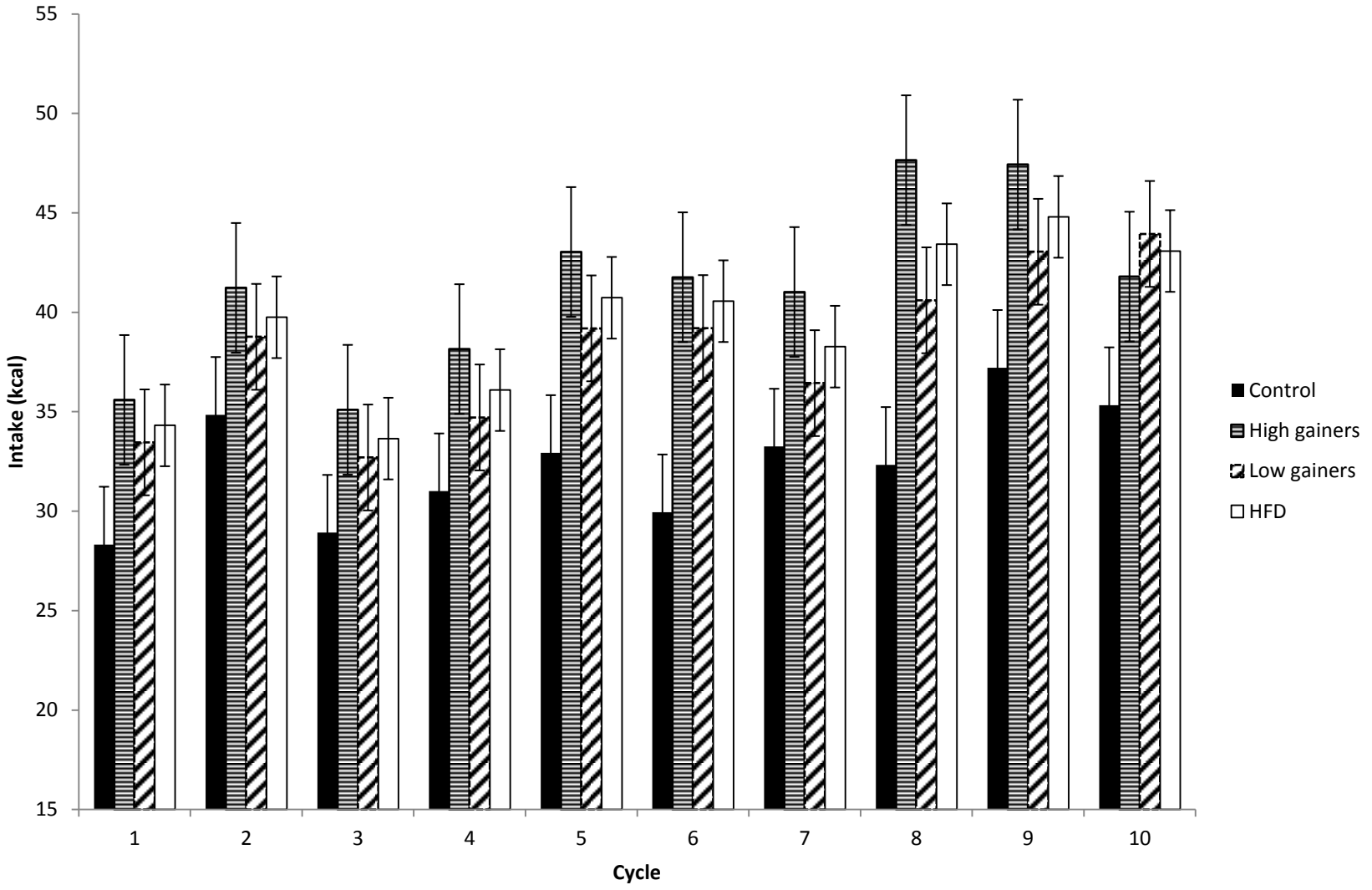


Figure 25b. Intake of respective maintenance food (kcal, LSmean ± SEM) over ten successive cycles for control group and HFD group, and high gainers and low gainers (within HFD group) 1 h after fast.

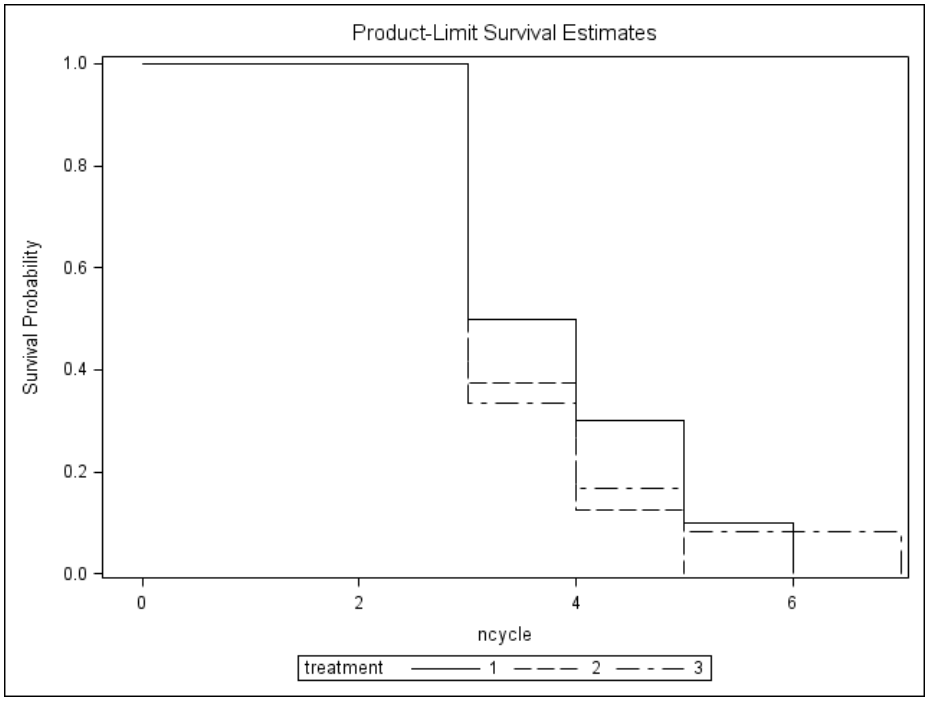


Figure 26. PROC LIFETEST Survival analysis testing time to first peaks for control group (treatment 1) vs. high weight gainers (treatment 2) vs. low weight gainers (treatment 3) using criteria of greater than one gram

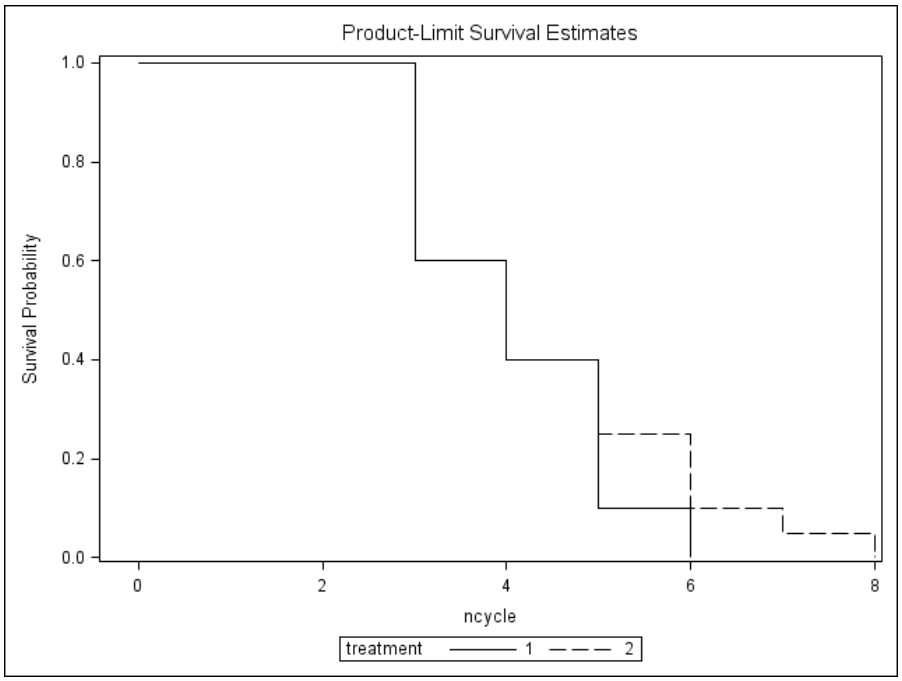


Figure 27. PROC LIFETEST Survival analysis testing time to first peaks for control group (treatment 1) vs. HFD group (treatment 2) using criteria of greater than two grams

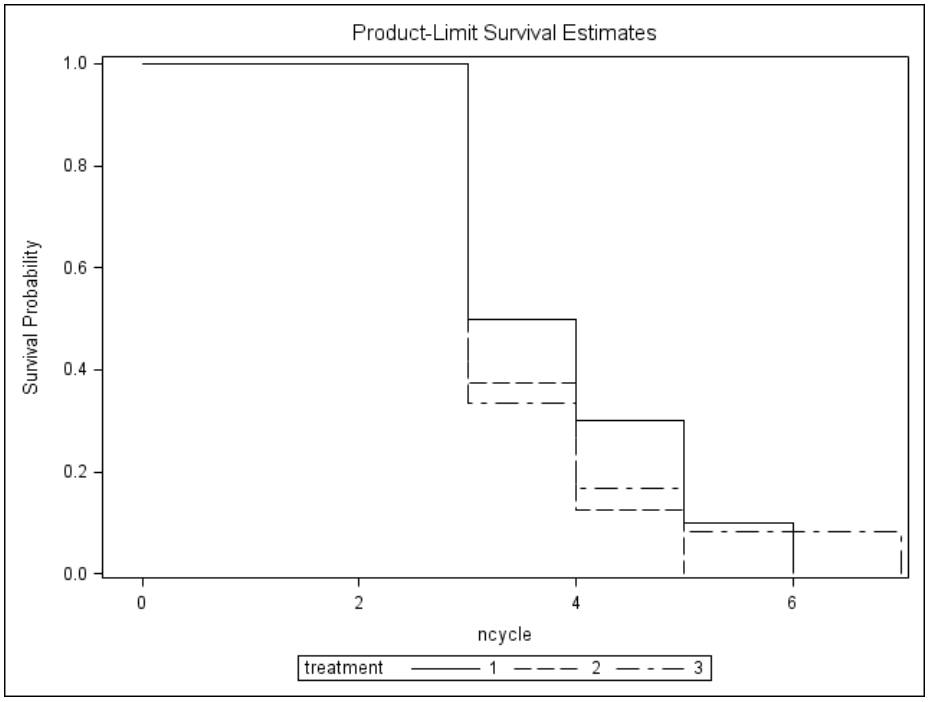


Figure 28. PROC LIFETEST Survival analysis testing time to first peaks for control group (treatment 1) vs. high weight gainers (treatment 2) vs. low weight gainers (treatment 3) using criteria of greater than two grams

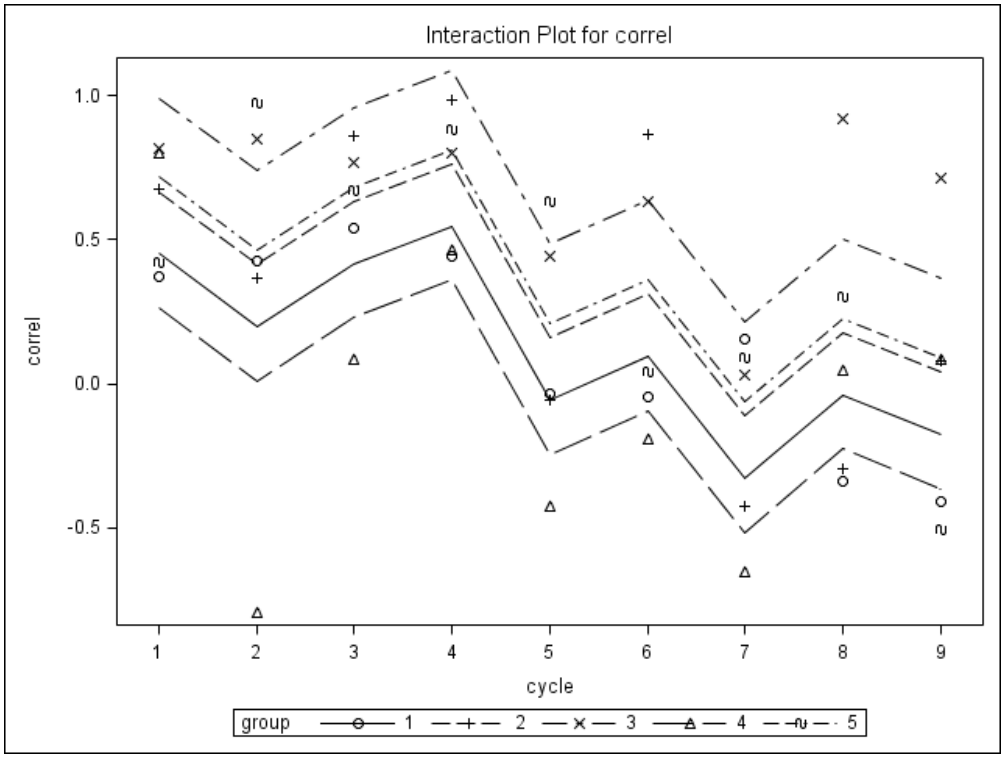


Figure 29. PROC GLM ANOVA for 'reward' hypothesis. 1 is control, 2 is high weight gainers/low fat gainers, 3 is low weight gainers/low fat gainers, 4 is low weight gainers/high fat gainers, and 5 is high weight gainers/high fat gainers

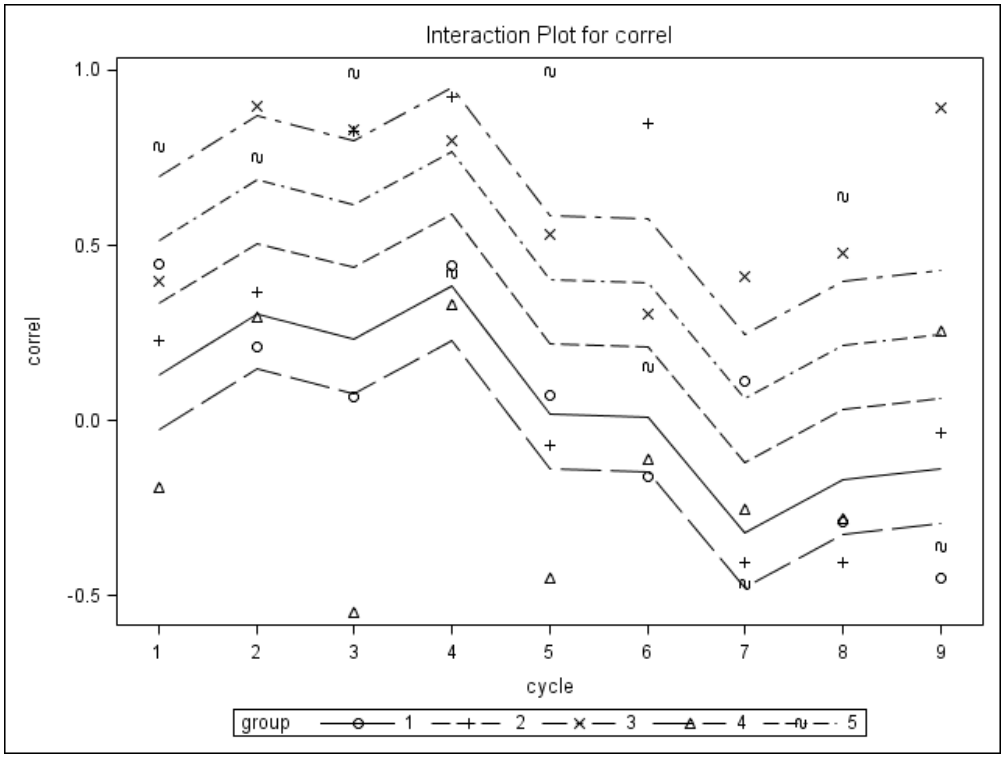


Figure 30. PROC GLM ANOVA for 'satiety' hypothesis. 1 is control, 2 is high weight gainers/low fat gainers, 3 is low weight gainers/low fat gainers, 4 is low weight gainers/high fat gainers, and 5 is high weight gainers/high fat gainers

Appendix 3. Statistical models and descriptions

(Some excerpts taken and modified from Mavereta Vagenas' MScA Project, 2013)

Food Intake During the Maintenance Period

Daily intake (g) of food during the maintenance period was calculated by subtracting the final amount of food left over from the initial amount provided. These values were then used to compute mean intakes (LS means) and standard errors for each cycle for each group (control and HFD).

The statistical model that was adopted for the analysis of the intake over the 37 day maintenance period was:

$$Y_{ijk} = \mu + diet_i + rat_{ij} + day_k + diet_i*day_k + e_{ijk}$$

Where Y_{ijk} was the intake (g) on the *i*th diet for the *j*th rat (where rat is nested within diet) on the *k*th day, μ was the overall intake (g) score of food, $diet_i$ was the fixed effect of the *i*th diet on the intake score of food (*i*= 1, 2; 1= control diet, 2= HFD), rat_{ij} was the random effect of the *j*th rat on the *i*th diet [*j*=1,…10 or 1,…20; $rat_{ij} \sim N(0, \sigma^2 rat_{ij})$], day_k was the fixed effect of the *k*th day on the intake score of food (*k*= 1,…37), $diet_i*day_k$ was the fixed effect of the combination of the *i*th diet and the *k*th day, over and above the main effect of the *i*th diet and the main effect of the *k*th day, and e_{ijk} was the random residual error associated with the *i*th diet for the *j*th rat (where rat is nested within diet) on the *k*th day [$e_{ijk} \sim N(0, R)$, where R is the variance-covariance matrix of the error terms]. The latter error statement assumes the rats were not independent of each other, i.e. there may have been correlations between them (Littell, Milliken & Stroup, 2006).

The amounts of maintenance food consumed (g) during the 37 days maintenance period prior to training were compared between the control group and HFD group across days by repeated measures analysis of variance (ANOVA) using the PROC MIXED procedure of SAS, seeking main effects of diet (chow vs. HFD) and day (1 to 37), and their interaction. Multiple pairwise comparisons were evaluated for statistical reliability using Scheffé's test.

To compare the energy consumption of both groups, the amounts of maintenance food consumed in grams were converted to kilocalories (kcal) by multiplying intake (g) by 3.4 calories per gram for the control diet and 4.2 calories per gram for the HFD. The amounts of maintenance food consumed (kcal) during the 37 days maintenance period prior to training were compared between the control group and HFD group across days as described above for amounts of maintenance food consumed (g).

The relationship between food intake (g and kcal) and body weight gain (g) was evaluated using a model, which began with a completely randomized design. The model progressed to undergo an analysis of covariance, fitting two regression coefficients and a classification variable, and their interaction:

$$Y_{ij} = \mu + diet_i + b_1*Intake_{ij} + b_2*diet_i*Intake_{ij} + e_{ij}$$

Where Y_{ij} was the total weight gain (g) after the 37 day maintenance period of the j^{th} rat (j= 1,…10 or 1,…20), with intake (g or kcal) on the *i*th diet (i= 1, 2; 1= control diet, 2= HFD), μ was the overall weight gain, $diet_i$ was the fixed effect of the *i*th diet on weight gain , b_1 was the regression coefficient of weight on intake of the *j*th rat on the *i*th diet, $intake_{ij}$ was the intake (g or kcal) of the *j*th rat on the *i*th diet (where rat is nested

within diet), b_2 was the regression coefficient for the interaction between diet and intake, $diet_i$*$Intake_{ij}$ was the fixed effect of the combination of the *i*th diet and the intake of the *j*th rat, over and above the main effect of the *i*th diet. We fitted such an interaction because we presumed there were different slopes for the two diets. Finally, e_{ij} was the random residual error associated with the *i*th diet for the *j*th rat (where rat is nested within diet [$e_{ij} \sim N(0, R)$, where R is the variance-covariance matrix of the error terms](Littell, Milliken & Stroup, 2006).

Body Weight and Composition

Throughout the 37 days (i.e. maintenance diet period) body weight (g) was recorded daily for all the rats and this data was used to create 3 new variables. The first variable, total weight gain was determined by subtracting initial weight (day 17) from final weight (day 53). The second variable, weight gain in grams per day was determined by dividing total weight gain by 37 days. The third variable, percent weight gain was determined by dividing total weight gain by initial weight (day 17) and then multiplying by 100. The fourth variable was final weight, measured on day 53 or the last day of the maintenance diet, preceding conditioning.

The other variables used for analysis were total body fat (g and % of total mass), total lean mass + BMC (g), total BMC (g), total mass (g), abdominal fat (g and % of total abdominal mass), abdominal lean mass + BMC (g), abdominal BMC (g), and total abdominal mass (g) measured at 3 time points throughout the experiment, baseline, pre-training, and post-training. At each time point, every rat had the average of duplicate or triplicate measures for each variable used (i.e. at baseline, rat 1 had body fat measured

twice in succession and the average of those two measurements was used as their baseline body fat). Additionally, these variables were used to create 3 new variables for each rat at each time point. Total BMC (g) was subtracted from total lean mass + BMC (g) to determine total lean mass (g). Abdominal BMC (g) was subtracted from abdominal lean mass + BMC (g) to determine abdominal lean mass (g). Abdominal fat (% of total body fat) was determined by dividing abdominal fat (g) by total body fat (g) and multiplying by 100. The next variable was fat pad mass (i.e. abdominal, retroperitoneal, and epididymal) measured in grams when rats were terminated (day 61 and 62).

Rats in the HFD group were then categorized as high weight gainers and low weight gainers based on weight gain in grams per day over the 37 day maintenance period. Rats that gained between 4.1 to 5.1 g/day were categorized as low weight gainers (n=8) and rats that gained between 5.4 to 6.4 g/day were categorized as high weight gainers (n=12). Based on this categorization, low weight gainers also gained less total weight (i.e 152.4 g to 190.5 g) compared high weight gainers (i.e. 201.5g to 236.6g) over the 37 day maintenance diet period. As well, percent weight gain in the low weight gainers was also lower (i.e. 42.0% to 54.6%) than in the high weight gainers (i.e. 52.4% to 62.7%).

Moreover, the Lee obesity index of each rat was calculated, using the following formula: $3\sqrt{[wt(g)]}/[naso\text{-}anal\ length\ (mm)] \times 1000$ (Lee, 1929). Weight and length (i.e. nose to anus) were measured on day 33 or 34 of the maintenance diet period for each rat and used in the previously mentioned formula. A Lee obesity index of greater than 310 is indicative of obesity (Hariri & Thibault, 2010). Previous research has found a high

correlation between Lee obesity index and adiposity (Bernardis & Skelton, 1965; Gold, Sawchenko & Kapatos, 1977)

The appropriate statistical tests were conducted to assess normality. Due to the fact that there is a fixed effect (i.e. diet) the observations or "raw data" cannot be assessed for normality. In this case, the residuals must be used for testing normality. A model was created for each of the following variables with the fixed effect of diet: total weight gain, percent weight gain, weight gain in grams per day, fat pad mass (g). The same model, with the fixed effect of diet was also used to assess normality in the body composition variables estimated by DXA at baseline, pre-training and post-training: total body fat (g and %), total lean mass + BMC (g), total lean mass (g), total BMC (g), total mass (g), abdominal fat (g, % of total body fat, and % of total abdominal mass), abdominal lean mass + BMC (g), abdominal lean mass (g), abdominal BMC (g), and total abdominal mass (g). The Shapiro-Wilk (S-W) test was conducted to assess normality. A P value above 0.01 was taken to mean data was normal. All variables were found to be normal, therefore no data transformation were required.

The statistical model that was adopted for the analysis of weight over the 37 day maintenance period began as a completely randomized design with the fixed effect of diet and random effect of rat. The model progressed to include a repeated measures design because rats were allocated to one of two diets while their weights were measured on repeated days. The final statistical model was:

$$Y_{ijk} = \mu + diet_i + rat_{ij} + day_k + diet*day_{ik} + e_{ijk}$$

Where Y_{ijk} was the weight (g) on the *i*th diet for the *j*th rat (where rat is nested within diet) on the *k*th day, μ was the overall weight (g), $diet_i$ was the fixed effect of the *i*th diet on rat weight (*i*= 1, 2; 1= control diet, 2= HFD), rat_{ij} was the random effect of the *j*th rat on the *i*th diet [*j*=1,...30; $rat_{ij} \sim N (0, \sigma^2 rat_{ij})$], day_k was the fixed effect of the *k*th day on weight (*k*= 1,…37), $diet_i * day_k$ was the fixed effect of the combination of the *i*th diet and the *k*th day, over and above the main effect of the *i*th diet and the main effect of the *k*th day, and e_{ijk} was the random residual error associated with the *i*th diet for the *j*th rat (where rat is nested within diet) on the *k*th day [$e_{ijk} \sim N (0, R)$, where R is the variance-covariance matrix of the error terms](Littell, Milliken & Stroup, 2006). The same PROC MIXED program was run twice with different covariance structures, once with Compound Symmetry (CS) and once with Auto-Regressive [AR(1)]. The CS structure assumes equal variance at all times and equal covariance between observations on the same subject at all pairs of times (Littell, Milliken & Stroup, 2006). The AR(1) structure assumes adjacent observations tend to be more highly correlated than observations further apart in time (Littell, Milliken & Stroup, 2006). The model with the lower Bayesian Information Criterion (BIC) was considered better fitting.

The weight recorded during the 37 days maintenance period prior to training were compared between the control group and HFD group across days by repeated measures analysis of variance (ANOVA) using PROC MIXED procedure of SAS, with the main effects of diet (chow vs. HFD) and day (1 to 37), and their interaction. Multiple pairwise comparisons were evaluated for statistical reliability using Scheffé's test.

The statistical model that was adopted for the analysis of each body composition variable began as a completely randomized design with the fixed effect of group and

random effect of rat. The model progressed to include a repeated measures design because rats were allocated to one of three groups while body composition was measured at three time points. The final statistical model was:

$$Y_{ijk} = \mu + group_i + rat_{ij} + time_k + group_i*time_k + e_{ijk}$$

Where Y_{ijk} was one of thirteen body composition variables [i.e. total body fat (g and %), total lean mass + BMC (g), total lean mass (g), total BMC (g), total mass (g), abdominal fat (g, % of total body fat, and % of total abdominal mass), abdominal lean mass + BMC (g), abdominal lean mass (g), abdominal BMC (g), and total abdominal mass (g)]. For the description of terms and parameters Y_{ijk} will be abdominal fat (g) for jth rat (where rat is nested within group), in the ith group at the kth time, μ was the overall abdominal fat, $group_i$ was the fixed effect of the ith group on rat weight (i= 1, 2, 3; 1= control diet, 2= high fat low gainers, 3 = high fat high gainers), rat_{ij} was the random effect of the jth rat on the ith diet [j=1,...30; $rat_{ij} \sim N (0, \sigma^2 rat_{ij})$], $time_k$ was the fixed effect of the kth time point (k= 1, 2, 3; 1 = baseline, 2 = pre-training, 3 = post-training), $group_i*time_k$ was the fixed effect of the combination of the ith group and the kth time, over and above the main effect of the ith group and the main effect of the kth time, and e_{ijk} was the random residual error associated with the ith group for the jth rat (where rat is nested within diet) on the kth time [$e_{ijk} \sim N (0, R)$, where R is the variance-covariance matrix of the error terms](Little, Milliken & Stroup, 2006). The same PROC MIXED program was run twice with different covariance structures, once with Compound Symmetry (CS) and once with Auto-Regressive [AR(1)]. The CS structure assumes equal variance at all times and equal covariance between observations on the same subject at all pairs of times (Littell, Milliken & Stroup, 2006). The AR(1) structure assumes adjacent

observations tend to be more highly correlated than observations further apart in time (Littell, Milliken & Stroup, 2006). The model with the lower Bayesian Information Criterion (BIC) was considered better fitting.

Each body composition variable recorded at baseline, pre-training and post-training were compared between the control group, HFD group, and low gainers and high gainers of the HFD by repeated measures analysis of variance (ANOVA) using PROC MIXED procedure of SAS, with the main effects of group (control vs. HFD low gainers vs. HFD high gainers) and time (baseline, pre-training, and post-training), and their interaction. Multiple pairwise comparisons were evaluated for statistical reliability using Scheffé's test.

A randomized complete block model, also called a two-way analysis of variance (ANOVA), mixed model was used to analyze all the body composition variables [i.e. total body fat (g and %), total lean mass + BMC (g), total lean mass (g), total BMC (g), total mass (g), abdominal fat (g, % of total body fat, and % of total abdominal mass), abdominal lean mass + BMC (g), abdominal lean mass (g), abdominal BMC (g), and total abdominal mass (g)] measured at baseline, pre-training, and post-training. Each variable was analyzed with the same statistical model:

$$Y_{ij} = \mu + rat_i + treatment_j + e_{ij}$$

This model was used to analyze each group of rats individually (i.e. control group, HFD rats, low gainers, and high gainers). To see whether the effect of rat is statistically significant the model was run twice, once with the random effect of rat and a second time dropping the random effect of rat. The model with the lower -2 Res Log Likelihood was

used. The following is an example of the terms in the model when analyzing the variable: body fat. The term Y_{ij} is the body fat of the *i*th rat on the *j*th treatment, μ is the overall mean body fat, rat_i is the random effect of the *i*th rat [*i* = 1,…10 or 1,…20 or 1,…8 or 1,...12; $rat_i \sim N(0, \sigma_{rat}^2)$], $treatment_j$ is the overall, fixed effect of the *j*th treatment (*j* = 1, 2, 3; 1 = DXA 1, 2 = DXA 2, 3 = DXA 3), and e_{ij} is the random residual error associated with the *i*th rat on the *j*th treatment [$e_{ij} \sim N(0, \sigma_e^2)$]. All values are reported as Least Square means ± Standard Error. Multiple comparisons were evaluated for statistical reliability using Bonferroni's test.

A one-way analysis of variance (ANOVA) was used to compare data for each body composition variable (i.e. body fat, abdominal fat, total and abdominal lean body mass + BMC, total and abdominal mass), fat pad mass, initial weight, final weight, percent weight gain, total weight gain, and weight gain in grams per day between all 30 rats, at different time points (i.e. baseline, pre-training, post-training). Each variable was analyzed with the same statistical model:

$$Y_{ij} = \mu + group_i + e_{ij}$$

Each body composition variable was examined twice with two different groupings; the first grouping was control diet and HFD, and the second grouping was control, low gainers and high gainers. The following is an example of the terms and parameters in the model when analyzing the variable body fat. The term Y_{ij} was the body fat of the *i*th rat (*i* = 1,…30) in the *j*th group, μ is the overall mean body fat, $group_i$ is the fixed effect of the *j*th group (*j* = control, HFD or control, low gainer, high gainer), and e_{ij} is the random residual error associated with the *i*th rat in the *j*th group [$e_{ij} \sim N(0, \sigma_e^2)$].

All values are reported as Least Square mean ± standard error. Multiple comparisons were evaluated for statistical reliability using Bonferroni's test. The previously mentioned statistical analyses were carried out using SAS version 9.2.

Pearson correlation coefficients were used to assess the bivariate association between fat pad mass measured in grams and abdominal fat measured in grams by post-training by DXA. This was done using IBM SPSS Statistics (version 19).

The mean coefficient of variation (%) was determined for each body composition variable (i.e. total fat (g and %), abdominal fat (g and %), total lean mass + BMC (g), abdominal lean mass + BMC (g)) at 3 time points (i.e. baseline, pre-training, and post-training). The coefficient of variation for each rat was calculated using triplicate DXA scan estimates for the body composition variables and the following equation: coefficient of variation = (standard deviation ÷ mean) x 100. These calculations were done on Microsoft Excel for Mac 2011 (Version 14.2.3).

Prism 5 for Mac OS X version 5.0d (GraphPad Software Inc.) was used to create a Bland-Altman Plot to compare two assay methods; abdominal area fat pad weighed at time of sacrifice (g) and abdominal fat as measured post-training by DXA. A plot was created with the Y-axis representing the difference between the two measurements and the X-axis representing the average of the two measurements (Bland & Altman, 1986).

Learning of Anticipatory Eating

Intakes (g) of test food during access for 1 h prior to the 8 h fast were calculated by subtracting the final amount of food left over from the initial amount provided. Also the

initial intake of control diet and HFD at the end of the 8 h fast following the training period was measured by re-weighing the food after access for 1 h. These values were then inserted into SAS as raw data to compute mean intakes (LS means) and standard errors for each cycle for each group (control and HFD).

The statistical model that was adopted for the analysis of the intake over the ten cycles during the training period (prior to the 8 h fast and during the 1 h access period after the fast) was the same as that for the 37-day maintenance period; however, the main effect of day was termed $cycle_k$ (k=1, …10). Furthermore, when the HFD group was divided into two groups (high-gainers and low-gainers), the main of effect of $diet_i$ included 3 variables such as i= 1, 2, or 3, where 1 represented the control group, 2 the high-gainer group and 3 the low-gainer group.

Learned intake (g) of the test food (i.e. the ability to increase intake in anticipation of a long fast) prior to the fast and initial intake (g) of maintenance diet during 1 h access after fasting were compared between the control group and HFD group across cycles by repeated measures ANOVA using the PROC MIXED procedure of SAS, seeking main effects of diet (control vs. HFD) and training cycle (cycles 1 to 10), and their interaction. Multiple pairwise comparisons were evaluated for statistical reliability using Scheffé's test. Orthogonal polynomial contrasts were used to analyze test food intake before the fast across cycles for linear, quadratic, cubic and quartic trends.

Using our classification for high weight-gainers (4.1 g/day to 5.1 g/day) and low weight-gainers (5.4 g/day to 6.4 g/day), the amounts of test food intake (g) prior to the fast and initial intake (g) of maintenance diet during 1 h access after fasting were

compared between the control group, high-gainers group (HFD), and low-gainers group (HFD), thus separating the diet effect into three groups rather than two. Analysis was computed as described above for intake, seeking main effects of diet (control vs. HFD-high gainers vs. HFD-low gainers) and training cycle (cycles 1 to 10), and their interaction.

Peak intakes during the training period were identified from cycle 3 to 10 using a threshold of 2.0 g, i.e. the logically minimum criterion for a peak was at least 2.0 g greater than two successive preceding days. Statistical significance of the peaks was analyzed using pairwise comparisons.

Identical analyses were then used to compare energy consumption of test food intake prior to the fast and initial intake of maintenance diet during 1 h access after fasting between the control and HFD group. Food consumed (g) was converted to kilocalories (kcal) by multiplying intake (g) by 3.4 calories per gram for the control diet and 4.2 calories per gram for the HFD. Using our classification for high-gainers and low-gainers, identical analysis was used to compare the three groups' intakes (g and kcal), as described above.

To test the difference between individuals' peaks and troughs between the control and HFD groups, peaks and troughs that occurred in each cycle were counted. Peaks and troughs were counted as significant if they fell under the criteria of being greater than two grams. Using PROC FREQ, a chi-squared test was executed across the timings of peaks/troughs, first across all the data (across both diets) then within each diet, looking for differences in the number of peaks for each cycle.

Using PROC GENMOD, a chi-squared test was executed between diets for each cycle to compare both groups testing the effects of diet and cycle, while specifying a binomial distribution. The statistical model that was adopted for this analysis was:

$$Y_{ij} = \mu + diet_i + cycle_j + e_{ij}$$

Where Y_{ij} was the number of peaks out of the number of rats on the *i*th diet for the *j*th cycle, μ was the overall number of peaks per number of rats, $diet_i$ was the fixed effect of the *i*th diet on the number of peaks per number of rats (*i*= 1, 2; 1= control diet, 2= HFD), $cycle_j$ was the fixed effect of the *j*th cycle on the number of peaks per number of rats (*j*=3,…10), and e_{ij} was the random residual error associated with the *i*th diet for the *j*th cycle [$e_{ijk} \sim N(0, R)$, where R is the variance-covariance matrix of the error terms]. The latter error statement assumes the rats were not independent of each other, i.e. there may have been correlations between them (Littell, Milliken & Stroup, 2006).

The same PROC FREQ and PROC GENMOD tests were executed again between diets, but reducing the ten cycles to five, i.e. combining peaks at cycle 3 with peaks at cycle 4, 5 with 6, 7 with 8, and 9 with 10 to see if greater numbers of peaks per unit of time would enhance the sensitivity of the analysis.

The PROC FREQ and PROC GENMOD procedures do not account for each rat being an individual entity. To account for such an issue, PROC GLIMMIX was used to further examine the data by analyzing each rat as a separate entity, i.e. looking at each cycle and whether a rat peaked or not, while testing the main effects of rat, diet, and cycle for a binomial distribution. For the former analyses, eight cycles were analyzed (3-10) because

the third cycle is the minimum analyzable based on the criterion for a peak/trough. The statistical model that was adopted for this analysis was:

$$Y_{ijk} = \mu + diet_i + rat_{ij} + cycle_k + e_{ijk}$$

Where Y_{ijk} was the dependent binary variable, i.e. whether the *j*th rat on the *i*th diet peaked or not (where rat is nested within diet) on the *k*th cycle, μ was the overall score of peaking, $diet_i$ was the fixed effect of the *i*th diet on the score of peaking (*i*= 1, 2; 1= control diet, 2= HFD), rat_{ij} was the random effect of the *j*th rat on the *i*th diet [*j*=1,…10 or 1,…20; $rat_{ij} \sim N (0, \sigma^2 rat_{ij})$], $cycle_k$ was the fixed effect of the *k*th cycle on the peaking score (*k*= 3,…10), and e_{ijk} was the random residual error associated with the *i*th diet for the *j*th rat (where rat is nested within diet) on the *k*th cycle [$e_{ijk} \sim N (0, R)$, where R is the variance-covariance matrix of the error terms]. The latter error statement assumes the rats were not independent of each other, i.e. there may have been correlations between them (Littell, Milliken & Stroup, 2006).

To test for the differences between the control and HFD groups' speed of learning, first peaks and troughs that occurred over the eight cycles were counted. This analysis was computed twice, once using the criterion of being greater than 2 grams and again using a criterion of being greater than 1 gram for an analysis of sensitivity. Peaks and troughs were counted as significant if they fell under the criteria and if they were followed by an inflection in intake (i.e. a fall after a peak or a rise after a trough). Data was analyzed using survival analysis, i.e. PROC LIFETEST to analyze the timing of peaks for each rat (control of HFD) over eight cycles or the linear rank statistics to test the effect of diet on timing of peaks. The data set contained 4 variables: ncycle (the

number of cycle at which a rat showed their first peak), status (censoring indicator variable: a rat was considered censored if it did not reach its first peak by cycle 10), treatment (control or HFD), and rat (individual rat number). Identical analysis was computed using the three groups including the high-gainers and low-gainers. The statistical model that was adopted for this analysis was:

$$Y_{ij} = \mu + \text{treatment}_i + e_{ij}$$

Where Y_{ij} was the time (cycle) to peak of the j^{th} rat with the i^{th} treatment, μ was the overall time to peak, treatment_i was the fixed effect of the i^{th} treatment (i= 1, 2; 1= control diet, 2= HFD), and e_{ij} was the random residual error associated with the jth rat in the ith treatment group [$e_{ij} \sim N(0, \sigma_e^2)$]. The same analysis was done taking into account our classification of high weight gainers and low weight gainers, i.e. 3 treatment groups.

To further analyse our classification of the HFD, we separated the group again based on global body fat gain to yield five groups: control, high weight gainers/low fat gainers, low weight gainers/low fat gainers, low weight gainers/high fat gainers, and high weight gainers/high fat gainers. All of the latter analyses were computed again taking into account the new classification.

Before testing hypotheses about how the learning occurs, a simulation of data was conducted, where the correlations were already known. Data was simulated with 20 rats and 9 periods, both as random effects. First there was made to be no correlation between the intake measurements before the fast and after the fast. The analyses was run to compute the correlation within each rat, i.e. amongst the 9 measurements for rat 1, then for rat 2, etc. then a correlation was computed within cycle, i.e. the correlation between

the before and after measurements. PROC GLM and MANOVA were used to compute the correlation adjusting for rat and cycle. Finally, using the same code the correlation was changed from zero to 0.5.

To test for how the learning occurs, we looked at the intakes and correlations at every cycle, not just at peaks. For our 'reward' hypothesis, the variables entering the analysis were rats' intakes of maintenance food at the 1st hour of refeeding on one trial and intake of test food on the next trial, while adjusting for cycle. For our 'satiety' hypothesis, the variables entering the analysis were rats' intakes of maintenance food at the 1st hour of refeeding and the intake of test food earlier that same day/trial, while adjusting for cycle. Reward minus satiety values were computed by taking the difference between the two r values. All the latter correlations were computed across cycles using PROC CORR. Means, medians and numbers of counts (k) were computed for each group and for the total. Identical analysis was computed to obtain a correlation value for each cycle (3-10) while adjusting for rat. Finally, identical analysis was computed in each cycle across each subset of rats, i.e. obtaining a correlation value for each rat in each cycle.

To test for differences within the 'reward' correlation coefficients, 'satiety' correlation coefficients, and between the two (reward minus satiety), ANOVAs were computed using the PROC GLM procedure. The statistical model that was adopted for this analysis was:

$$Y_{ijk} = \mu + group_i + cycle_j + e_{ijk}$$

Where Y_{ijk} was the correlation for a rat in the *i*th group for the *j*th cycle, μ was the overall correlation for rats, $group_i$ was the fixed effect of the *i*th group on the

correlation of a k^{th} rat (*i*= 1,...5; 1= control, 2= high weight gainers/low fat gainers, 3= low weight gainers/low fat gainers, 4= low weight gainers/high fat gainers, and 5= high weight gainers/high fat gainers), $cycle_j$ was the fixed effect of the *j*th cycle on the correlation of the k^{th} rat (*j*=3,…9), and e_{ij} was the random residual error associated with the k^{th} rat in the *i*th group for the *j*th cycle [$e_{ijk} \sim N(0, R)$, where R is the variance-covariance matrix of the error terms]. The latter error statement assumes the rats were not independent of each other, i.e. there may have been correlations between them (Littell, Milliken & Stroup, 2006). All values are reported as Least Square means ± Standard Error. Multiple comparisons were evaluated for statistical reliability using Bonferroni's test.