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EFFECTS OF HIGH MEAL FREQUENCY ON BODY WEIGHT LOSS, APPETITE REGULATION AND PYY LEVELS

by

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B.Sc.Nutrition, Université de Moncton, 2004

THESIS

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Abstract

Whether increasing meal frequency leads to greater body weight loss, better appetite control and higher levels of PYY remains to be determined. The purpose of this study is to investigate the effect of high MF (HMF) on body weight loss, appetite and PYY in healthy obese men and women. Sixteen obese individuals were randomized to an 8-week equicaloric energy restriction (-700 kcal/day) that either consisted of HMF (3 meals/day and 3 snacks/day; age= 34.63±9.50 y and BMI = 37.1±4.6 kg/m²) or low MF (LMF) (3 meals/day; age=36.3±7.4 y and BMI=34.8±4.0 kg/m²). Baseline energy needs were determined with indirect calorimetry. Appetite (VAS) and body composition (DEXA) were assessed before and after weight loss. Body weight was significantly decreased in both groups ($p<0.001$), but no significant difference was found between conditions ($p>0.05$). Significant higher levels of fullness at time 120min and at time 300min was noted for the LMF group ($p< .05$). PYY levels were comparable across conditions and remained unchanged over the intervention. These findings suggest that increasing MF under conditions of equicaloric energy restriction does not increase weight loss. Further, no favorable effects of increased MF on appetite and on PYY levels were noted.

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List of abbreviations

BMI	Body mass index, kg/m2
BMR	Basal metabolic rate
CDA	Canadian Diabetes Association
cm	Centimeters
DIT	Dietary-induced thermogenesis
EE	Energy expenditure
EI	Energy intake
FFM	Fat free mass
FM	Fat mass
g	Gram
GLP-1	Glucagon-like peptide
GI	Gastrointestinal
h	Hour
HMF	High meal frequency
iAUC	Incremental area under the curve
Kcal	Kilocalories
Kg	Kilogram
KJ	Kilojoule
LMF	Low meal frequency
m	meters
MF	Meal frequency
MJ	Megajoule
mL	Mililiters
mm	Milimiters
min	Minute
NPY	Neuropeptide Y

NS	Non significant
Pg	Picogram
PYY	Peptide YY
REE	Resting energy expenditure
RMR	Resting metabolic rate
TEF	Thermic effect of food
TEFQ	Three eating factor questionnaire
μL	Microliter
yrs	Years

Chapter I - Introduction

1.1 Obesity

The prevalence of obesity has increased markedly over the years. Obesity and its related complications are a leading health threat and obesity has consequently been characterized has a global epidemic by the World Health Organization (WHO, 1998). The most recent Canadian health report revealed that 5.5 million individuals, representing 23% of adults are obese and 36% are overweight (CCHS, 2004). Overweight and obesity are based on body mass index (BMI) which is a measure of body weight in relation to height; they are classified has a BMI of ≥ 25 and BMI of ≥ 30 respectively (WHO, 1998). BMI is highly correlated with body fat and used to indicate health risk (Canada, 2003). Obesity is a complex multi-factorial condition in that environmental, behavioral, physiological, and genetic factors interact (Bouchard, 1991; Jequier & Tappy, 1999). The increase in industrialization, urbanization and mechanization in most countries are associated with changes in diet and behavior (WHO, 2002). High fat energy-dense foods, eating out and binge-eating pattern have been linked to increasing body fat (WHO, 2002) (see **Figure 1**). The decline in daily physical activity is also a major factor contributing to obesity. Most epidemiological studies show a smaller risk of weight gain, overweight and obesity among people who engage in regular amount of physical activity (Fogelholm & Kukkonen-Harjula, 2000; Saris et al., 2003). The environment we live in has become "obesogenic" (obesity-promoting) and can increase the drive to eat and promote sedentary lifestyle as a result lead to excess body weight (Blundell, Lawton, Cotton, & Macdiarmid, 1996; Stubbs, Ritz, Coward, & Prentice, 1995; WHO, 2002; Wynne, Stanley, McGowan, & Bloom, 2005) **(Figure 2).**

1.1.1 Health complications

Obesity and its complications have become a major focus of public health policies. Abdominal obesity, which is associated to the increase in waist circumference is strongly associated with the metabolic syndrome (Despres et al., 1991), which comprise 6 major components: abdominal obesity, atherogenic dyslipidemia (mostly high levels of triglycerides and low levels of HDL cholesterol), raised blood pressure, insulin resistance ± glucose intolerance, proinflammatory state (elevations of C-reactive protein) and prothrombotic state (increase plasma plasminogen activator (PAI)-1 and fibrinogen) (ATPIII, 2002). Because obesity is associated with dyslipidemia and high blood pressure, it often conveys a greater risk of cardiovascular diseases (CVD) (ATPIII, 2002; Carmelli, Zhang, & Swan, 1997; Després, 2001). Obesity also contributes to increased risks for type 2 diabetes (Després, 2001), pulmonary dysfunction, arthritis (Mokdad et al., 2003) and some type of cancers (Carmelli et al., 1997). Furthermore, psychosocial problems, functional limitations and disabilities are as well linked with obesity (Canada, 2003; Visscher et al., 2004).

The numerous health complications associated with obesity strengthens the need to find strategies to promote weight loss in order to achieve a healthy body weight. Several nutritional approaches to overcome excess adiposity that have been studied only results in limited success. Relapse to initial level of adiposity is common after body weight loss (Anderson, Konz, Frederich, & Wood, 2001; Wadden, 1993). Despite the observation that energy restricted diets can produce significant body weight loss (Anderson, Luan, & Hoie, 2004; Wadden, 1993) and considerable improvements of the determinants of the metabolic profile (Wing & Jeffery, 1995), the fact remains that body weight loss maintenance often poses a challenge.

1.2 Energy Balance

The maintenance of body weight requires a long-term maintenance of energy balance (Jequier & Tappy, 1999). Energy balance is achieved when energy intake (EI) equals energy expenditure (EE), and weight gain occurs when EI exceeds EE or when EE is lower than EI (Macias, 2004). Energy is expended or lost through heat, is used to accomplish work, or is stored in the form of lipids, glycogen or tissue proteins. When EI is greater than EE, there is an accumulation of body fat stores and other stores as well as tissue protein through muscle and glycogen stores leading to increasing body weight (Marieb, 1999).

1.2.1 Total Daily Energy Expenditure

The components that contribute to the variation in total daily EE (24 h EE) are basal metabolic rate (BMR), thermic effect of feeding (TEF) and energy cost of physical activity (Donahoo, Levine, & Melanson, 2004; Ravussin & Bogardus, 1992; Verboeket-van de Venne, Westerterp, & Kester, 1993). BMR represents the minimum caloric requirement needed to maintain essential vital functioning (Donahoo et al., 2004) such as respiration, thermoregulation, blood circulation and accounts for approximately 70% of daily EE in sedentary individuals (Ravussin & Bogardus, 1992; Ravussin, Lillioja, Anderson, Christin, & Bogardus, 1986). BMR is typically measured immediately upon waking after 8 hours of sleep and a 12 h fast. Resting energy expenditure (REE) measurement is a little less stringent and is what is normally performed in research projects. The second component of daily EE is TEF which can also be referred to diet-induced thermogenesis (DIT). TEF is the energy required for digestion, absorption, and storage of food (D'Alessio et al., 1988; Leibel, Rosenbaum, & Hirsch, 1995) and accounts for approximately

10% of daily EE (Ravussin & Bogardus, 1992). Lastly, the energy cost of physical activity (activity thermogenesis) is the most variable component (Ravussin & Bogardus, 1992) and usually accounts for 10-30% (Ravussin & Bogardus, 1992).

1.2.2 Energy Intake

Macronutrient composition, meal size, feeding frequency, caloric density of meals and organoleptic properties (sight, smell, taste, and texture) play an important role in the determination of EI (Jequier & Tappy, 1999). Macronutrient composition and feeding frequency will be further discussed later in the review of literature. For this section, we will mostly focus on meal size and caloric density.

Large portion size can easily contribute to excess EI (Rolls, Morris, & Roe, 2002). One study examined the effect of large portion of foods on EI and observed that subjects consumed 30% more energy when offered large portion compared to smaller portion of foods independent of the serving method (pre-portioned or self-served) and of the subject characteristics (Rolls et al., 2002). Larger snack packages were also found to induce higher EI at the time of the snack and also at the subsequent meal (Rolls, Roe, Kral, Meengs, & Wall, 2004). Large portion of foods especially with a high energy density can even more lead to excessive EI (Ledikwe, Ello-Martin, & Rolls, 2005). The energy density (kj/g) (Ledikwe et al., 2005) and volume (Rolls et al., 1998) of food also contribute to higher EI. Energy density is dependent not only on fat content, but also on water and fiber content of foods (Rolls & Bell, 1999). Water has the greatest impact on energy density since it adds weight without adding energy (Ledikwe et al., 2005). Because of its high energy content, fat (37.7 kj/g) influences the energy density of a food more than carbohydrate or protein (16.7 kj/g) (Ledikwe et al.,

2005). Consumption of food high in energy density is associated with higher level of EI compared to similar food with a lower energy density independent of the macronutrient content or palatability (Bell, Castellanos, Pelkman, Thorwart, & Rolls, 1998).

The literature presented in this thesis will emphasize on the effects of weight loss on EI and EE as well as the different methods currently used for achieving body weight loss accentuating dietary manipulations.

Chapter II – Review of literature

2.1 Effects of Weight Loss

Weight loss is the result of a negative energy balance (i.e., EI lower than EE). EE and EI are indisputably affected during and after weight loss. In order for weight loss to occur, EE has to be increased over EI, or EI decreased below EE. These 2 different methods will be further discussed.

2.1.1 Energy Expenditure

Individuals with high levels of 24h EE have been shown to be more successful at losing weight (Astrup et al., 1995). 24 h EE is positively related to body weight (Weyer et al., 2000); as a result, obese individuals are known to have higher EE than lean individuals in part because of larger adipose tissue mass (Leibel et al., 1995) and fat free mass (FFM) (Leibel et al., 1995; Ravussin, Burnand, Schutz, & Jequier, 1982). In fact, the best available determinant of 24h EE is FFM (Doucet et al., 2001; Leibel et al., 1995; Ravussin et al., 1982; Ravussin et al., 1986) and was found to explain 81% of the variance between individuals (Ravussin et al., 1986). However, it is important to note that there is a large variability in 24 h EE among individuals even when adjusted for FFM, fat mass (FM), age and sex (Ravussin & Bogardus, 1992; Ravussin et al., 1982). A later longitudinal study in Pima Indians found that change in body weight alone explained approximately 58% of the variability in the change in 24 h EE and changes in FFM, FM, and spontaneous physical activity together explained 63 % of the variability in 24 h EE (Weyer et al., 2000). It is important to mention that changes in total relative EE are not related with the degree of adiposity (Leibel et al., 1995). A decrease in body weight greater or equal to 10% from initial weight was found to be associated with

a reduction in total EE of 6±3 and 8±5 kcal/Kg of FFM in lean in obese individuals, respectively. These results suggest that relative REE is similar among lean and obese individuals, which is not the case for absolute REE values (Leibel et al., 1995).

Many studies have reported a decrease in EE after body weight loss (Doucet et al., 2003; Doucet et al., 2001; Leibel et al., 1995), which could in part be explained by the changes in body mass (Leibel et al., 1995). In fact, body weight loss most often reduces FFM (Doucet et al., 2003) and FM (Doucet et al., 2003; Doucet et al., 2001); the extent of the decrease is generally 75 to 80 % FM and 20 to 25 % FFM (Jequier & Tappy, 1999; Webster, Hesp, & Garrow, 1984). EE is not only found to be reduced in obese individuals after weight loss but also in non-obese individuals (Leibel et al., 1995), therefore a decrease in EE is expected independently from initial body weight. In fact, one study demonstrated that a 10 % increase in body weight from initial weight resulted in an increase in total EE, non-resting EE and in TEF and a 10 to 20 % weight loss below the initial weight resulted in a decrease in total EE, non-resting EE and RMR (Leibel et al., 1995). It is important to note that these changes in EE were not proportional to the changes in body weight. The fact that a change in body weight is associated with compensatory changes in EE complicates the maintenance of a body weight that is different from the usual weight, which may account for the poor long-term efficacy of obesity treatments.

2.1.2 Appetite

Appetite is a complex phenomenon arising from sequence of interactions among peripheral, central mechanisms and several other complex factors and therefore very difficult to predict (Cameron & Doucet, 2006b; Jequier & Tappy, 1999). Hunger drives for the search for and the

ingestion of food. Termination of food intake occurs when hunger is suppressed, which is called satiation and is often determined by meal size. The absence of hunger between meals is referred to satiety. Termination of the period of satiety triggers feelings of hunger, which normally leads to food intake (Blundell et al., 1996; de Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004). A high level of hunger is usually related to a low level of satiety, which influence meal initiation (de Graaf et al., 2004). Increased BMI has been associated with delayed satiation (Delgado-Aros et al., 2004). It has been reported that overweight and obese individuals require approximately 225kcal more than normal weight individuals to reach maximum satiety (Delgado-Aros et al., 2004); however there is no such evidence yet available during energy restriction. One major problem arising after energy restriction and body weight loss is an increase in appetite (Doucet et al., 2000), which may favor weight regain in reduced-obese individuals (Pasman, Saris, & Westerterp-Plantenga, 1999). Body weight loss is associated with changes in appetite sensations and the best predictors of body weight loss are decreased fasting desire to eat, hunger, and prospective food consumption (Drapeau et al., 2006). On the other hand, fullness is the most useful appetite sensation to predict total EI (Drapeau et al., 2005).

Because body weight loss complicates the control of appetite and EI, appetite sensations must be considered in body weight loss interventions. As a reduction in EI is often associated with an increased in appetite sensations, this poses an immense challenge while attempting to increase compliance to an energy restricted program leading to body weight loss.

2.2 Weight Loss Interventions

With the rising rates of obesity, there is an increased interest in investigating different approach to weight management. For this thesis we will emphasize more on dietary strategies.

2.2.1 Physical Activity

Subjects who participate in formal exercise program under controlled supervision are likely to achieve weight loss and decrease FM (Doucet, Imbeault, Almeras, & Tremblay, 1999; Kraemer et al., 1999; Ross et al., 2000; Ross et al., 2004). However, some reports suggest that exercise alone is not sufficient in order to lose weight without the addition of a certain caloric restriction (Donnelly et al., 2003; Garrow & Summerbell, 1995; Gordon, Scott, & Levine, 1997; Votruba, Horvitz, & Schoeller, 2000). On the other hand, studies comparing exercise-induced weight loss to diet-induced weight loss, found fat reduction to be greater after the exercise-induced weight loss in both men (Ross et al., 2000) and women (Ross et al., 2004). Garrow & Summerbell (1995) reported results of a meta-analysis of twenty-eight publications designed to compare the effect of exercise with or without dieting. The analysis demonstrated how weight loss can be limited when submitted to an exercise program alone. Aerobic exercise without dietary restriction resulted in an average weight loss of 3 kg in men in 30 weeks and of 1.4 kg in women in 12 weeks (Garrow & Summerbell, 1995). A systematic review based on observational studies noted that an increase in EE of physical activity of approximately 1500-2000kcal/week was associated with improved weight maintenance (Fogelholm & Kukkonen-Harjula, 2000). It has been suggested that having access to home exercise equipment was shown to facilitate the maintenance of short-bout of exercise which could improve long-term weight loss (Jakicic, Winters, Lang, & Wing, 1999). Physical activity is known to

promote weight stability after weight loss (Prentice & Jebb, 2004). Doucet and colleagues (1999) showed that after an initial weight loss, more physically active reduced obese individuals retained a greater proportion of their initial weight loss (Doucet et al., 1999).

2.2.2 Dietary Strategies

Manipulations of dietary fat, carbohydrate, protein, the glycemic index of food, fiber, as well as the meal frequency and portion size are all important components in dietary treatments for obesity (WHO, 2002). Generally, studies aim for either non-specific macronutrient energy restriction or specific macronutrient energy restriction. The two are discussed in greater details below.

2.2.2.1 Non- Specific Macronutrient Energy Restriction

Non- specific macronutrient energy restriction relates to diets low in total calories. This approach is based on decreasing EI below EE and therefore creating an energy deficit to lose body weight (Finer, 2001). For rapid weight loss over a short period of time, very low energy-diets (200-800 kcal/day) and low energy-diets (800-1500 kcal/day) are often used (Astrup, 1999). However, it is difficult to obtain all of the essential nutrients when following very low energy-diets and micronutrient supplements are often required (Astrup, 1999). As for low energy-diets, they are considered as balanced deficit diets (Wadden, Foster, & Letizia, 1994) and can be achieved without micronutrient supplementation (Astrup, 1999). One study demonstrated that a 1200 kcal/day and a 400 to 800 kcal/day diets combined with behavior modification caused body weight loss of approximately 8.5 kg in 20 weeks and 20 kg in 12-16 weeks, respectively (Wadden, 1993). However only 2/3 in the low energy diet of the weight was maintain 1 year following the weight loss program

and 1/2 to 2/3 for the very low energy diet. Both diets were associated with increasing weight regain over time (Wadden, 1993). Other energy restriction studies were also able to find a reduction in body weight, FM and in BMI (Doucet et al., 2001; Doucet et al., 2002), though a reduction in FFM was also observed (Doucet et al., 2002). There are clear indications that non-specific macronutrient energy restriction induces weight loss, but the major concern is the reduction in FFM, poor compliance to the diet and consequently poor long-term weight maintenance results (Finer, 2001; Wadden et al., 1994).

2.2.2.2 Specific Macronutrient Energy Restriction

Specific macronutrient energy restriction diets target one or more macronutrients with or without energy restriction. Many different macronutrient combinations have been investigated; however, there is still some debate about which macronutrient composition best promotes body weight loss.

The palatability of dietary fat (Poppitt, McCormack, & Buffenstein, 1998; Saris & Tarnopolsky, 2003) and its low effect on satiety and satiation (Poppitt et al., 1998) as well as its high energy density (Rolls, 2000) promote passive over-consumption and positive energy balance causing excessive body fat storage (Blundell et al., 1996). Some studies have showed that low-fat *ad libitum* diets and low-energy diets result in similar body weight reduction (Jeffery, Hellerstedt, French, & Baxter, 1995; Shah, McGovern, French, & Baxter, 1994). Reduction in dietary fat consumption without imposed energy restriction was shown to cause body weight loss in overweight individuals and to prevent body weight gain in normal weight individuals (Astrup, Grunwald, Melanson, Saris,

& Hill, 2000; Astrup, Ryan et al., 2000). In addition, a randomized intervention study demonstrated that following a one-year weight loss program, obese subjects who consumed an *ad libitum* low-fat and high-carbohydrate diet had regained on average 5.9 kg less than a group who cut down on all calories equally (Toubro & Astrup, 1997). A number of meta-analyses on the relationship between freely available low-fat diets and body weight control showed that a reduction in dietary fat intake is directly associated with weight loss. In fact, a reduction of 10% from initial dietary energy from fat was associated with a spontaneous weight loss of 3.2 kg more in the intervention than in the control group. Weight loss was dependent on the pre-treatment body weight so that for each additional 10 kg of initial body weight an additional 2 - 2.6 kg weight loss was reported compared with control groups (Astrup, Grunwald et al., 2000). However, a review analysis from Astrup et al. (2002) demonstrated that a 10% reduction in dietary fat is predicted to only produce a 4-5 kg weight loss in individuals with a BMI of 30 kg/m^2 (Astrup, Buemann, Flint, & Raben, 2002). Overall, we may conclude that *ad libitum* low fat diets can indeed cause body weight loss; however it can be a limited approach in order to lose large amount of body weight in obese individuals.

Most low-fat diets discussed in the previous section are rich in dietary carbohydrate; therefore a specific section on low-carbohydrate diets is needed. Nowadays, the carbohydrates are more refined than they used to be, which increases their energy content in foods, therefore reducing their potential to induce satiety (Prentice & Jebb, 2004). The effect of high-sucrose diet versus a high-starch and high-fat diet on freely available EI demonstrated that EI was lower on the starch-diet. High-starch diets are known to have an increased satiating effect because of high-fiber content and volume and also have a reduced palatability compared to sucrose and high-fat diets (Raben, Macdonald, & Astrup, 1997). On the other hand, the CARMEN multi-centre study investigated the

long-term effect of changes in dietary carbohydrate/fat ratio and simple versus complex carbohydrate. This study found that freely available intake of low fat (reduction of 10%) resulted in significant decrease in body weight but the increase in either simple or complex carbohydrate did not make any significant difference on the amount of weight loss (Saris et al., 2000). Furthermore, the so called "Atkins Diet", which is a low-carbohydrate, high-fat and high-protein diet was compared to the conventional energy restricted high-carbohydrate and low-fat diet. Although the initial weight loss was higher in the low-carbohydrate diet of approximately 4 % after the first six months, the difference in body weight reduction after one year was not significant from the conventional diet (Foster et al., 2003). Overall, high-starch diets seem to have a higher satiety potential compared to high-sucrose diets. Nonetheless, low-carbohydrate diets do not seem to differ from low-fat diets in terms of body weight loss in the long-term.

There is increasing interest in high-protein diets as a strategy to lose weight because of its important role in the appetite regulation and thermogenesis. In general, it is well documented that protein and carbohydrate have higher satiety signals than fat; however, protein also has a more potent satieting effect than carbohydrate (Poppitt et al., 1998; Westerterp-Plantenga, Lejeune, Nijs, van Ooijen, & Kovacs, 2004). It is interesting to note that people tend to be less hungry (Poppitt et al., 1998) and to consume less energy when following a high protein diet compared to other macronutrients (Poppitt et al., 1998; Skov, Toubro, Ronn, Holm, & Astrup, 1999). In fact, Poppitt et al. (1998) found that a high-protein meal decreased EI by 12.3% to 20.8% relative to high alcohol, fat and carbohydrate meals (Poppitt et al., 1998). In some cases, high-protein diets are found to induce greater body weight loss than high-carbohydrate diets (Baba et al., 1999; Dumesnil et al., 2001; Skov, Toubro, Ronn et al., 1999). The replacement of some dietary carbohydrates by dietary

proteins (25%) combined to a low-fat diet (< 30%) with no imposed energy restriction was studied in obese individuals. The high-protein group (25% as protein and 45 % as carbohydrate) lost significantly more weight after a 6-month intervention compared to the high-carbohydrate group (12 % as protein and 58% as carbohydrate). Thirty five percent of the subjects lost >10 kg in the high-protein group compared to 9% in high-carbohydrate group (Skov, Toubro, Ronn et al., 1999). In a study comparing the American Heart Association phase 1 diet (low-fat-high-carbohydrate diet) to the Montignac diet (low-glycaemic index-low-fat-high-protein diet) consumed *ad libitum* observed a spontaneous decrease in EI (25%) only after 6 days of following the Montignac diet (Dumesnil et al., 2001). Furthermore, weight regain after energy restriction leading to body weight loss was found to be 50% lower in individuals consuming 18% of total energy from protein compared to 15% consisting from FFM only (Westerterp-Plantenga et al., 2004). Overall, protein has an important role in the appetite control which could increase compliance to energy restricted diets leading to body weight loss.

2.2.2.3 Meal Frequency

Reduced meal frequency (MF) is thought to contribute to the development of obesity (Farshchi, Taylor, & Macdonald, 2005; WHO, 2002). Some reports have observed an inverse relationship between the number of meals consumed in a day and the degree of adiposity. Several studies have noticed a decrease in body weight when eating smaller quantities more frequently, suggesting that high MF combined with a regular meal pattern could be a strategy to prevent obesity (Bellisle, McDevitt, & Prentice, 1997; Drummond, Crombie, Cursiter, & Kirk, 1998; Metzner, Lamphiear, Wheeler, & Larkin, 1977; Speechly, Rogers, & Buffenstein, 1999; Summerbell, Moody,

Shanks, Stock, & Geissler, 1996). Drummond and colleagues (1998) suggested that eating more frequently is likely to lead to high carbohydrate consumption instead of high fat consumption, which could favor body weight control (Drummond et al., 1998). On the other hand, one particular study found no significant difference in anthropometric measurements or body fat composition when subjects were eating 6 times a day with regular intervals (> 1 hour between 2 meals) compared to a chaotic meal pattern (between 3 to 9 meals a day) at irregular intervals (Farshchi et al., 2005). High eating frequency was associated with leanness in men, but not in women, which is though to be influenced by the level of compensation (Drummond et al., 1998). Energy compensation response varies among individuals and with different levels of dietary restraint (Drummond et al., 1998). It appears that dietary unrestrained, obese and non-obese males are better compensators than women by reducing the size of subsequent eating episodes and therefore adjusting EI when submitted to higher MF (Drummond et al., 1998; Rolls et al., 1994). The inconsistencies in the body weight results among epidemiological studies could come from the fact that obese individuals often underreport their food consumption, which can affect the relationship between body weight and EI (Farshchi et al., 2005), giving the impression that low eating frequency is positively related to adiposity (Bellisle et al., 1997). In fact, an inverse relationship between high feeding frequency and BMI in adolescents was lost when invalid dietary records from under-reporters and dietary restraint were excluded (Summerbell et al., 1996).

Very few experimental studies have been conducted to investigate the effect of MF on body weight loss through energy restriction. A review analysis by Bellisle and colleagues (1997) revealed that most studies were performed in the 1970s and found that MF had no significant impact on weight loss during energy restriction (**see Table 1**) (Bellisle et al., 1997). Among these studies only

2 had an elaborated description of their protocol (Garrow et al., 1981; Verboeket-van de Venne & Westerterp, 1993) from which only one had the main purpose of investigating the rate of weight loss under different feeding frequency (Verboeket-van de Venne & Westerterp, 1993). One experiment prescribed an energy content of 1000 kcal/day for 4 weeks where seven subjects were eating 2 meals/day and seven others 3-5 meals/day. Their results showed no effect of feeding frequency on the rate of body weight loss, FM and in FFM (Verboeket-van de Venne & Westerterp, 1993). In addition, in cross-over design comparing different amounts of proteins combined with different frequency of eating being 1 meal/day or 3 meals/day or 5 meals/day in obese women on a 800 kcal/diet for 3 weeks, no evidence that higher MF could affect fat loss was found. However, they reported that while on a low energy diet, subjects found it more tolerable when the energy was distributed in many small meals (Garrow et al., 1981). The duration of these studies seem to be an issue. Experimental studies that have looked at the effect of feeding frequency on the rate of weight loss with or without energy restriction were all conducted between 14 days to a maximum of 4 weeks. Therefore, we might possibly note changes in body weight after a longer period of time. Many aspects between meal patterning and body weight control remain unclear.

The evidence, although controversial that negative relation between high MF and body weight often observed, could in part be explained by lower EI (Drummond et al., 1998; Farshchi et al., 2005; Speechly et al., 1999; Westerterp-Plantenga, Kovacs, & Melanson, 2002) and feeling less hungry (Speechly et al., 1999). Generally, it seems that EI in obese and lean individuals is lower when following a regular meal pattern with higher frequency than during an irregular meal pattern with lower frequency (Drummond et al., 1998; Farshchi et al., 2005; Speechly et al., 1999; Westerterp-Plantenga et al., 2002). In a study by Speechly & Buffenstein (1999), participants ate

their breakfast in either one meal or five small hourly meals. The amount of food they consumed in an *ad libitum* lunch was then measured. The single meal group consumed a larger lunch (26.6% more) than the multiple-meal group, leading the investigators to conclude that appetite and caloric intake is best controlled by the consumption of smaller and more frequent meals(Speechly & Buffenstein, 1999). Others have found similar results, but only for men, since women tended to have higher EI when eating more frequently (Drummond et al., 1998). This sex difference in EI could again be explained by the fact that women are known to be poor compensators compared to men, however it could also be influenced by the level of physical activity (Drummond et al., 1998). Higher levels of physical activity may promote higher EI to meet increased energy requirements (Drummond et al., 1998). Thus, further studies should take into account daily physical activity in subjects submitted to higher eating frequency when trying to make relationships between MF and EI. The finding of reduced appetite (Farshchi et al., 2005) and reduced EI (Taylor & Garrow, 2001) after frequent meal consumption is not always consistent among studies, which could again be a result of underreporting (Farshchi et al., 2005). One study suggested that increased periodicity of eating could also favor the release of gastric hormones associated to the appetite control (Speechly & Buffenstein, 1999), however other studies are needed to confirm this postulation. Indeed, studies should be looking at manipulating MF as well as macronutrient composition and meal timing to favor the release of gastrointestinal peptides known to influence food intake in order to better control appetite sensations upon energy restricted diets leading to body weight loss.

Most reports, did not find any association between high MF and EE (Taylor & Garrow, 2001; Verboeket-van de Venne et al., 1993). In many cases, high MF was found to have no effect on RMR

(Farshchi et al., 2005; Kinabo & Durnin, 1990; Verboeket-van de Venne et al., 1993), TEF (Kinabo & Durnin, 1990; Verboeket-van de Venne et al., 1993) and on physical activity EE (Verboeket-van de Venne et al., 1993). Only one study noted a reduction in TEF with irregular MF (Farshchi, Taylor, & Macdonald, 2004).

2.3 Hormonal Profile and Body Weight Loss Program Outcome

There is increasing evidence that body weight loss is followed by metabolic and/or endocrine adaptations that could influence the appetite in the reduced-obese state (Cameron & Doucet, 2006a; Doucet et al., 2000) Some hormones are responsible for meal initiation and others for its termination (Wynne et al., 2005). As previously reviewed (Woods, 2004; Wynne et al., 2005), several peptides synthesized and secreted within the gastrointestinal (GI) tract are known to modulate food intake, such as ghrelin, polypancreatic peptides family (e.g., PYY), proglucagon products (e.g., GLP-1), and cholescystokinin. These peptides are short-term modulators of food intake (Wynne et al., 2005), which respond to nutrients within the gut and interact with specific receptors to limit meal size (Havel, 2001). In contrast, insulin (Schwartz, Boyko, Kahn, Ravussin, & Bogardus, 1995) and leptin (Considine et al., 1996) are long-term regulators of food intake and fluctuate with body energy reserves (Havel, 2001). However, some tend to consider ghrelin both a long-term and short-term regulator of food intake (Cummings et al., 2002).

Some of these peptides are known to fluctuate with acute and chronic food deprivation. Changes in levels of these peptides may influence appetite control upon energy restriction leading to body weight loss (Cameron & Doucet, 2006a). Following body weight loss, levels of leptin were shown to decrease (Infanger et al., 2003; Racette, Kohrt, Landt, & Holloszy, 1997; Wadden et al.,

1998) as well as after acute energy deprivation (Doucet, Pomerleau, & Harper, 2004; Wadden et al., 1998), which was associated with increased appetite sensations (Doucet et al., 2004). In contrast, ghrelin was shown to increase in response to sustained energy restriction leading to body weight loss (Cummings et al., 2002). It has been suggested that the pre-prandial rise in plasma ghrelin levels could serve as a meal initiation signal (Cummings et al., 2001) as elevated levels were associated with increased appetite and food intake (Druce et al., 2005). Very few studies have been done on the effects of energy restriction and dietary induced body weight loss on peptide yy (PYY). One study reported fasting total PYY to be increased after successful weight loss in children (Roth et al., 2005). However, following a 2 or 3 day fast, fasting circulating total PYY was shown to be reduced by as much as 50% (Chan, Stoyneva, Kelesidis, Raciti, & Mantzoros, 2006). Since elevated levels of PYY are associated with a decrease in food intake (Batterham et al., 2003; Batterham et al., 2002), its reduction upon energy restriction could compromise appetite control. Elevated levels of GLP-1 are also associated with lower EI (Flint, Raben, Astrup, & Holst, 1998) and were shown to be reduced after body weight loss (Adam, Jocken, & Westerterp-Plantenga, 2005). In brief, these results suggest that a reduction (or increase) in any of the above peptides following energy restriction and body weight loss could compromise the adherence to energy restricted diets during and after body weight loss.

Of interest for this thesis are the short-term signals. These satiety signals arise from the GI tract and related organs during an eating episode and create a sensation of fullness which cause food intake to be reduced and therefore affect the size of individual meals (Cummings & Shannon, 2003; Havel, 2001; Woods, 2004). Among the several satiety signals, PYY is the hormone of interest for this project. Recently, literatures have been supporting the impact of PYY on food intake suggesting

that this peptide favors appetite control. Whether structuring food intake and meal patterning in a way that could favor the release of PYY in order to increase compliance during and after body weight loss remains to be determined.

2.3.1 Peptide YY

PYY is a 36 amino-acid peptide belonging to the pancreatic polypeptide family along with pancreatic peptide (PP) and neuropeptide Y (NPY). It was named PYY because of the presence of an amino acid terminal (Y) tyrosine and a carboxyl terminal tyrosine amide (Y). There are two forms of PYY in the circulation: PYY (1-36) and PYY (3-36) (Wynne et al., 2005) which is produced by the action of the enzyme dipeptidyl peptidase-IV (DPP-IV) (Batterham & Bloom, 2003) . PYY (3-36) differs from PYY (1-36) in that it is enzymatically cleaved at the N-terminus, resulting in a truncated 34 amino acid form (Grandt et al., 1994). PYY (1-36) is the major form in the fasting state and is known to increase appetite and body weight gain in rats (Ballantyne, 2006; Grandt et al., 1994) while PYY (3-36) is the major form in the gut and in the circulation following a meal, and comprises about 63% of total plasma PYY (Grandt et al., 1994).

PYY is synthesized and secreted from endocrine L-cells in the distal gut (Adrian et al., 1985) with greater extent from the ileum and colon (Ekblad & Sundler, 2002) in response to food consumption (Adrian et al., 1985). In general, PYY levels increase within 15 min, peak around 60-180 min and remain elevated for up to 6 h (Adrian et al., 1985). The rise in PYY levels is influenced by the number of calories and by the macronutrient content of foods, (le Roux et al., 2006; Pedersen-Bjergaard et al., 1996) such as after fat and protein ingestion, levels of PYY were found to peak at 2

h and while following carbohydrate ingestion, PYY levels peaked at 30 min but returned to basal levels after only 60 min (Adrian et al., 1985). Fat was known to be the major releaser of PYY when compared to protein or carbohydrate (Fu-Cheng et al., 1995); however in a recent study a high protein meal resulted in the greatest increase in PYY (Batterham et al., 2006). Some studies have shown an attenuated postprandial PYY responses in obese subjects (Batterham et al., 2003; le Roux et al., 2006) and lower fasting endogenous PYY concentrations (Batterham et al., 2003; le Roux et al., 2006). In fact, one particular study showed that after normal weight and obese subjects received a random order either of 500 ml liquid meal (250,500, and 1000 kcal) or a 900 ml meal (1000, 2000, and 3000 kcal), obese subjects had an attenuated PYY response across all range of meals with different caloric content compared to normal weight individuals. Nevertheless, PYY and fullness levels still increased after every meal (see **Figure 3 and Figure 4**) (le Roux et al., 2006). PYY (3-36) is thought to be an important factor in the regulation of food intake (Small & Bloom, 2004), since its peripheral infusion was reported to decrease appetite in humans (Batterham et al., 2003; Batterham et al., 2002) and to inhibit food intake by > 30% over 24h in both normal weight and obese individuals (Batterham et al., 2003; Batterham et al., 2002). However, a recent study showed that pharmacological levels are needed to achieve these effects (Degen et al., 2005). The anorectic effects of PYY (3-36) is though to be in part mediated through reduced levels of ghrelin (Batterham et al., 2003) since its infusion was shown to attenuate the pre-meal rise in ghrelin (Batterham et al., 2003). PYY has been shown to exert several biological functions, including vasoconstriction, inhibition of gastric acid secretion, reduction of pancreatic and intestinal secretion, and inhibition of gastrointestinal motility (Batterham & Bloom, 2003). The inhibition of gastrointestinal motility acts as an "ileal break" to cause a sense of satiety, and is thought to be partially responsible for the

decrease in food intake (Korner & Leibel, 2003) (see **Figure 5**). PYY mediate its effects through the NPY receptors of which there are several subtypes (Y1, Y2, Y4, and Y5); PYY (1-36) exerts its effects through at least three Y receptor subtypes (Y1, Y2, and Y5), whereas PYY (3-36) is more selective for the Y2 receptor (Y2R) highly expressed on NPY neurons in the arcuate nucleus (ARC) (Batterham & Bloom, 2003; Broberger, Landry, Wong, Walsh, & Hokfelt, 1997; Larhammar, 1996).

In conclusion, appetite seems to increase following weight loss an effect that may be partially mediated by an increase or decrease in hormones known to influence feeding and for that reason it is important to have a dietary regimen that has a high satiating effect, so people can make better food choices and control their food intake. Furthermore, by better understanding the actions of PYY on appetite and by developing a nutritional strategy that could optimize their beneficial effects on EI, our subjects might have better success at loosing greater amount of body weight. As for MF, experimental studies that have looked at the effect of feeding frequency on the rate of weight loss with or without energy restriction were all conducted between 14 days to a maximum of 4 weeks. Therefore, we might possibly note changes in appetite ratings and in body weight after a longer period of time. If increasing the frequency of eating has beneficial effects on appetite ratings in an obese population, this may allow an improved probability for those individuals to lose weight on a restrictive dietary regimen. Overall, the integration of the literature presented in this review suggests that post-prandial secretion in PYY could optimize compliance during weight loss, and that meal frequency could help in this manner. A better understanding of increased MF on body weight loss, appetite regulation and on PYY is warranted.

2.4 Statement of the Problem

To date there have been few effective dietary treatments for obesity. Despite considerable research, debate remains on the energy content, meal patterning and the optimal macronutrient distribution for effective weight loss. Even though low fat, low carbohydrate and low calorie diets were found capable to induce weight loss, their effectiveness is still quite limited as the prevalence of obesity is continually rising. Among the different weight loss strategies, high MF with optimal meal timing to promote elevated levels of PYY combined with an energy restriction could perhaps promote successful weight loss, and possibly weight maintenance, although it will not be addressed in this study. However, to our knowledge, no study has adequately addressed whether increasing MF leads to enhanced compliance to the dietary restriction and over time to greater weight loss through a less pronounced increased in hunger, desire to eat, prospective food consumption, and higher levels of fullness, an effect that may be partially mediated to a more favorable gut hormone profile, such as PYY.

2.5 Purpose of the Study

Little research has been done on meal timing and frequency which are 2 major components to dietary manipulations. Furthermore, few recent studies have concentrated on the efficacy of increased MF on weight loss and appetite regulation during energy restriction. This project is designed to investigate the effect of high MF on body weight loss, appetite regulation and on the profile of peptide YY in apparently healthy obese men and women.

2.6 Objectives

The main objective of this study was to investigate whether using an increased meal frequency pattern (HMF) (3 meals + 3 snacks/day) with snacks being individually timed (SEE DESCRIPTION IN METHODS) could lead to greater weight loss than the conventional LMF pattern (3 meals/day) in response to an equal caloric restriction. The secondary objectives of this study were to: (a) examine the daily level of PYY between the two groups and; (b) examine the daily variation in appetite between the two groups.

2.7 Hypotheses

We hypothesized that: (a) a greater weight loss will be observed in the HMF group because of higher levels of fullness and possibly to a greater compliance to the energy restriction; (b) higher daily levels of fullness will be observed in the HMF group likely because of higher levels of PYY throughout the day; (c) higher daily PYY levels will be observed in the HMF group likely because of shorter period of time between each eating occasion.

2.8 Delimitations

The recruitment for this study was limited to obese (30 kg/m^2 < BMI < 45 kg/m^2) men and women between 18 and 55 years old, non-diabetic, non-smokers, not pregnant, sedentary and weight stable for $\geq$6 months ($\pm$2 kg). Subjects were apparently healthy and free from any illnesses and medication that might have influenced the outcome of the program. As well, only pre-menopausal women with a regular menstrual cycle (28-35 days) were recruited because of hormonal changes that occur in women during and after menopause.

2.9 Limitations

This study was limited to eight participants in each group due to human, financial and material resources limits. Results are not applicable beyond the specific population from which the sample was selected. Statistical power is thus a limitation for results interpretation.

2.10 Relevance of the Study

The dietary strategy for this study which is increased MF with individually timed snacks and the addition of a caloric deficit could facilitate weight loss among obese individuals. Furthermore, the anticipated results could help us understand the effects of HMF on body weight loss, body composition, appetite ratings and on the profile of PYY in apparently healthy obese men and women.

Chapter III - Methodology

3.1 Study Description

The study protocol was approved by the Ethical Research Committee of the University of Ottawa (see **Appendix 1** for the Approval Letter) and informed consent was obtained from all participants. The research participants were recruited primarily through ads posted all around the University of Ottawa campus and Montfort Hospital, and newspaper ads. Information concerning the inclusion/exclusion criteria of the study was gathered through a pre-screening questionnaire (**Appendix 2**) that was administered during our first telephone contact. Participants were informed that the study involved identifying the effects of MF on body weight loss, appetite regulation and PYY levels through an energy restriction approach to treat obesity.

3.2 Subjects

Eighteen subjects (9 men and 9 women) were recruited; of these participants, sixteen completed the study (8 men and 8 women), whose results are presented in this study. They were obese (30 kg/m^2 < BMI < 45 kg/m^2), non-diabetic, non-smokers, not pregnant, sedentary (<30 min of continuous exercise performed $\leq$2 times/wk), weight stable for $\geq$6 months ($\pm$2 kg) and aged between 18 and 55 years. Only pre-menopausal women with a regular menstrual cycle (28-35 days) were recruited including those using oral contraceptives. Apart from being characterized by an increased adiposity, subjects were apparently healthy, that is free from any illnesses and medication that could have influenced the outcome of the program. This information was first gathered through a telephone interview with subjects who responded to the media ads. Participants that met the

inclusion criteria were asked to present themselves to the laboratory for a first initial visit, in which pre-test evaluation took place.

3.3 Study Design

This study was conducted as a randomized approach with two parallel treatment groups. Participants were randomized to 1 of the 2 groups as follows: the high meal frequency (HMF) (3 meals + 3 snacks/day) and the low meal frequency (LMF) (3 meals/day). Women and men were stratified equally in both groups.

3.4 Experimental Protocol

Both groups were provided with a meal plan according to the recommendations from the Canadian Diabetes Association (CDA) (CDA, 2003). The nutritional approach to lose weight corresponded to a reduction of EI of 2.9 MJ/day (700kcal/day). Both groups were prescribed to the same energy restriction for a period of 8 weeks. Energy needs were calculated based on each participant's REE result multiplied by a physical activity level of 1.4 (OPDQ, 2000) to estimate daily EE. Mean REE for LMF and HMF was 1856 kcal and 1922 kcal respectively. To fix the EI for the program, 2.9MJ (700 kcal) were subtracted from each participant's total energy needs per day as described above (REE*1.4 – 700 kcal). In order to achieve the energy restriction and to follow the CDA guidelines, the food exchange system from the CDA was explained to each participant by a Registered Dietitian. In this system, foods are categorized into six groups as follows: starch foods; fruits & vegetables; milk products; protein foods; fat & oils; and extras (composed of food item

containing ≤ 2.5g carbohydrate per ½ cup). Participants had the flexibility to select their food within the meal plan that was individually designed for them based on their daily caloric prescription. The macronutrient composition was reflective of the Dietary References Intakes for Canadians (NRC, 2005). Carbohydrate was accounted for 55% of calories, preferably from low glycemic index foods with high fiber content ranging from 25 to 35 grams per day. Fat had to provide 27% of calories and saturated fat, trans-fat and cholesterol had to be as low as possible. Finally, 18% of calories had to come from protein. As for meal timing, it varied among participants. A time period of at least 4 hours but no more than 6 hours between main meals was instructed for both LMF and HMF groups. The last meal of the day had to be consumed at least 3 hours before bedtime and 2 hours for snacks. Ingestion of the pre-lunch and pre-dinner snacks for the HMF group was set at specific times for each subject. This time was determined before weight loss based on the delay to achieve peak fullness in response to a test meal of 300 kcal (2 whole wheat toast, 1 tablespoon natural peanut butter and 125 ml 2% milk) at baseline. Meal timing for snacks was determined by subtracting the time to achieve peak fullness from main meals. For example, if breakfast was served at 8:00 and peak fullness was achieved after 30 minutes (8:30) and remained elevated for 120 minutes (10:30), we would subtract 120 minutes from lunch time (12:00). Therefore, mid-morning snack would have been served at 10:00. This was done so participants in the HMF would initiate lunch and dinner at the time where the snack exerted its maximal fullness (and possibly PYY) effects. A "meal" had to be composed from at least 3 food groups from the Canadian Food Guide and a "snack" was represented by one source of carbohydrate (grain products or fruits and vegetables) and one source of protein (meat and substitutes or milk products).

3.5 Measurements

Measurements during this protocol included anthropometry (body weight, waist circumference, % body fat, lean mass and fat mass), appetite, eating behavior, EE and PYY levels. In order to minimize the hormonal effects on main outcomes, we aimed measurements for women to be performed between days 1 - 5, where ovarian hormones are at their lowest levels (Marieb, 1999). However, the timing was difficult to gauge and therefore most measurements were done between days 1 – 10 of their menstrual cycle with the exception of 2 women that were between days 1 - 5. A series of measures were performed before, during and after the weight loss program. See **Figure 6** for details. Furthermore, subjects from both groups had to come to our laboratory for 3 control sessions during which they were weighed and their waist circumference was measured and a 24-h dietary recall with a Registered Dietitian was filled out to assess their adherence to the meal plan. Further, 3 telephone controls were also done on the weeks when subjects did not have to come to the laboratory.

3.5.1 Initial Visits

A first initial visit was required to measure body weight and height to assure subjects were within the requirements for the study. At initial visit 1, a food appreciation questionnaire (**Appendix 3**) was given to every participant to make sure they did not express aversion to any foods served during the snack test meal and for the pre-post sessions. A 3 day food journal was given at initial visit 1 to observed habitual food intake of every participant in order for the dietitian to make proper individuals recommendations. At the end of the session, participants had to give informed consent before going any further with the procedures (**Appendix 4**). Once the informed consent was

received, subjects had to return to the laboratory for a second visit, where a REE measurement was performed. Next, a meal plan to favor weight stabilization was designed based on the CDA recommendations for 3 days prior to the beginning of the weight loss program. The energy requirements for the 3 day weight stabilization were calculated based on the results from the REE measurement with an activity level of 1.4.

3.5.2 Baseline Assessments

Fullness levels were measured following a standardized snack test meal in order to determine the fullness peak and the time fullness remained elevated for every participant. This session helped to determine meal timing for the HMF group. Weight, waist circumference and body composition were also measured.

3.5.3 Pre- and Post-Diet Sessions

A pre- and post-diet session was needed to measure appetite ratings and PYY between the 2 groups on their respective dietary regimen (HMF versus LMF). During the pre- and post sessions, subjects were served foods according to their respective meal plan. Meals and/or snacks were served according to each participant's energy prescription (**Table 2**). Even if the energy prescription was equicaloric, MF, meal timing and portion size varied among participants.

3.6 Laboratory Procedures

3.6.1 Resting Energy Expenditure

Early in the morning following a 12-h overnight fast and after a 30 min resting period in the supine position, a measurement of REE was done. Oxygen consumption and carbon dioxide production was assessed by indirect calorimetry with an open-circuit ventilated hood system (Deltatrac II Metabolic Monitor, Sensor Medics Corporation, Yorba Linda, CA, USA). Coefficient of variation and correlation for the Deltatrac II metabolic cart was 2.3% (R = 0.98). The metabolic cart was calibrated against 95% O_2 / 5% CO_2 reference gas at the beginning of each day. A plexiglass hood was placed over the participant's head through which fresh air was drawn. The expired air was sampled for analysis and percentages of oxygen and carbon dioxide determined for 30 minutes. The first and last 5 minutes of measurement were discarded, and the values of VO2 and VCO2 for the middle 20 minutes were averaged for the calculation of the rate of REE.

3.6.2 Anthropometric Measures

Body weight was determined with a standard beam scale (HR-100; BWB-800AS, Tanita Corporation, Arlington Heights, IL., USA), whereas height and waist circumference was measured with a tape. These values were used to determine BMI. Body weight was measured after voiding, while clothed in a hospital gown and after having removed all accessories (watches/bracelets, chains, eye glasses, etc). Height was measured with the participant's bare feet together, with their heals, buttocks, back, and head against the wall, and following a normal inspiration. Waist circumference was measured directly on the skin, in duplicate (and averaged), at the mid-point between the last floating rib and the top of the iliac crest.

3.6.3 Body Composition

Body composition was determined using dual-photon x-ray absorptiometry (DEXA) which measures percent fat, fat mass and lean mass (Lunar Prodigy, General Electric, Madison, WI, USA). Coefficient of variation and correlation for DEXA was 1.8% (R = 0.99). During this measurement, the participant clothed in a hospital gown, was asked to lie in the supine position, while a low intensity x-ray scanned their entire body. This measurement was done immediately after anthropometric measures. The radiation associated with it is less than 0.5 millirem.

3.6.4 Standardized Snack Test Meal

This meal was designed to reflect the definition of a snack according to the study guidelines (2 starches, 1 protein, 1 fat and 1 milk). Following a 12-h overnight fast, subjects had to come to the laboratory for a standardized snack test meal, which had to be consumed in 15 min. The standardized snack consisted of 2 whole wheat toast (140 kcal), 1 tablespoon of no sugar added peanut butter (95 kcal) and 125 ml of 2% milk (60 kcal) for a total of 295 kcal. Calories were determined using a nutritional program for analysis (The Food Processor® SQL Version 9.6, ESHA Research, Salem, OR., USA).

3.6.5 Appetite

Following a 12-h overnight fast, subjects had to come to the laboratory for appetite measurements. Appetite ratings were measured immediately before and after as well as every 15 min until 180 min at baseline session and every hour for a period of 360 min at pre-and post-diet sessions. This was done using a pen and paper on a 150-mm visual analogue scale (VAS) adapted

from Hill and Blundell (A. J. Hill & Blundell, 1986) (**Appendix 5**). Briefly, desire to eat, hunger, fullness and prospective food consumption (PFC) was rated. Questions were asked as follows: 1) "How strong is your desire to eat?" (Very weak- Very strong); 2)"How hungry do you feel?" (Not hungry at all- As hungry as I have ever felt); 3) "How full do you feel?" (Not full at all- Very full), and 4) "How much food do you think you could eat?" (Nothing at all- A large amount).

3.6.6 Attitude in relation to food

The Three-Factor Eating Questionnaire (TFEQ) (Stunkard & Messick, 1985) was administered at initial visit 1 (**Appendix 6**). Eating behavior was assessed with the TFEQ which measures dietary restraint, disinhibition and hunger. In this study, the only variable of interest was the participant's level of restraint.

3.6.7 Blood Samples: PYY measurement

Upon arrival at the laboratory, a qualified nurse inserted an intravenous catheter in the antecubital vein of the participant's non-dominant arm from which blood could be drawn at different times throughout the session. Approximately 2.5mL of blood was collected into 3mL lavender vacutainer tubes containing EDTA. PYY concentration was measured before and after the weight loss trial. Following a 12 h fast, blood was drawn every hour for a period of 6 hours while subjects were following their respective meal plan. Immediately after collection, the vacutainer tube was rocked gently several times for anti-coagulation purposes and aprotinin (180μL) and DPP-4 (30μL) was added to inhibit the activity of proteinases, after which the samples were centrifuged at 3000 rpm for 15 min at 4^{o}C. Plasma was then transferred into 2mL tubes and immediately stored at –80^{o}C

until assayed. Total PYY was assayed in duplicate using an enzymatically amplified "one-step" sandwich-type immunoassay (Total Peptide YY ELISA DSL-10-33600, Diagnostic Systems Laboratories, Inc., Webster, Texas). The inter- and intra-kit coefficient of variation was 10.9% and 4.1%, respectively. The analyses of PYY for each individual were always performed within the same kit.

3.7 Statistical Analysis

SPSS software 14 (SPSS Inc., Chicago, IL, USA) was used for all analyses. A Mixed Model ANOVA with one between factor (MF) was used to determine the effects of the treatment on all dependent variables. Because sex was randomized equally in both experimental arms, we also performed a Mixed Model ANOVA with 2 between factors (MF and sex) even if this sub-analysis was underpowered. Significant effects of this model were tested with appropriate *post hoc* comparison tests. Postprandial changes in PYY and in appetite ratings were also expressed as the incremental area under the curve (iAUC) calculated by the trapezoid method. One-tailed Pearson's correlations were also used to assess the relationship between variables (PYY and appetite measures). For all statistical tests, alpha was set at $p = .05$ to determine significance of any observed differences. Data in the text and in **Table 3** are displayed as means and standard deviations ($M \pm$ SD), while the Y error bars in all figures are expressed as standard error of the mean (SEM).

Chapter IV - Results

4.1 Participant characteristics

The 16 apparently healthy obese men and women who volunteered for this study were aged 35.4 ± 8.2 yrs (mean ± SD) with a BMI of 35.9 ± 4.2 kg/m^2 and body weight 107.5 kg ± 21.3 at baseline. **Table 3** presents the descriptive characteristics of subjects before and after weight loss according to the frequency group. The TEFQ was administered at initial visit 1 to screen for dietary restraint. The mean results were 8.5±4.8 and 7.2±2.7 for LMF and HMF respectively, indicating both groups were not dietary restraint according to the cut-off point 10 (Stunkard & Messick, 1985). No significant difference was found in the level of restraint between experimental conditions. Age was not significantly different between LMF and HMF. Pre-treatment body weight, BMI, waist circumference, fat mass, % body fat and lean mass levels were higher in the HMF group but not statistically significant. All descriptive statistics for participants were normally distributed, except for some PYY concentrations.

4.2 Anthropometric measures

The mean weight loss for the LMF and HMF group was -5.2kg and -4.5kg respectively of their initial body weight. No effect of the intervention was noted on body weight between experimental conditions ($p > .05$). As expected, a significant effect of time on body weight, fat mass, lean mass, BMI and waist circumference was observed in both groups ($p < .01$) (**Table 3**). A significant intervention-by-gender-by-meal frequency interaction appeared for lean mass ($p < .01$).

Post hoc analyses revealed that male in the HMF group lost significantly more lean mass ($p < .05$) over the intervention (-5.4kg) than all other groups. It should be noted that one male subject in the HMF group was excluded from body composition analysis, as results from DEXA were questionable. Total Body weight loss for this subject as measured with the standard beam scale was 4.9kg. Body composition analysis from the DEXA revealed a fat mass lost of 10.99kg, lean mass gained of 6.79kg and percent fat mass lost of 6.8% compared to the mean fat mass lost of 2.01kg, mean lean mass lost of 3.01kg and mean percent fat mass lost of 0.11% for the remaining 7 subjects in the HMF group. When this subject was included in the body composition analysis, the significant difference was lost ($p > .05$). However, it should be noted that this subject was included for all other analyses. No further significant difference was observed between experimental conditions. **Table 4** presents results pertaining anthropometric measures in response to weight loss among experimental conditions in absolute and relative values.

4.3 Appetite parameters

Visual analogue scale measurements are illustrated in **Figure 7**. As expected, all appetite measures were significantly modulated in the post-prandial period ($p < .05$). However, no significant difference was found in the feeling of hunger, prospective food consumption or desire to eat between experimental conditions ($p > .05$). On the other hand, a significant main effect was observed for the intervention-by-meal frequency interaction ($p < .05$) for fullness. Further analyses were executed to follow-up the interaction at post-treatment, which revealed significantly higher ($p < .05$) levels of fullness at time 120min and at time 300min for the LMF group. As for desire to eat, a significant

interaction was found for intervention-by-gender ($p < .05$) and for iAUC ($p < .05$), which resulted in overall higher levels of desire to eat for male at post-treatment ($p < .05$). At baseline session, mean fullness level at fasting was 19.6±24.2 mm and 36.1±23.7 mm for the LMF and HMF group respectively. Mean fullness peak for the LMF group was 35.8±42.4 minutes and remained elevated for 117.1±47.0 min. As for HMF group mean fullness peak was 20.0±20.9 min and remained elevated for 106.0±54.4 min.

4.4 Peptide YY

Analysis for PYY was performed with twelve participants distributed equally in both conditions. Mean fasting levels at pre-treatment was 303.1 pg/mL and 300.4 pg/mL and at post-treatment 271.0 pg/mL and 277.4 pg/mL in LMF and HMF respectively. No significant difference was found between fasting levels in both experimental conditions at both interventions ($p > .05$). At pre-treatment, peak PYY occurred at 120 min and 60 min (LMF and HMF respectively) and peaked at 120 min at post-treatment in both groups. At peak in pre-treatment, PYY levels increased by (17%) in the LMF group, while an increase of 26.3% was seen for the HMF group from fasting levels. At peak in post-treatment, PYY levels increased by 31.6% and by 63.7 % in LMF and HMF respectively. The increase from fasting levels to peak PYY levels was not statistically different between the 2 groups ($p > .05$). As expected, a significant post-prandial effect of time was noted for both LMF ($p < .01$) and HMF ($P < .01$) (**Figure 8**). No further significant difference was observed between experimental conditions (over 6h and iAUC) ($p > .05$). Fasting PYY was not correlated with fasting subjective ratings of appetite (fullness, hunger, desire to eat and prospective food

consumption) at pre- and post-treatment ($p > .05$). No significant correlation was seen between the iAUC for each appetite parameter and iAUC PYY at both interventions ($p > .05$).

4.5 Resting Energy Expenditure

As mentioned in our methods, REE was not a primary outcome for this study; however because the measurement needed to be taken to determine each participant's energy need, we nonetheless report them herein. As expected there was a significant decrease after the intervention for both experimental conditions ($p < .05$). The extent of the decrease was -6.5±7.0% (111.4 kcal) and -2.5±7.8% (65.3 kcal) for LMF and HMF respectively which was not statistically different ($p > .05$). Interestingly, a significant main effect for the intervention-by-gender-by-MF interaction was noted ($p < .05$). Follow-up analyses showed that female in the HMF group had a significant increase ($p < .05$) in REE (+35.6 kcal) at the end of the intervention as opposed to a decrease as seen in the other groups.

Chapter V - Discussion

Recently, particular interest has been focused on MF, satiety and PYY as therapeutic targets for obesity. Despite considerable research in dietary strategies for weight loss, debate remains on the energy content, meal patterning and the optimal macronutrient distribution for effective weight loss. As previously reviewed, lower EI and body weight are associated with higher MF (Drummond et al., 1998; Farshchi et al., 2005; Speechly et al., 1999); however very weak evidence exists in experimental studies. Termination of a meal depends on the balance between hunger and fullness or satiety following the consumption of a meal (de Graaf et al., 2004; Nicolaidis & Even, 1985). Many hormonal signals are generated during a meal, which some are known to contribute to satiety including PYY. However, to our knowledge, no study has adequately assessed whether increasing MF leads to enhanced compliance to the dietary restriction and over time to greater weight loss through a less pronounced increased in hunger, desire to eat, prospective food consumption, and higher levels of fullness. We therefore investigated the effect of high MF on body weight loss, appetite regulation and on the profile of PYY in apparently healthy obese men and women. We hypothesized that: (a) a greater weight loss will be observed in the HMF group because of higher levels of fullness and therefore greater compliance to the energy restriction; (b) higher daily levels of fullness will be observed in the HMF group likely because of higher levels of PYY throughout the day; (c) higher daily PYY levels will be observed in the HMF group likely because of shorter period of time between each eating occasion. It is important to note that the main purpose of this study was to compare the effect of the treatment between the two experimental conditions (LMF and HMF); however because gender was randomized equally in both experimental arms, we decided to further

our analyses and therefore compare between gender as well even though the analysis was underpowered.

The majority of studies on the effect of MF on body weight are of epidemiological type, where most observed an inverse relationship between the number of meals consumed in a day and the degree of adiposity. In other words, eating smaller quantities more frequently was associated with a decrease in body weight (Bellisle et al., 1997; Drummond et al., 1998; Metzner et al., 1977; Speechly & Buffenstein, 1999; Summerbell et al., 1996). Very few experimental studies have been done on the effect of MF on body weight loss through energy restriction. Most were performed in the 1970's and 1980's (Bellisle et al., 1997) where many methodological aspects were unclear and their main purposes different from ours. Among these studies only 2 had an elaborate description of their protocol (Garrow et al., 1981; Verboeket-van de Venne & Westerterp, 1993) from which only one had the main purpose of investigating the rate of weight loss under different feeding frequency (Verboeket-van de Venne & Westerterp, 1993). Results reported in this study showed that, after a comparable body weight loss, that is -5.2kg and -4.5kg of initial body weight in LMF and HMF group respectively: (1) men in the HMF group lost significantly more lean mass when compared to the other groups; (2) male had significant higher overall levels of desire to eat at post-treatment; (3) the LMF group had significant higher levels of fullness at time 120min and 300min at post-treatment; (4) PYY concentrations were similar between conditions over the experiment. These results refute all of our study hypotheses.

5.1 Body Weight

Body weight was reduced in a similar fashion in response to the treatment (-5.4% and -3.9% in both LMF and HMF respectively). As expected, a significant effect of time on body weight, FM, lean mass, BMI and waist circumference was observed in both experimental conditions ($p < .01$) (**Table 3**). Interestingly, we noted that men in the HMF group lost more lean mass than the other groups. Since very few studies have been done on the effect of MF on body weight, it is difficult to explain why men in the HMF group were the ones to lose more lean mass over the study. Our findings are similar to those of Verboeket-van de venne & Westerterp (1993) and Garrow et al. (1981), in the way that higher MF does not seem to affect the rate of weight loss. In Verboeket-van de venne & Westerterp (1993), the experiment was only performed on obese women under an energy restriction of 1000 kcal/day for 4 weeks. Seven subjects were on 2 meals/day and seven others were on 3-5 meals/day. Their final results showed no significant difference in weight loss, FM and in FFM (Verboeket-van de Venne & Westerterp, 1993). As for Garrow et al. (1981), they recruited 35 women and 3 men which had to follow an 800 kcal/diet for 3 weeks. This cross-over design were comparing different amount of protein combined with different frequency of eating being 1 meal/day or 3 meals/day or 5 meals/day. No evidence was found that higher MF could affect fat loss, but subjects find it more tolerable to follow the energy restriction while distributed in many short meals (Garrow et al., 1981).

It is possible that we may have underestimated the time needed to observe any significant differences in anthropometric measures between experimental conditions. Most weight loss studies are performed between 12-15 weeks (Brinkworth et al., 2004; Doucet et al., 2000; Doucet et al.,

2001; Doucet et al., 2002; Farnsworth et al., 2003; Wadden, 1993) while others are performed over 6 months (Skov, Toubro, Bulow et al., 1999; Skov, Toubro, Ronn et al., 1999). Even though we were limited on time as this research was a master's project, the duration was twice as long in our study when compared to the 2 previous studies on the effect of rate loss among different frequency of feeding. Nonetheless, our results suggest no significant effect of MF on body weight loss contrary to what was stated in our first hypothesis. Another possible reason why we may not have observed any significant differences in body weight across conditions could be attributed to the fact that the study was carried out in a free-living setting and subjects were assumed to be compliant with their meal plan. We initially thought that increasing MF could have nonetheless helped subjects being more compliant in the HMF group. While following the participants in their weight loss process, we are fully aware that compliance was far from being perfect. A number of subjects in the HMF group verbally reported to be difficult to be compliant with meal timing and frequency because of hectic schedule interfering with meal and snack planning. On the other hand, some participants in the LMF group verbally reported to feel hungry between main meals and tended occasionally to ingest snacks which were extra calories not calculated in the initial meal plan.

5.2 Appetite

The main reason why we stated in our first hypothesis that a greater weight loss will be observed in the HMF group is because of perhaps higher levels of fullness associated with snacks and therefore greater compliance to the energy restriction over time. In epidemiological associations between habitual MF and appetite regulation, it seems that EI and hunger in obese individuals are

lower when following a regular meal pattern with higher frequency than during an irregular meal pattern with lower frequency (Drummond et al., 1998; Farshchi et al., 2005; Speechly et al., 1999; Westerterp-Plantenga et al., 2002). We did not measure EI for this study since dietary records are often unreliable in most obese subjects, especially when given a prescribed diet (Bellisle et al., 1997; Farshchi et al., 2005; Summerbell et al., 1996). However, we did measure the four appetite parameters and unexpectedly found higher levels of fullness at time 120min and 300min for the LMF group at post-treatment (**Figure 7**). Lunch during the experiment was served at time 240min for all subjects and snack for the HMF group was served on average at 133.9min with a mean caloric content of 202.8±49.1kcal. The assumption put forward is that the LMF group responded better to the amount of calories served at breakfast and at lunch as compared to the HMF group. Lunch was composed of 557.1±210.4kcal and 365.4±136.1kcal and breakfast was composed of 698.7±122.6 kcal and 561.3±166.4 kcal in the LMF and HMF group, respectively. Even if a snack was served before lunch in the HMF group, this was not sufficient to cause a marked increase in satiety. We therefore concluded that since breakfast and lunch were higher in calories in the LMF group, the effect of fullness lasted longer. We also noted that men had higher overall levels of desire to eat after the intervention, which may be placing men at risk for increase EI and therefore increase weight gain in the long term. However, fullness is the appetite sensation the most strongly associated with total EI and the most useful to predict long-term EI (Drapeau et al., 2005). Similar results were observed in a body weight loss study conducted by Doucet et al. (2000); however both men and women had higher levels of desire to eat after the intervention which was correlated to changes in fasting plasma cortisol in men (Doucet et al., 2000). Statistical analysis showed that the increase between fasting and peak fullness level was not significant after the standardized snack test meal of 300 kcal. In

other words, if there was no difference in the feeling of fullness after a snack of 300 kcal, having a snack between meals of that caloric content would make no difference on appetite. The only study that has examined the effect of similar calories on fullness was performed by le Roux et al. (2006). However, they only show fullness score at 180 min after the consumption of the meal and they do not mention if the difference between fasting level is significant from the peak(le Roux et al., 2006). Hence, we need more information on the effect of different amount of calories on fullness to better adjust meal timing and frequency for future studies.

5.3 Energy Compensation

According to Drummond et al. (1998), energy compensation varies among individuals and with different levels of dietary restraint, with unrestrained male being better compensators than unrestrained females by reducing the size of subsequent eating episodes and therefore adjusting EI when submitted to higher MF (Drummond et al., 1998). Therefore, if women are known to have more difficulty than men to adjust their EI when submitted to higher MF; women might have responded better to the 3 meals/day and as a result favoring the LMF group to lose more weight, even if no such difference was noted after the intervention. Longer studies with more subjects are needed to confirm this postulation.

Appetite is a complex phenomenon arising from sequence of interactions among peripheral, central mechanisms and several other complex factors and therefore very difficult to predict (Cameron & Doucet, 2006b; Jequier & Tappy, 1999). Hunger drives the search for and the ingestion of food. Termination of food intake occurs when hunger is suppressed, which is call satiation often determined by the meal size. The absence of hunger between meals is referred to satiety.

Termination of the period of satiety trigger the feeling of hunger, which leads to food intake (Blundell et al., 1996; de Graaf et al., 2004). Even though we tried as much as possible to control the appetite, we are fully aware that humans are easily influenced by their environment and tend to eat for other reasons than just to meet their energy needs. In addition, because the study was done in a free-living context, it is extremely possible that external factors took over the satiety signals, ultimately influencing food intake and compliance to the meal plan. It is evident that food intake is not always the results of hunger. Many situations such as social activities may lead to food or drink consumption (De Castro, 1997; Jequier & Tappy, 1999).

5.4 Peptide YY

Our third hypothesis, which stated that higher daily PYY levels will be observed in the HMF group, likely because of shorter period of time between each eating occasion, was also rejected. Satiety signals from the GI tract that limit meal size are secreted in proportion to ingested calories and accumulate during a meal, ultimately providing an integrated signal and causing eating to stop (Havel, 2001). It has been suggested that the gut hormone PYY suppresses appetite not only by the ileal break mechanism (Korner & Leibel, 2003) but may also be a modulator of central satiety signal (Batterham et al., 2002; Gale, Castracane, & Mantzoros, 2004). Our results differs with previous literature which demonstrate a positive relationship between administration of pharmacologic PYY concentrations (Batterham et al., 2002; Batterham et al., 2006) and endogenous PYY, and satiety (Batterham et al., 2002; Batterham et al., 2006; Guo et al., 2006). Neither fasting PYY concentrations nor the post-prandial PYY responses (over 6 h and iAUC) were associated with

fasting and post-prandial satiety (over 6 h and iAUC). Despite the fact that we found higher levels of fullness at time 120min and 300min for the LMF group at post-treatment (**Figure 7**), we did not observe the same outcome for PYY concentrations. In contrast, PYY levels (at pre-treatment) increased by 8.8% and by 26.3% (LMF and HMF respectively) 60 min after the consumption of breakfast and increased by 18.9% and 24.7% 60 min after the consumption of lunch. As for the post-treatment session, PYY increased by 28.3% and by 48% (LMF and HMF respectively) 60 min after the consumption of breakfast and by 9.2% and decreased by 8.3% 60 min after the consumption of lunch. Interestingly, at breakfast in both interventions and at lunch at pre-treatment only, the HMF group had a higher increase in PYY even though the amount of calories ingested was lower compared to the LMF group (amount of calories consumed previously reported in discussion). Moreover, the snack served before lunch was not sufficient to cause a marked increase in PYY levels; nevertheless it seemed to have maintained PYY levels in the HMF group especially at post-treatment (**Figure 8**). The temporal pattern of PYY in this study is difficult to explain. In experimental studies, the temporal pattern of PYY is usually studied over 3 h (Batterham et al., 2006; Fu-Cheng et al., 1995; Guo et al., 2006; le Roux et al., 2006) with only one recent study that have gone up to 8 h, but this was done in mice (Batterham et al., 2006). In most studies, PYY levels remained above or equal to fasting level, conversely to what was found at pre-treatment in this project. However, this was not the case at post-treatment, where PYY remained above fasting level for as much as 6 h (**Figure 8**).

Furthermore, we did not observe any significant difference in post-prandial PYY concentrations between experimental conditions over the weight loss program. It is known that PYY

is secreted in proportion to caloric intake (le Roux et al., 2006; Stock et al., 2005). By looking at **Figure 7** and **8**, we can notice a similar increase in fullness and PYY levels after breakfast and lunch in both experimental conditions. However, after the consumption of the snack (mean = 133.9min), we noted an increase in fullness levels (NS), which was not observed for PYY. Indeed, the caloric content of the snack was not enough to make a significant difference in PYY levels, especially when we know that obese individuals have reduced plasma PYY levels and attenuated PYY response (Batterham et al., 2003; Guo et al., 2006; le Roux et al., 2006; Stock et al., 2005). In fact, overweight and obese individuals require approximately 225 kcal more than normal weight individuals to reach maximum satiety (Delgado-Aros et al., 2004). Findings suggest that lower endogenous postprandial PYY levels may be related to reduced satiety (le Roux et al., 2006). We were aware that a single satiating signal such as PYY was unlikely to entirely account for compliance to a weight loss regimen. Indeed, some have postulated that PYY is responsible for approximately 10% of the satiating signal, therefore it may be unreasonable to rely only on PYY when we know that other satiety signals resulting from a meal exist (cholecystokinin, amylin, GLP-1, nutrient signals, distention signals, etc.) (Young, 2006). However, it should be noted that in some surgery studies, the pronounced post-prandial secretion of PYY has been postulated to be the main contributing factor to increased appetite control and success of this weight loss intervention (Chan, Mun, Stoyneva, Mantzoros, & Goldfine, 2006; Korner et al., 2005). Satiety deficit reported by obese individuals does not only derive from attenuated PYY levels and thus appears more likely that low plasma PYY is a consequence rather than a cause of obesity (le Roux et al., 2006; Young, 2006). Whether it is possible to structure food intake and meal patterning in a way that could favorably alter endogenous PYY (3-36) to regulate satiety and body weight in humans still remains to be investigated.

In summary, our three hypotheses were rejected. Contrary to what was hypothesized, the HMF group did not lose more weight than the LMF group, suggesting that increasing MF under conditions of equicaloric energy restriction does not increase weight loss. Similarly, no favorable effects of increased MF on appetite and PYY levels were noted. We are unable to conclude from our study that increasing MF while following a weight loss regimen will lead to greater weight loss when compared to lower frequency.

General conclusions and Perspectives

6.1 Conclusions

In conclusion, it is apparent in this study that HMF does not affect changes in body weight, appetite and PYY levels differently from LMF in apparently healthy obese men and women under caloric restriction. These results are contrary to what was originally hypothesized, which was higher MF would favor greater weight loss, lesser increase in appetite and higher levels of PYY. It is still unclear if whether smaller and more frequent meals could benefit weight loss while promoting a healthy lifestyle. Scientific investigations that have been done on the effect of MF on body weight loss have resulted in mixed conclusions. In order to obtain clear results, the number of meals we eat in a day needs to be subjected to rigorous scientific control. Until then, no clear recommendations relating to MF can be put forward. In addition, more studies are needed to understand the role of endogenous PYY in the regulation of appetite and energy balance in humans. The fact that obese individuals have lower endogenous postprandial PYY levels which may relate to reduce satiety make them more vulnerable to relapse and therefore results in a vicious cycle.

6.2 Recommendations and Future Perspectives

In retrospect, there are undoubtedly some limitations in our study. One is that the standardized snack test meal to determine meal timing was based on a standard amount of calories (300 kcal) instead being calculated on the basis of body weight. If we would to start this study over, prior to the beginning of the intervention, a pilot study would have been of great help to determine the effect of different amount of calories on PYY and satiety levels. For example, testing the effect of realistic caloric content snack (100 kcal, 200 kcal, 300kcal), to see if this study could in fact make

a significant difference in the outcome measures. However, this would have required more funds and might have been time consuming for a masters project. The standardized snack test meal was not appropriate for everyone. Given that we used the fullness response following the intake of 300kcal to determine meal timing for the HMF group, it was clearly not reflective of individual caloric intake. Nonetheless, this standardized snack test meal was based on results available in the literature that had showed this amount of calories to elicit an increase in fullness and PYY secretion (le Roux et al., 2006). However, customized standard snack test meal for each individual would have been more accurate to determine everyone's meal timing. Additionally, one thing we could do differently is not to follow the CDA recommendations to stimulate satiety and PYY. Instead of 3 snacks and 3 meals daily, we could replace snacks by meals; that is 6 small meals daily. By having the same distribution of calories at every meal, it could favor the maintenance of PYY and ultimately increase satiety. Another strategy to consider in the future would be to start the energy restriction at a lower rate and increase the restriction in time to have a gradual change in appetite instead of reducing energy consumption by 700 kcal from the beginning. The downside to this method is the time frame; as the energy deficit would increase gradually, it would take longer to reach goals.

In addition, even though many efforts were concentrated on explaining to participants how important it was to follow the protocol, a few were not 100% compliant. Future studies may reconsider the educational method and tools used in this study in order to maximize compliance. For example, some suggested using a card system to identify food categories, pictures for portions and ideas for recipes.

As a final point, the power of statistical analyses in this study was somewhat weak with a large variation resulting from 10-90%. A larger sample size might result in different conclusions. However, we cannot ignore the fact that if the study would have been extended over a longer period of time, we might after all have observed a difference in the outcome variables with only eight subjects in each group. Therefore, it may be relevant for future related studies to be pursued over a longer period of time.

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Statement of contribution of collaborators

Marie-Josée Cyr

I am the first author of my thesis and work associated with completing the requirements for the Master's of Sciences program at the University of Ottawa. I participated in all of the data collection processes such as participant recruitment, pre-screening, initial sessions, experimental sessions and the overall coordination of the study. I was the Registered Dietitian of the study who designed the meal plan and did the nutritional sessions such as education, recommendations and follow-ups. I was further responsible of writing and preparing all proper documents for the Research Ethics Board concerning amendments to protocols. I wrote an application and obtained additional funding from the Consortium National de Formation en Santé (CNFS), which covered the foods needed for this study. In addition, I was responsible for the all data entry, data and statistical analyses, interpretation of results, and writing the thesis. I plan on writing an article as first author, and submitting it for publication.

Dr Eric Doucet

Dr Eric Doucet is my Master's Thesis Supervisor. He guided me in developing my research proposal. He was involved in many aspects of this study including the revision of all documents sent to the Research Ethics Board and he helped in the interpretation of the results, and in revising the final thesis. He further helped me write an abstract for submission to the European Congress of Obesity (ECO) for April 2007. He was my main source of knowledge and expertise throughout this study.

Table 1

Summary of studies comparing weight loss on energy restricted nibbling v. gorging. Figure from (Bellisle et al., 1997)

Reference	Subjects	Weight-loss regimen	Meal pattern (no. of meals daily)	Results	Statistical significance
Bortz *et al.* (1966)	Six women: 19–56 years Obese	2·5 MJ/d for 60 d (20 d on each meal pattern)	Three One Nine	Not listed* – 0·23 kg/d – 0·24 kg/d	NS
Finkelstein & Fryer (1971)	Eight women†: 20–22 years BMI 27 kg/m^2	7·1 MJ/d for 30 d followed by 5·9 MJ/d for 30 d	Three Six	– 6·1 kg – 5·5 kg	NS
Young *et al.* (1971)	Eleven men: 20–25 years BMI 34 kg/m^2	7·5 MJ/d for 14 weeks‡	One, three and six	Frequency effect (greater *v.* lesser) – 0·32 kg BW – 0·12 kg FM	 NS NS
Debry *et al.* (1973)	Eight men, twenty-four women: 16–65 years 120–220 % IBW	5·0–7·5 MJ/d§ 42 % energy from carbohydrate	Three Seven	– 78 g/d	$P < 0·025$ N > G
Debry *et al.* (1973)	Eight men, twenty-eight women: 16–65 years 120–220 % IBW	5·0–7·5 MJ/d§ 16 % energy from carbohydrate	Three ‖ Seven Seven Three	Rp 0·238 Rp 0·220 Rp 0·461 Rp 0·188	NS $P < 0·025$ N > G
Garrow *et al.* (1981)	Fourteen women: 18–56 years BMI 38 kg/m^2	3·4 MJ/d for 3 weeks¶	One Five	– 255 (SE 29) g/d – 224 (SE 20) g/d	NS
Verboeket-van de Venne & Westerterp (1993)	Fourteen women**: 20–58 years BMI 30 kg/m^2	4·2 MJ/d for 4 weeks	Two†† Three to five Two Three to five Two Three to five	– 4·1 kg BW – 4·7 kg BW – 2·3 kg FM – 2·7 kg FM – 1·8 kg FFM – 2·0 kg FFM	 NS NS NS

N, nibbling; G, gorging; BW, body weight; FM, fat mass; FFM, fat-free mass; IBW, ideal body weight; Rp, change in BW expressed as % IBW.

* Graphical summaries of each subject's weight curve shows no difference.

† Four subjects followed each meal pattern.

‡ After 14 d run-in on three meals daily subjects were randomly assigned to one, three or six meals daily for 5 weeks then randomly reassigned to these meal patterns for a further 5 weeks.

§ Adjusted to each subject's 'needs'. Three meal schedule was used for first month of diet, followed by seven-meal schedule for second month.

‖ Seventeen subjects had 1 month with three meals followed by 1 month with seven meals; nineteen subjects had order reversed.

¶ Meal frequency effects were tested for 7 d each during weeks 2 and 3 in randomized order.

** Seven subjects assigned to each meal pattern.

†† Two meals, 1·7 MJ lunch, 2·5 MJ supper; three to five meals, 1·25 MJ breakfast, 1·25 MJ lunch, 1·7 MJ supper with flexibility to use some lunch or supper as snacks.

Table 2

List of foods available for the study

Food Groups	List of foods
Starch	Whole wheat bread
	Crackers (wheat thin)
Fruits and Vegetables	Orange juice-100% juice
	Carrots
	Apple
	Dry raisins
Milk Products	Milk 2% MF
	Yogurt 1.5 % MF
Protein	Sliced turkey (not deli)
	Peanut butter (no sugar added)
	Cheddar cheese 19% MF
Fat	Miracle whip-calorie wise
	Margarine - non-hydrogenated

* Quantities of foods were adapted for each participant according to their energy prescription.

Table 3

Subject's characteristics before and after weight loss

Variables	Before wt loss (pre)				After wt loss (post)						
	LMF	Range	HMF	Range	LMF	Range	HMF	Range	Meal frequency	Time	Meal frequency x Time
Age (yr)[a]	36.3 ± 7.4	18	34.6 ± 9.5	31	-		-		NS	-	-
Wt (kg)	100.9 ± 16.6	44.7	114.0 ± 24.6	68.4	95.6 ± 17.4	46.4	109.5 ± 23.5	67.5	NS	***	NS
BMI (kg/m2)	34.7 ± 3.9	10.8	37.0 ± 4.5	13.4	32.9 ± 4.5	12.4	35.6 ± 4.6	13.6	NS	***	NS
Waist circumference (cm)	111.2 ± 12.3	34	119.7 ± 15.1	44.7	103.7 ± 12.5	32.5	113.0 ± 16.6	52.5	NS	***	NS
Fat mass (kg)[b,c]	38.3 ± 5.6	17.1	46.7 ± 10.5	29.8	35.2 ± 7.4	21.8	44.7 ± 10.3	29.3	NS	***	NS
Lean mass (kg)[b,c]	58.3 ± 13.6	35.4	61.5 ± 18.5	47.6	56.2 ± 13.8	35.7	58.5 ± 17.0	45.7	NS	***	NS
% Body fat[b,c]	38.9 ± 5.3	12.5	42.3 ± 5.3	16.6	37.7 ± 6.6	15.9	42.2 ± 5.1	15.4	NS	NS	NS

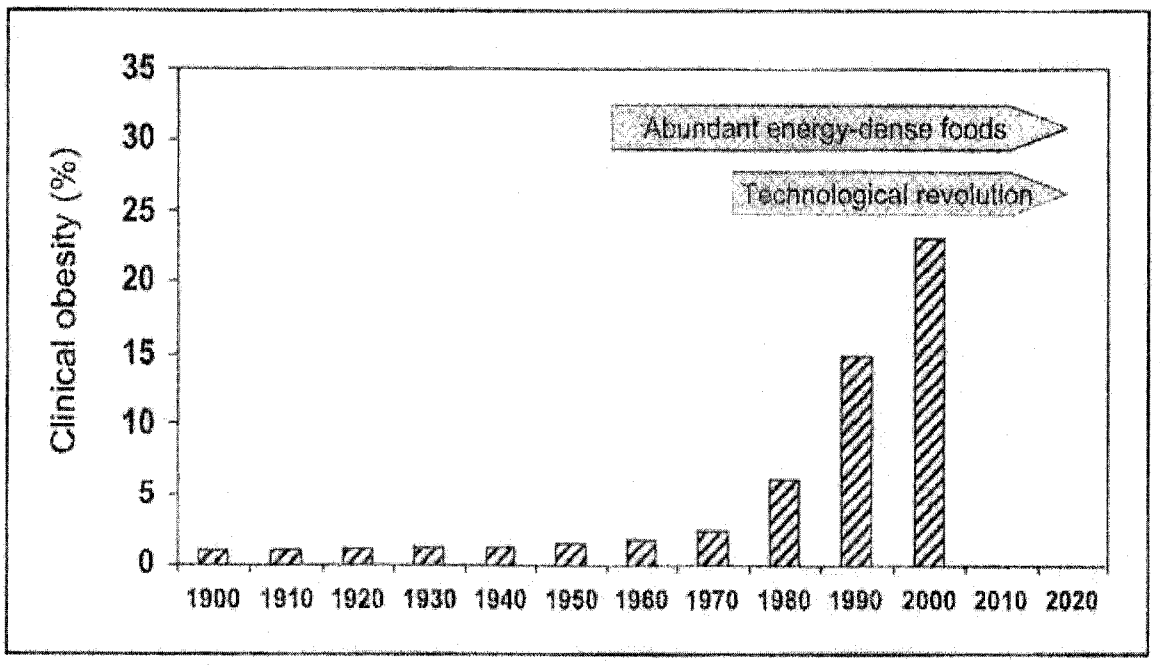
Note. Values are the means±SD; n= 4 men and 4 women in LMF and n= 4men and 4 women in HMF
*, **, *** Significant effect of meal frequency, time or their interaction $p<0.05$, $P<0.01$ and $p<0.001$ respectively
Time: pre- and post-treatment
NS, not significant
[a]Age at the onset of the program
[b] n= 8 LMF, n= 7 HMF. Missing values in HMF group
[C] Body weight of one subject in HMF >300 lbs, therefore body was scanned in two. Fat and lean mass from left and right sides were added. % fat mass calculated from mean of left and right sides.

Table 4

Reduction of anthropometric measures in absolute and relative values

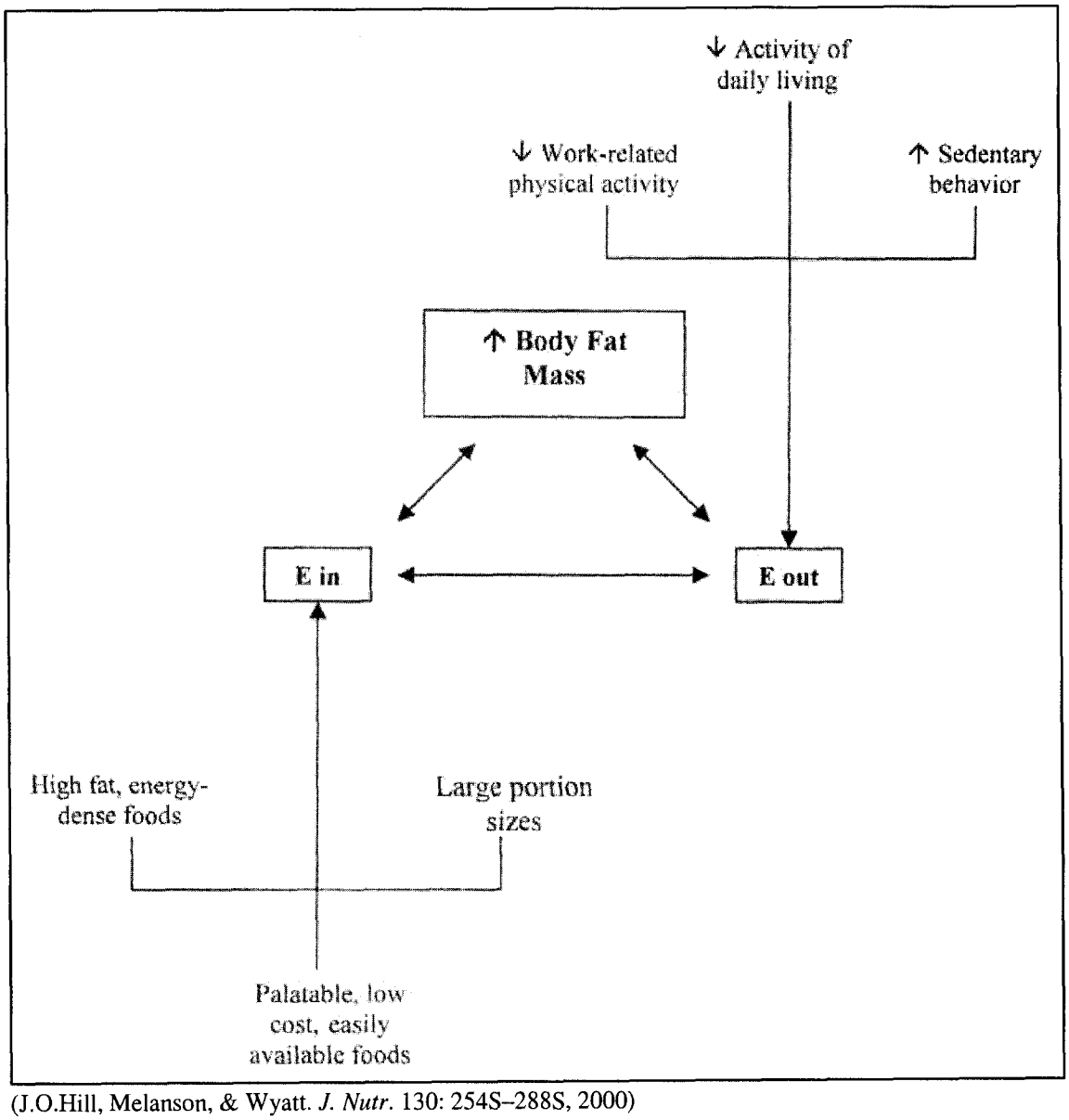
Variables	LMF				HMF			
	Absolute changes	Range	Relative changes (%)	Range	Absolute changes	Range	Relative changes (%)	Range
BMI (kg/m2)	1.87±0.9	2.9	5.55±0.9	2.9	1.48±0.7	1.9	4.07±0.7	1.9
WC (cm)	7.51±3.5	8.9	6.78±3.1	6.5	6.72±3.7	10.6	5.79±3.4	8.9
% Fat mass	1.28±2.1	6.2	3.66±5.9	17.5	0.11±2.6	8.4	0.14±5.9	19
Fat mass (kg)	3.08±2.5	8.5	8.69±7.5	24.3	2.01±3.5	10.6	4.46±5.6	16.5
Lean mass (kg)	2.18±1.7	4.8	3.93±3.3	10.5	3.01±4.2	14.1	4.50±6.0	20.7
Body weight (kg)	5.27±3.0	10.1	5.41±3.1	9.8	4.55±2.4	6.6	3.97±2.0	5.8

Note. Values are the means±SD; n= 4 men and 4 women in LMF and n= 4men and 4 women in HMF
*BMI: body mass index; WC: waist circumference



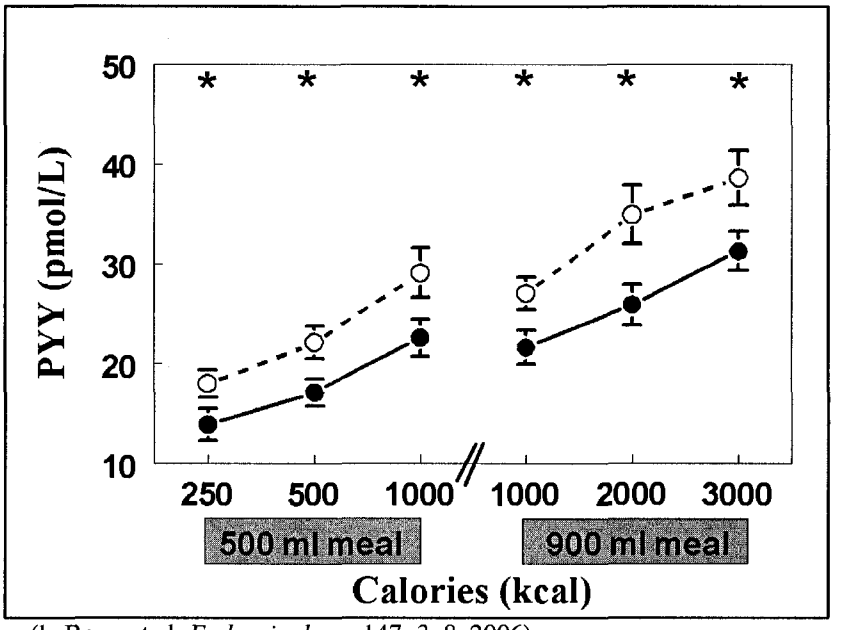
(Prentice & Jebb. *Nutr Rev*. 62: S98–104, 2004)

Figure 1. Rapid ecologic change in the late 20th century as the prime cause of obesity.



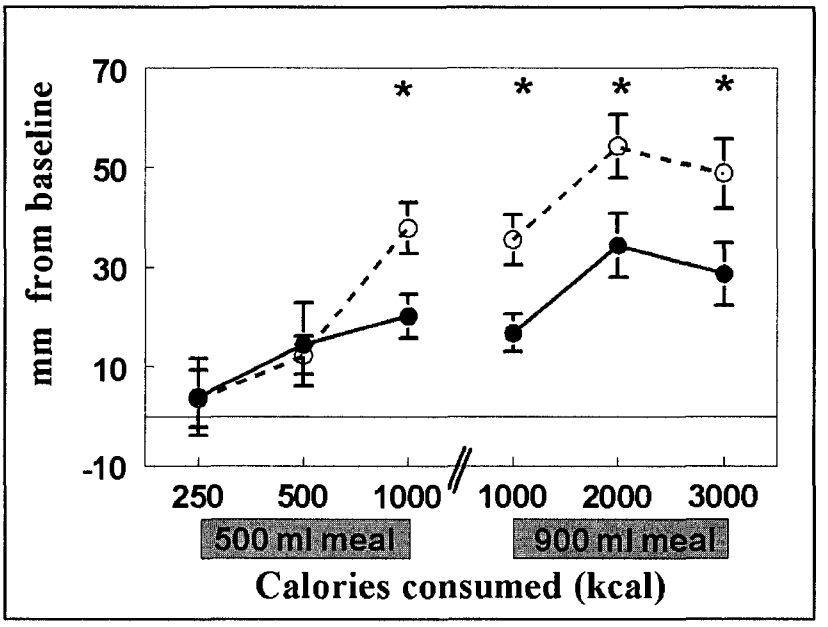
(J.O.Hill, Melanson, & Wyatt. *J. Nutr.* 130: 254S–288S, 2000)

Figure 2. The effect of environmental factors on energy balance. Figure adapted from (J. O. Hill, Melanson, & Wyatt, 2000).



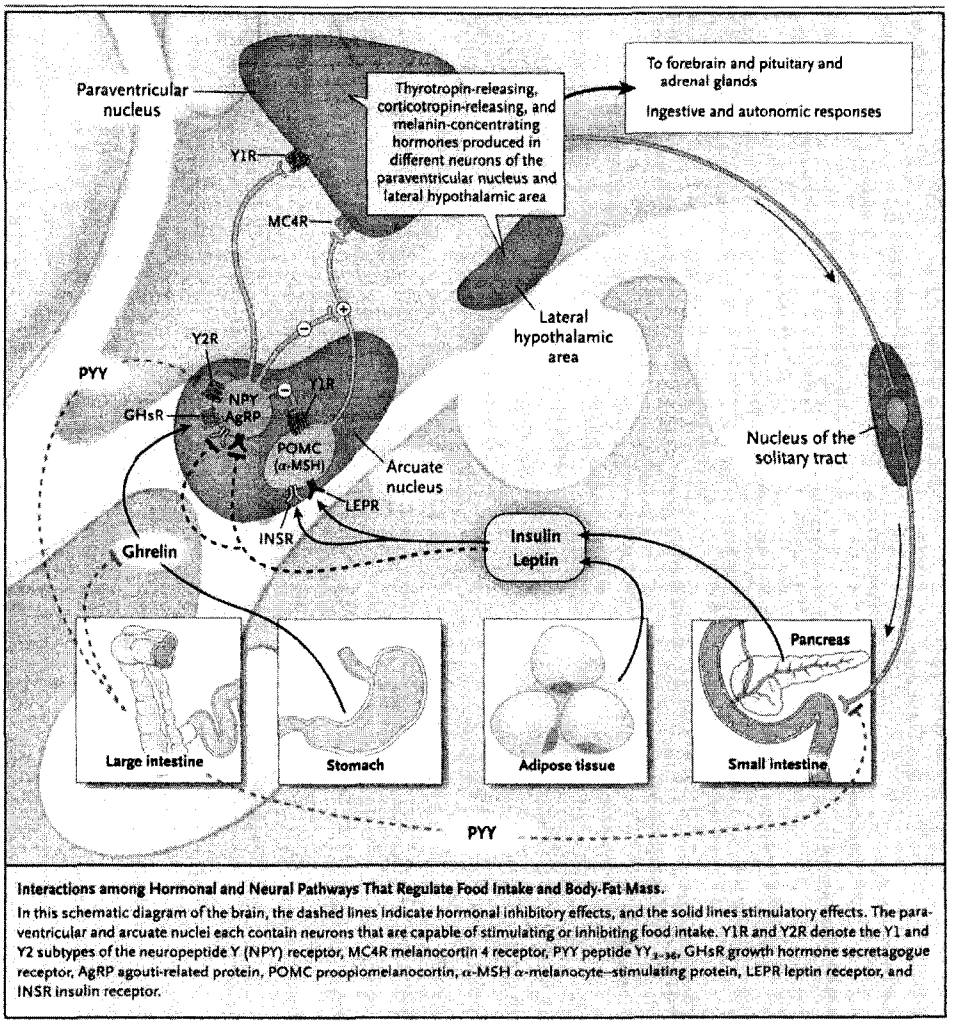
(le Roux et al. *Endocrinology*. 147: 3–8, 2006)

Figure 3. Demonstrates the peak PYY levels in obese (•) and normal weight (O) subjects at 90 minutes following the meal.



(le Roux et al. *Endocrinology*. 147: 3–8, 2006)

Figure 4. Shows the fullness score at 180 minutes after the meal, measured by VAS in the obese (•) and normal weight (O) subjects.



Interactions among Hormonal and Neural Pathways That Regulate Food Intake and Body-Fat Mass.

In this schematic diagram of the brain, the dashed lines indicate hormonal inhibitory effects, and the solid lines stimulatory effects. The paraventricular and arcuate nuclei each contain neurons that are capable of stimulating or inhibiting food intake. Y1R and Y2R denote the Y1 and Y2 subtypes of the neuropeptide Y (NPY) receptor, MC4R melanocortin 4 receptor, PYY peptide PYY_{3-36}, GHsR growth hormone secretagogue receptor, AgRP agouti-related protein, POMC proopiomelanocortin, α-MSH α-melanocyte–stimulating protein, LEPR leptin receptor, and INSR insulin receptor.

(Korner and Leibel. *N.Engl.J.Med.*. 349: 926–928, 2003)

Figure 5. Interactions among Hormonal and Neural Pathways that Regulate Food Intake and Body-Fat Mass.

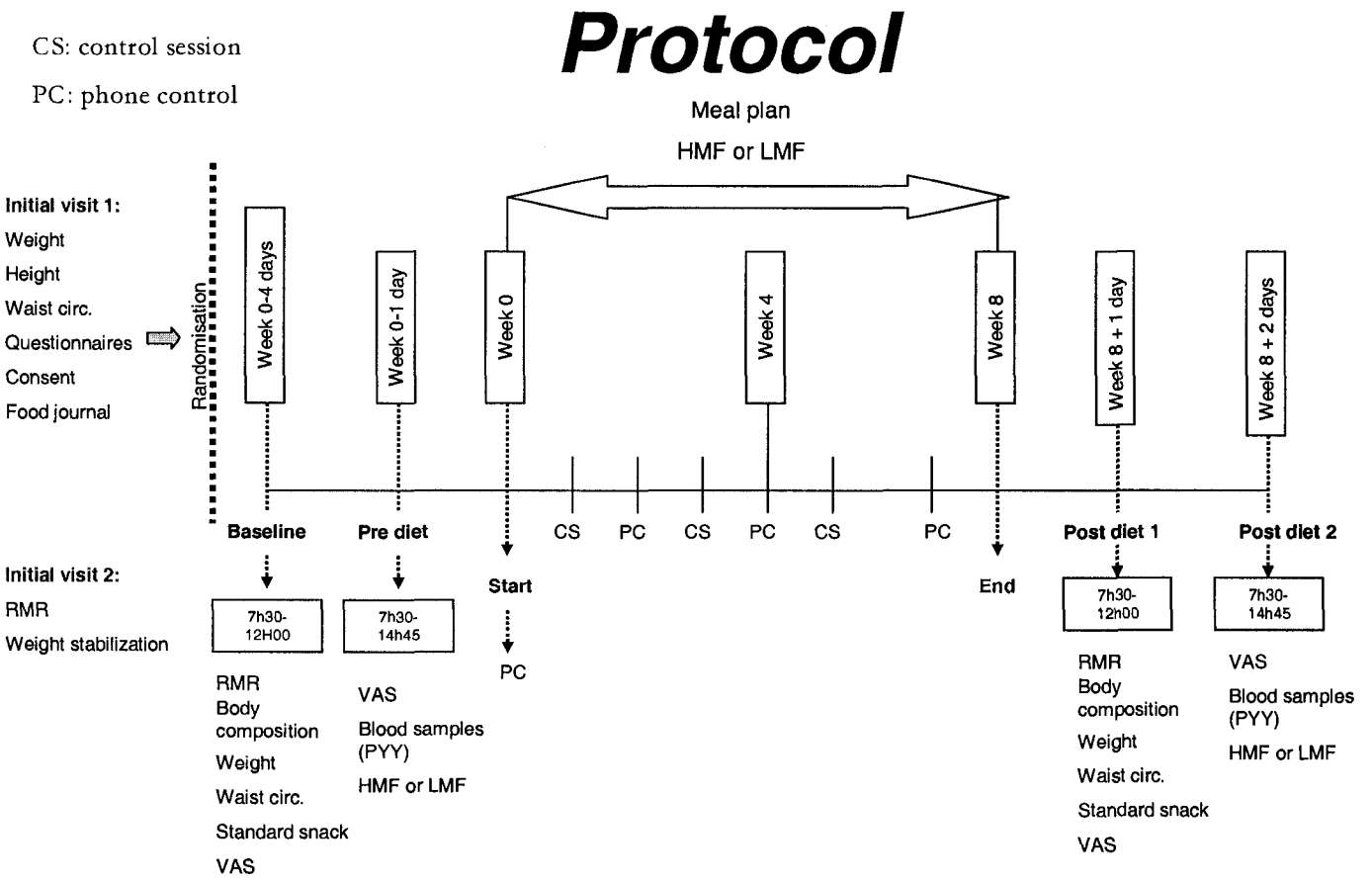
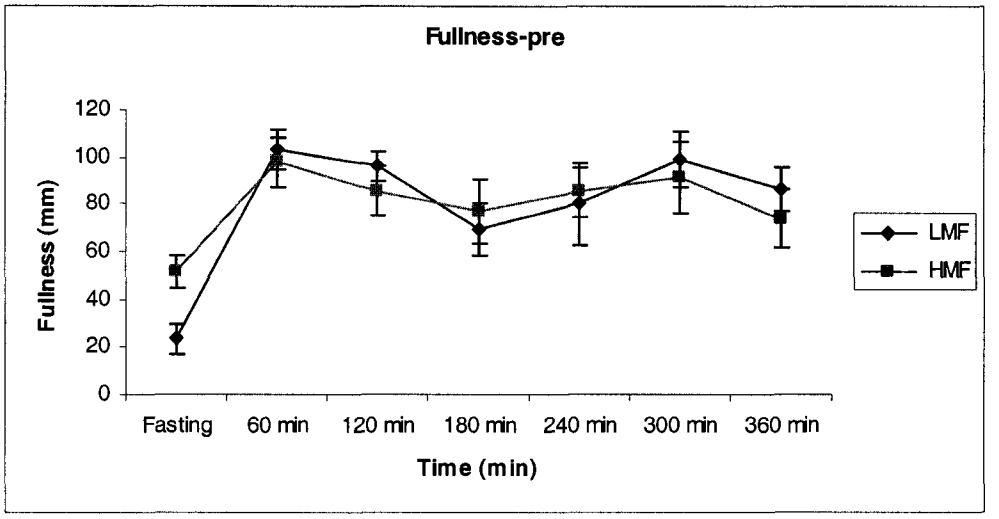


Figure 6. Scheme of the protocol.


Fullness-pre
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Fullness (mm)
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Time (min)
LMF
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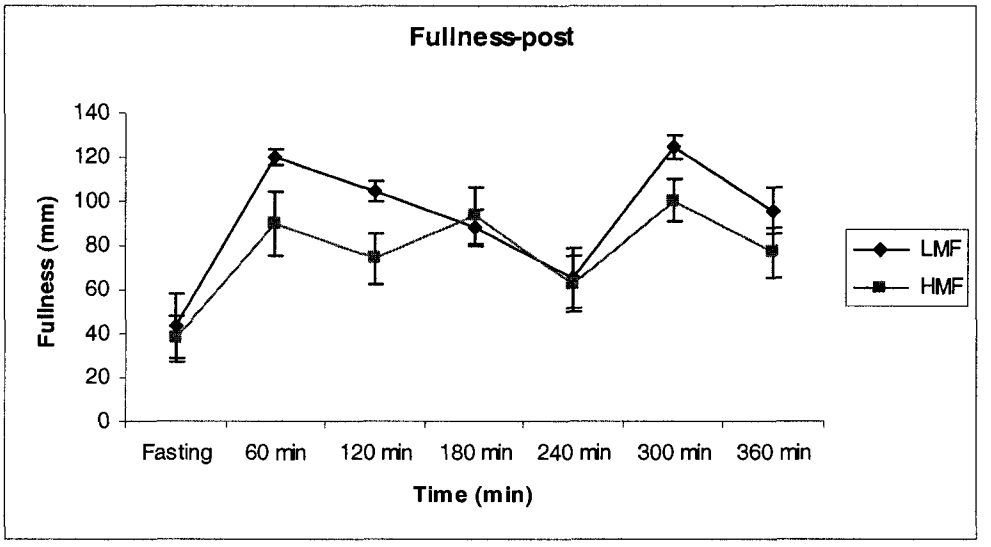
Fullness-post
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Fullness (mm)
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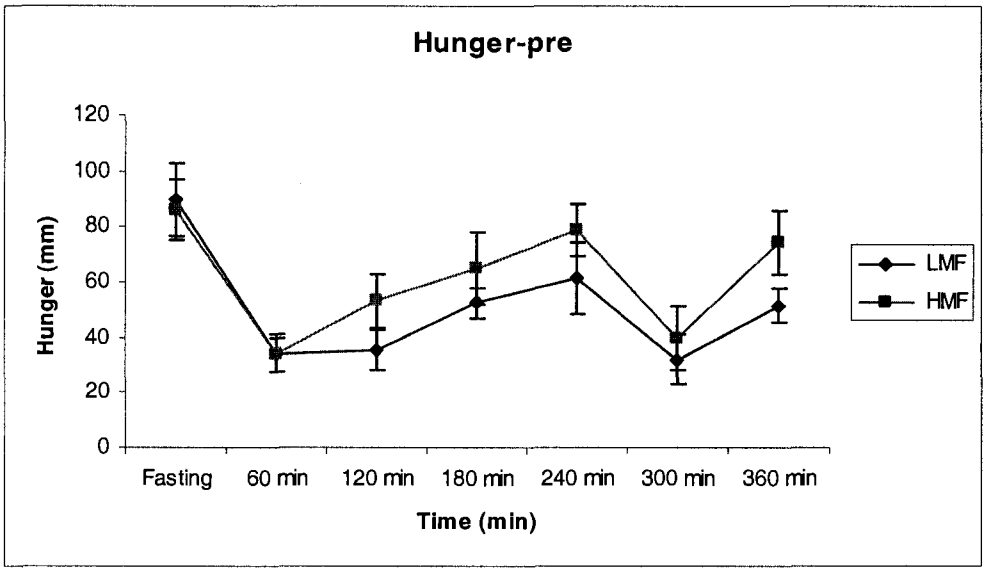
360 min
Time (min)
LMF
HMF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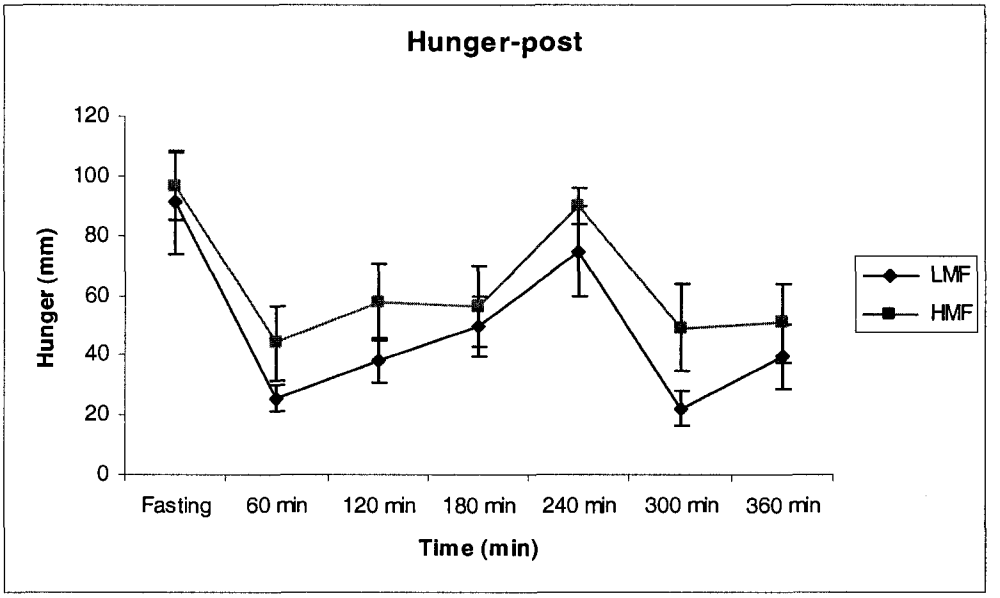
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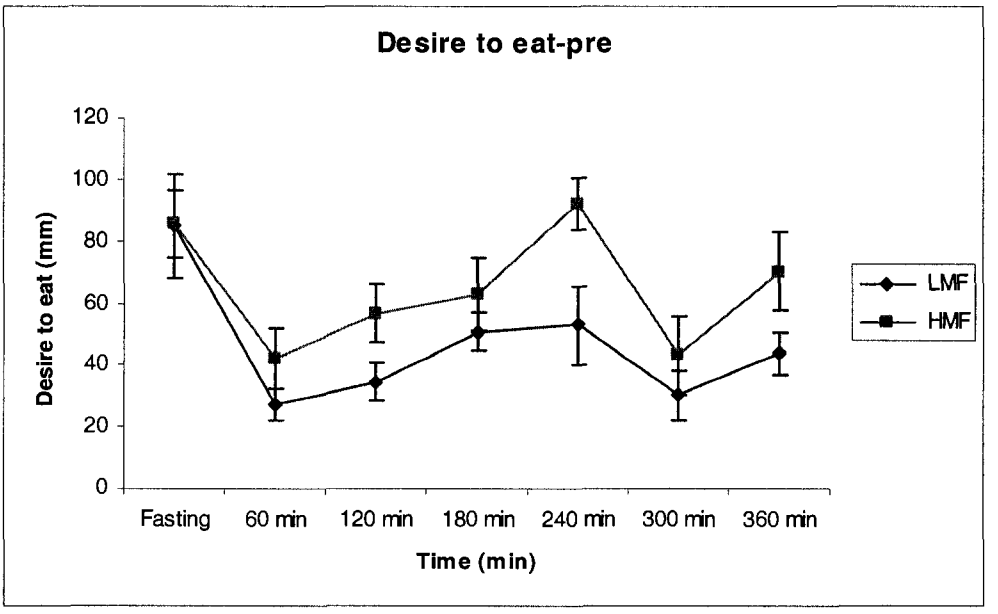
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Desire to eat (mm)
Fasting
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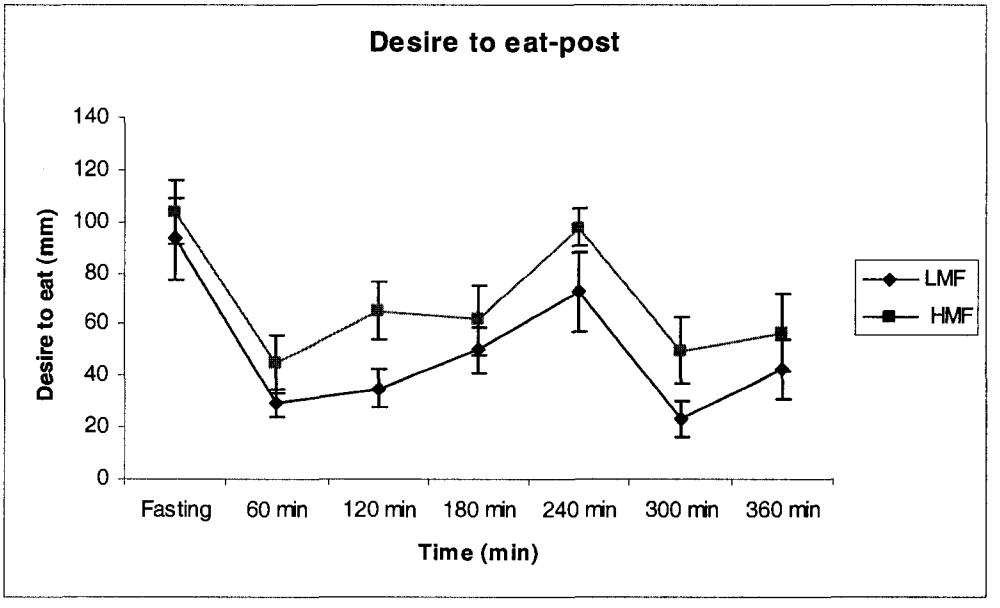
360 min
Time (min)
LMF
HMF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Desire to eat (mm)
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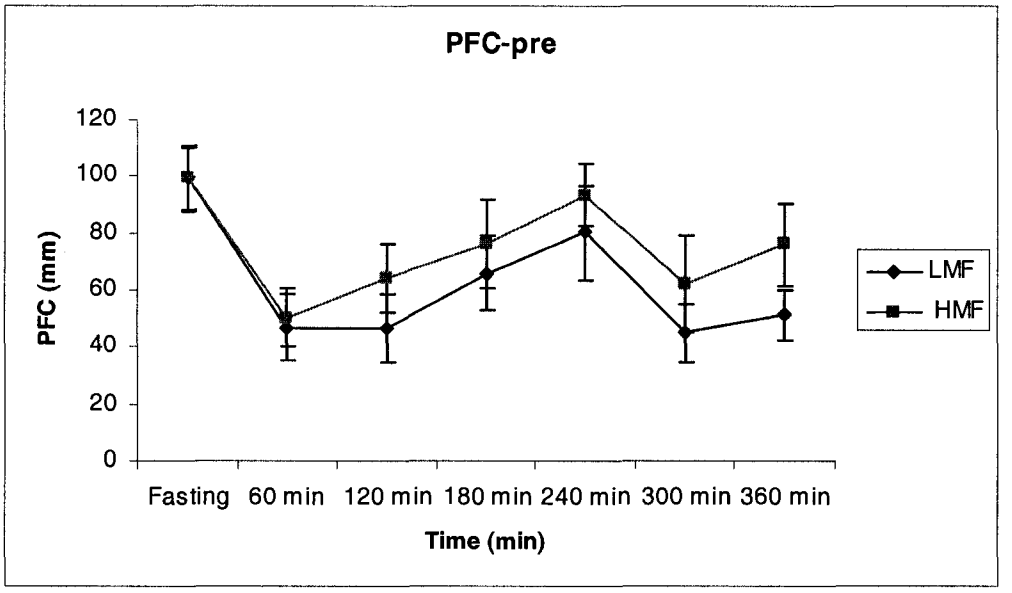


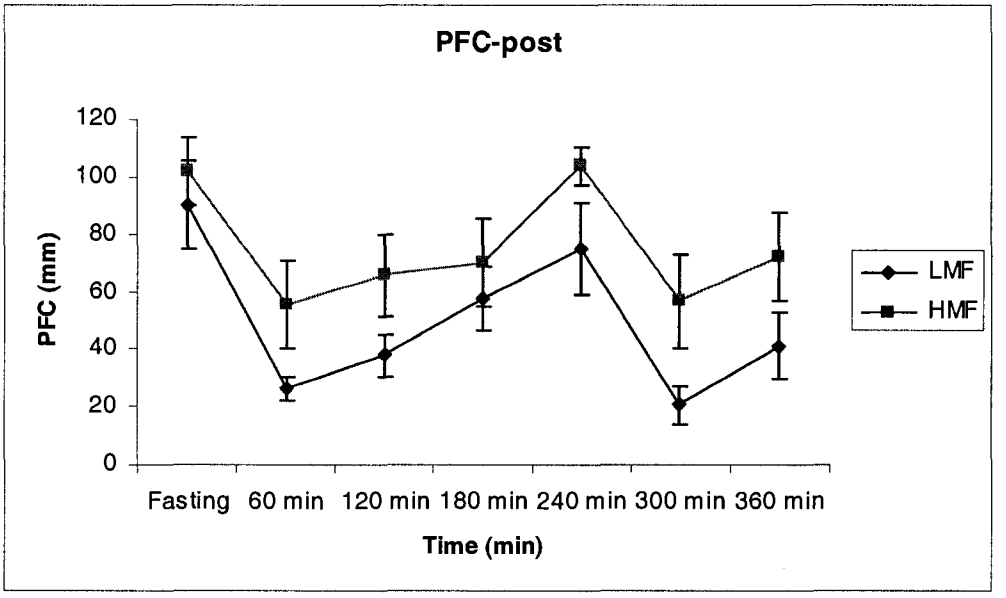
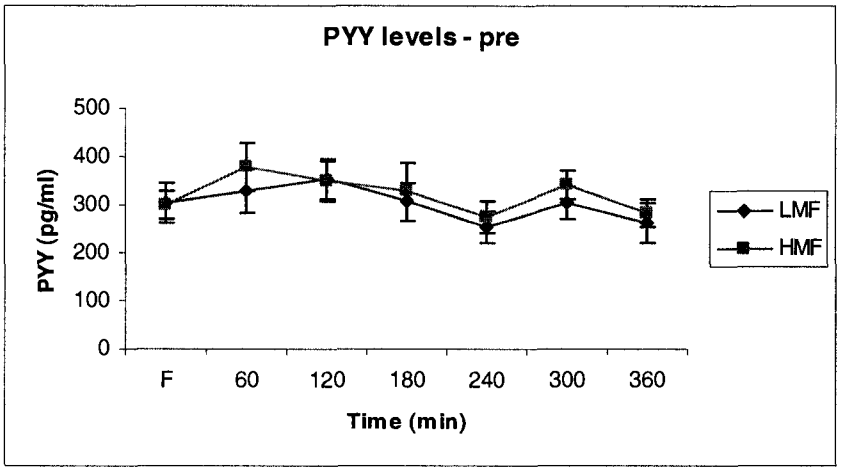


Figure 7. The four parameters of appetite measured through Visual Analogue Scale (VAS). Significant effect of time for all parameters pre- and post-treatment ($p < .05$).



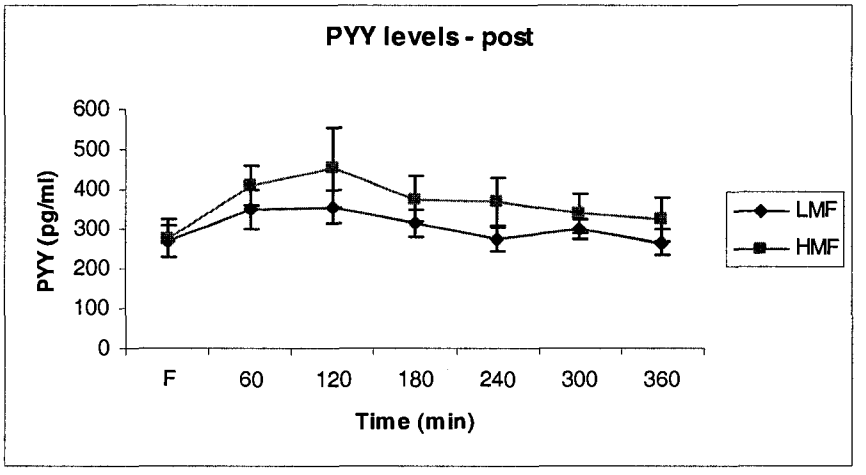


Figure 8. Fasting PYY and 6 h postprandial PYY concentrations at pre- and post-treatment. Significant effect of time ($p < .05$) in both conditions.

APPENDIX A

Ethics Board Approval Letter

Université d'Ottawa University of Ottawa
Service de subventions de recherche et déontologie Research Grants and Ethics Services

Le 16 janvier 2006

Éric Doucet
École des sciences de la réadaptation
Université d'Ottawa
125 Université, pièce 353
Ottawa, ON K1N 6N5

Marie-Josée Cyr
65 Boulevard Fournier, apt. 614
Gatineau, QC J8X 3P6

Objet: Effects of High Frequency Meals on Body Weight Loss, Appetite Regulation and PYY Levels (dossier H 12-05-06)

Chers Docteur Doucet et Mlle Cyr,

Vous trouverez ci-joint le certificat d'approbation éthique du Comité d'éthique de la recherche (CÉR) en Sciences de la Santé et Sciences pour votre projet de recherche.

Au cours de votre étude, toute modification au protocole ou aux formulaires ne peut être introduite sans l'approbation préalable écrite du CÉR. Vous devez aussi aviser, dans les plus brefs délais, le CÉR de tout événement ou expérience indésirables vécus par les participants.

Cette attestation d'approbation déontologique est valide jusqu'au 16 janvier 2007. Veuillez soumettre un rapport annuel à la Responsable de l'éthique en recherche en janvier 2007 pour soit fermer le dossier ou faire demande d'extension. Ce rapport se trouve à l'adresse suivante : http://web9.uottawa.ca/services/rgessrd/deontologie/application_dwn_f.asp

Une copie de cette approbation sera soumise aux Services de la Recherche, si nécessaire.

Si vous avez des questions, n'hésitez pas à me contacter au poste 5387.

Veuillez agréer mes sentiments les meilleurs.

Rita D'Alessandro
Responsable de l'éthique en recherche
Pour Dr Daniel Lagarec, Président du CÉR

Université d'Ottawa University of Ottawa

Service de subventions de recherche et déontologie Research Grants and Ethics Services

COMITÉ D'ÉTHIQUE DE LA RECHERCHE
EN SCIENCES DE LA SANTÉ ET SCIENCES

ATTESTATION D'APPROBATION ÉTHIQUE

La présente attestation certifie que le Comité d'éthique de la recherche en Sciences de la Santé et Sciences de l'Université d'Ottawa a examiné la demande d'approbation éthique pour le projet de recherche **Effects of High Frequency Meals on Body Weight Loss, Appetite Regulation and PYY Levels (dossier H 12-05-06)** présentée par Marie-Josée Cyr et supervisée par Éric Doucet de l'École des sciences de la réadaptation de l'Université d'Ottawa. Le Comité d'éthique a déterminé que la demande respectait les principes éthiques établis par l'Énoncé de politique des trois conseils et par les règles de procédure des Comités d'éthique de l'Université d'Ottawa. Le Comité d'éthique a donc accordé une catégorie 1a (approbation) à ce projet. La présente attestation est valide pour un an à partir de la date indiquée ci-dessous.

Rita D'Alessandro
Responsable de l'éthique en recherche
Pour Dr Daniel Lagarec, Président du CÉR en
Sciences de la Santé et Sciences

16 janvier 2006
Date

APPENDIX B

Inclusion/Exclusion Screening Questionnaire

PRE-SCREENING QUESTIONNAIRE

Legend: E = exclusion

Questions	Answer	
	Yes	No
➢ Where did you hear about this study?		
➢ What is your age? Between 18 and 55 years?	☐	E
➢ What is your weight?		
➢ What is your height?		
➢ BMI 30kg/m^2 < BMI < 45 kg/m^2 ?	☐	E
➢ What is your waist circumference? ≥ 102cm M or ≥ 88 cm F	☐	E
➢ Do you smoke?	E	☐
➢ Have you been weight stable (±2 kg) for the past 6 months?	☐	E
➢ Do you practice physical activity less than twice a week?	☐	E
-How many minutes per week?		
-Which activities?		
➢ Are you vegetarian?	E	☐
➢ Do you have any food allergies?	☐	☐
If yes, which ones?		
➢ Have you had a medical checkup in the last 12 months?	☐	☐
➢ Do you take any medications?	☐	☐
-If yes which ones?		
➢ Do you suffer from diabetes?	E	☐
➢ Do you suffer from heart problems? Which ones?	E	☐
➢ Do you suffer from hypertension?	E	☐
➢ Do you have any thyroid gland problems?	E	☐
➢ Do you suffer from any other health problem not mentioned in this questionnaire? If yes, which ones?	☐	☐
➢ Would you accept that we take blood samples at these visits?	☐	E

Le candidat rejoint les critères d'inclusion : Oui ☐ Non ☐

Si non, pourquoi :

_____

➢ If you do not correspond to the inclusion criteria, do you accept that we call you for future studies? ☐ ☐

When are you available?

Jour	**a.m**	**p.m**	**evening**
Monday	☐	☐	☐
Tuesday	☐	☐	☐
Wednesday	☐	☐	☐
Thursday	☐	☐	☐
Friday	☐	☐	☐
Saturday	☐	☐	☐
Sunday	☐	☐	☐

APPENDIX C

Food Appreciation Questionnaire

FOOD APPRECIATION

1- Ask the participant to give his level of appreciation of each of the foods of the buffet and the other meals.

2- Specify that on the appreciation scale, number 1 represents a food that he does not like at all and that number 5 represents a food that he likes a lot.

Meat and substitutes	I do not like at all				I Like it a lot
Natural peanut butter	1	2	3	4	5
Almonds	1	2	3	4	5
Turkey breast, slice	1	2	3	4	5
Milk products					
Cheddar cheese < 20 % fat	1	2	3	4	5
Yogurt < 2% fat (all flavors, plain)	1	2	3	4	5
Partially skim milk (2%)	1	2	3	4	5
Grain products					
Crackers (wheat thin)	1	2	3	4	5
Whole wheat bread	1	2	3	4	5

Fruits and vegetables					
Carrot	1	2	3	4	5
Raisins	1	2	3	4	5
Apple	1	2	3	4	5
100% pure orange juice	1	2	3	4	5

APPENDIX D

Consent Form

Université d'Ottawa · University of Ottawa

Faculté des sciences de la santé
École des sciences de l'activité physique

Faculty of Health Sciences
School of Human Kinetics

CONSENT FORM

EFFECTS OF HIGH FREQUENCY MEALS ON BODY WEIGHT LOSS, APPETITE REGULATION AND PYY LEVELS.

Masters thesis research project

Principal Investigator: Éric Doucet (Ph.D)
Research Coordinator and masters Student: Marie-Josée Cyr (Dt.P)
Faculty of Health Sciences, University of Ottawa
School of Human Kinetics

1. INVITATION TO PARTICIPATE: You are invited to participate in the above named research study conducted by Marie-Josée Cyr Dt.P, masters candidate supervised by Éric Doucet Ph.D.

2. PURPOSE OF THE STUDY: The aim of this study is to 1) investigate whether using an increased meal frequency pattern (3 meals + 3 snacks/day) will lead to greater weight loss than the conventional low meal frequency pattern (3 meals/day) in response to an equal caloric restriction; 2) examine the daily PYY levels between the two groups; 3) examine the daily variation in appetite between the two group. Therefore, if you wish to take part in this study, the intended duration of your participation will be of 12 weeks, including 9 visits to the research unit.

3. BACKGROUND: Few studies have concentrated on the efficacy of increased meal frequency on weight loss and appetite regulation. High meal frequency with a regular pattern has potential role in increasing the level of compliance while following an energy restriction via PYY levels, which favors better control in appetite. Peptide YY is a gut hormone released in the blood after meals and is recognized to inhibit food intake. Peptide YY is secreted from the pancreas and in the gastrointestinal tract. With this research, we want to study if high meal frequency will lead to a greater weight loss than the conventional low meal frequency. Measurements during this protocol will include body composition, energy expenditure, appetite and psychological assessments. Results obtained from this study will enable us to better understand the effects of meal frequency on body weight loss, appetite regulation and PYY level. We aim eventually to be able to design weight loss-weight maintenance programs that have a greater resolution potential.

4. DESCRIPTION OF THE STUDY: Initial visits: You will be asked to visit the research unit for an initial visit (*Initial visit 1*) of approximately 2 hours during which the study as well as the consent form will be explained to you. You can then bring the consent form home so further reading and discussion with family members is made possible. Other questionnaires will also have to be filled out at that moment and your height, weight and waist circumference will be measured for screening purposes. Results from these measurements will then be analyzed in order to determine if you correspond to the inclusion criteria of this present study. If you do correspond and agree to participate in this study, you will be asked to come to the research unit for a second initial visit *(initial visit 2)* which will last for approximately 3 hours. During this visit, your resting metabolic rate will be measured in order to determine your basal energy needs. The dietitian will give you a weight stabilization diet for 3 days. After this, you will have to come back to the research unit for 2 other sessions before the beginning of the weight loss program. Follow-ups will also be necessary during the weight loss program. This research project consists of two 8 week experimental diets (either high or low frequency meals). These sessions of testing are described in details below.

BASELINE ASSESSMENTS DAY (WEEK 0 – 4 days) AND POST DIET SESSION 2 (WEEK 8 + 2 DAYS)

A. Arrival at the laboratory 7h30.

B. Resting (7h30-8h00) – You will have to rest comfortably in the reclining bed for a 30 minute period.

C. Resting Metabolic Rate (8h00-8h30) - After a 30 minute resting period in the supine position a measurement of resting energy expenditure will be done. The measurement of resting metabolic rate takes place early in the morning after an overnight fast. A plexiglass hood will be placed over your head through which fresh air will be drawn. The expired air will be sampled for analysis and percentages of oxygen and carbon dioxide determined for 30 minutes. By measuring the flow rate, we will be able to determine the amount of oxygen that is consumed and derive energy expenditure. This test requires that you lie quietly and relaxed in bed for around 30 minutes. There are no risks associated with this procedure.

D. Body composition (8h30-9h00) - Body weight, height and waist circumference will be measured. A method called dual-photon x-ray (DEXA) will be used to measure bone density, percent fat and percent lean body mass. You will have to lie on an examination table, fully clothed, while a low intensity x-ray will scan the entire body. The measurements takes 20 minutes. The only risk is a minimal x-ray exposure of less than 0.5 millirem. This exposure is less than the naturel background from 1 day of exposure to sunlight.

E. Standardized breakfast test meal and appetite ratings (9h00-12h00) - After an overnight fast, you will be served a variety of foods at 8h30. You will be asked to eat a standardized breakfast test meal, which will consist of whole bread, natural peanut butter and milk (8h30-9h00). Appetite ratings will be measured before and after breakfast and every 15 minutes for a period of 180

minutes. This will be done using a pen and paper on a visual analogue scale. Briefly, desire to eat, hunger, fullness and prospective food consumption (PFC) will be rated. Questions will be asked as follows: 1) "How strong is your desire to eat?" (Very weak- Very strong); 2)"How hungry do you feel?" (Not hungry at all- As hungry as I have ever felt); 3) "How full do you feel?" (Not full at all- Very full), and 4) "How much food do you think you could eat?" (Nothing at all- A large amount).

O. END OF BASELINE AND POST DIET SESSION 2 12H00

PRE-DIET SESSION (WEEK 0 – 1 day) AND POST-DIET SESSION 1 (WEEK 8 + 1 DAY)

A. Arrival at the laboratory 7h30.

B. Insertion of cathether and PYY blood samples (7h30-14h45) – You will rest comfortably in a reclining bed. An intraveneous cathether will be placed in a vein in your arm. Blood samples will be drawn every 30 minutes to measure PYY levels. Eighteen samples of 5 ml will be taken for a total of 90 ml. Insertion of the cathether and blood samples will be performed by a registered nurse.

C. Appetite ratings (7h30-14h45) - Appetite ratings will be measured every 30 minutes for 8 hours. Food will be served to you according to your respective meal plan (high or low meal frequency).

D. Breakfast (8h00- 8h30) - A breakfast will be served consisting of toast, peanut butter, yogurt and a fruit.

E. AM Snack (10h15-10h30) - A midmorning snack will only be served to the high meal frequency group and will consist of crackers, cheese and milk.

F. Explanation of the experimental diet (10h30-11h00) - The dietitian will go through the meal plan as well as portion sizes. Any questions or concerns about the diet will be answered.

G. Lunch (12h00-12h30) - A lunch will be served consisting of a sandwich, vegetables, crackers and milk.

H. PM Snack (14h15-14h30) - An afternoon snack will be served to the high meal frequency group consisting of a fruit, nuts and yogurt.

I. END OF PRE-DIET SESSION AND POST DIET SESSION 2 14h45

CONTROL SESSIONS (WEEK 1 3 AND 5)

A. Arrival at the laboratory 8h00

B. Anthropometric measurements (8h00-8h10) - Body weight will be determined with a standard beam scale, whereas height and waist circumference will be measured with a tape.

C. 24 hour dietary recall (8h10-8h30) - The participant will be asked to fill out a 24 hour dietary recall with the help of the dietitian and any questions regarding their expiremental diet will be answered.

D. END of CONTROL SESSIONS 8h30

TELEPHONE CONTROLS (WEEK 0, 2 4 AND 6)

A. The dietitian will call the participant to assess his adherence to the expiremental diet and to answer questions.

5. POSSIBLE RISKS/DISCOMFORTS:

The risks associated with this project are low and minimal. The measure of body composition (DEXA) presents a low risk to you. However, it is important to underline that this apparatus will expose you to a minimal radiation (the equivalent of a day in the sun - 0.02-0.05 mRem). The blood samples also present very few risks. However, a small local hematoma (a bruise at the venal puncture) could develop during the few days following the blood sampling. Since the catheter has to be worn for a few hours for certain measures (satiety hormone PYY), it is possible that you will experience a certain discomfort. It is important to note that the risks of infection, of phlebitis (inflammation of the vein) and vaso vagal shock (loss of consciousness) are very low, but still remain a possibility. As for any weight loss program, the energy restriction could make you feel week and/or tired at the beginning of the program and it might take a certain numbers of days for your body to adapt to the energy restriction. Hunger will also be an important side-effect throughout. It is important to note that weight relapse is common after weight loss.

6. BENEFITS:

Your participation in this study will allow you to gather information on your body composition as well as on other health indicators (e.g. resting metabolic rate, glucose profile...). Further, you will most likely lose body weight which will lead to improvements of blood lipids such as total cholesterol and LDL cholesterol, blood glucose and quality of life. In addition, certain notions and practical advice on healthy eating will also be offered to you.

7. MONETARY COMPENSATION:

Parking at the research center is free for participants, as are all scientific tests. You will receive a compensation of $100.00 which will be paid in increments of 25$ at the beginning of visit initial 2, baseline session, pre-diet and post-diet. You will not be compensated for a session for which you did not show up.

8. CONFIDENTIALITY AND ANONYMITY:

In order to guarantee the confidentiality and anonymity of participants, all precautions and necessary measures will be taken to ensure that results and personal information of participants is kept under the strictest of confidentiality.

- Only the following persons will have access to the material: Principal Investigator, Research Coordinators, and Nurse. Any other individuals involved in the study will not have access to participant's personal information and results.
- The names of participants will not appear on any reports. A number code will be used to identify participants on all research documents.
- All material and information which can be linked to participants will not be made public and will be kept under the strictest confidentiality.
- Participants will not be identified in any way in publications or reports.
- The data collected will be kept in a locked cabinet in the Behavioral and Metabolic Research Unit with restricted access where all participant's folders will be kept. In addition, the computer files will be protected by a password.
- Blood samples will be kept in the research unit's laboratory freezer. Blood samples will be identified by a number code which will not be retraced.
- Data will be destroyed and any blood samples eliminated five years after publication of study results.

9. VOLUNTARY PARTICIPATION

- You are free to refuse to participate and if you choose to participate, you are free to withdraw from the study at any time for any reason. At any moment during this study, the best interests of participants will always prevail upon the objectives of the study.
- The participants will be made aware of new findings that might influence their decision to take part in the present study.

Any information about your rights as a research participant may be addressed to: Protocol officer for ethics in research, University of Ottawa, :

If I have any questions about the conduct of the research project, I may contact the research coordinators

There are two copies of the consent form, one of which I may keep.

Please choose one of the following options:

If I choose to withdraw from the study, I want that all data gathered from me until the time of withdrawal be destroyed ☐

Even if I withdraw from the study, I accept that the data gathered from me be used for this study ☐

RESEARCHER' SIGNATURE

Eric Doucet, Ph.D.: _____ Date: _____

RESEARCH COORDINATORS' SIGNATURES

Marie-Josée Cyr, M.(Sc).(candidate): _____ Date: _____

PARTICIPANT'S SIGNATURE:

I agree to participate in this study,

_____ _____ Date: _____

Printed Name Signature

APPENDIX E

Visual Analogue Scales

T :

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

1.How strong is your desire to eat?

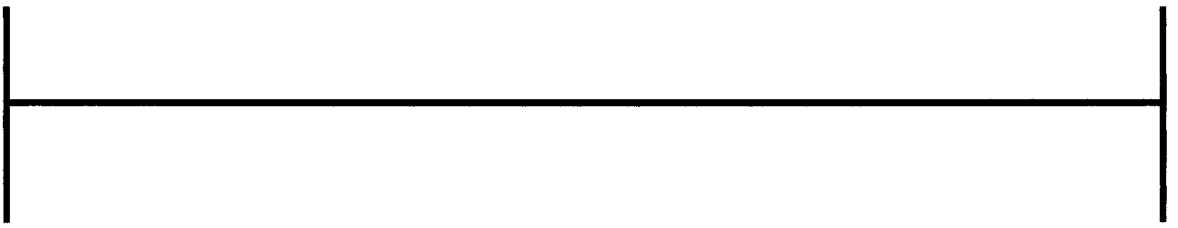
Very weak **Very strong**

T :

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

2.How hungry do you feel?



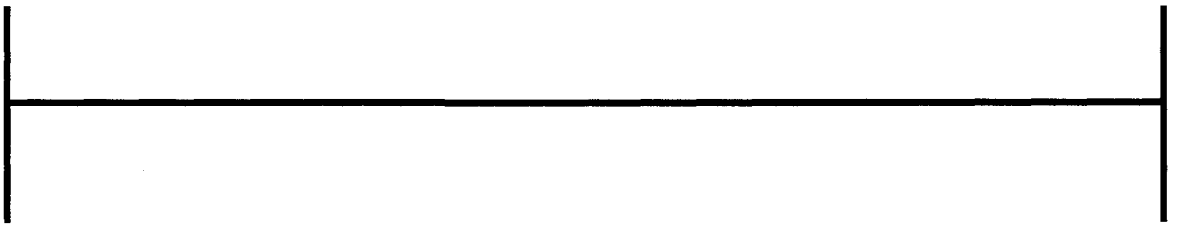
Not hungry at all **As hungry as I ever felt**

T :

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

3.How full do you feel?



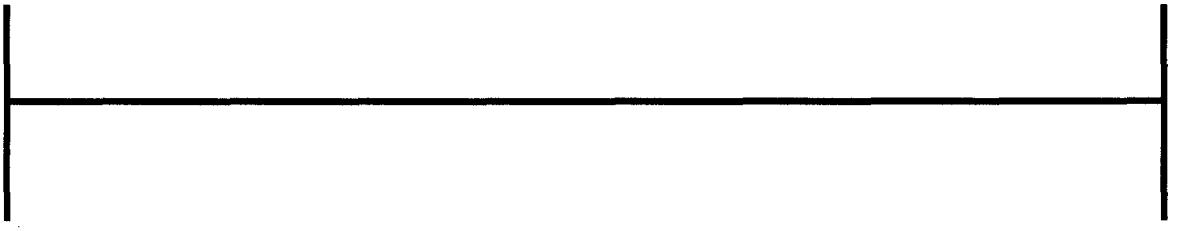
Not full at all **Very full**

T :

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

4.How much food do you think you could eat?



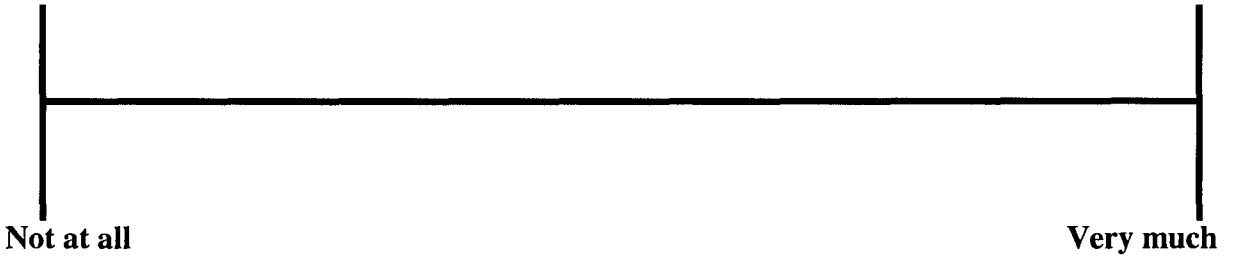
Nothing at all **A large amount**

T :

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

5.How did you appreciate this meal?



APPENDIX F

Three Eating Factor Questionnaire

FOOD HABITS QUESTIONNAIRE

(Stunkard et Messick, 1984)

This questionnaire contains a certain number of propositions.

If you agree with the proposition or if you feel like it can be applied to you, check the case TRUE which corresponds to the proposition.

If you disagree with the proposition or if you feel like it cannot be applied to you, check the case FALSE which corresponds to the proposition.

You have the choice to answer or not certain questions.

		TRUE	FALSE
1.	**When I smell a sizzling steak or see a juicy piece of meat, I find difficult to keep from eating, even if I have just finished a meal.**	❑	❑
2.	**I usually eat too much at social occasions, like parties and picnics.**	❑	❑
3.	**I am actually so hungry that I eat more than 3 times per day.**	❑	❑
4.	**When I have eaten my quota of calories, I am usually good about not eating any more.**	❑	❑
5.	**Dieting is so hard for me because I just get too hungry.**	❑	❑
6.	**I deliberately take small helpings as a means of controlling my weight.**	❑	❑
7.	**Sometimes things just taste so good that I keep on eating even when I am no longer hungry.**	❑	❑

8.	**Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I had enough or that I can something more to eat**	❑	❑
9.	**When I feel anxious, I find myself eating.**	❑	❑

		TRUE	FALSE
10.	**Life is too short to worry about dieting.**	❑	❑
11.	**Since my weight goes up and down, I have gone on reducing diets more than once.**	❑	❑
12.	**I often feel so hungry that I just have to eat something.**	❑	❑
13.	**When I am with someone who is overeating, I usually overeat too.**	❑	❑
14.	**I have a pretty good idea of the number of calories in common food.**	❑	❑
15.	**Sometimes when I start eating, I just can't seem to stop.**	❑	❑
16.	**It is not difficult for me to leave something on my plate.**	❑	❑
17.	**At certain times of the day, I get hungry because I have gotten used to eating then.**	❑	❑
18.	**While on a diet, if I eat food that id allowed, I consciously eat less for a period of time to make up for it.**	❑	❑
19.	**Being with someone who is eating often makes me hungry enough to eat also.**	❑	❑
20.	**When I feel « blue », I often overeat.**	❑	❑
21.	**I enjoy eating too much to spoil it by counting calories or watching my weight.**	❑	❑
22.	**When I see a real delicacy, I often get so hungry that I have to eat right away.**	❑	❑

	TRUE	FALSE
23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.	❑	❑
24. I get so hungry that my stomach often seems like a bottomless pit.	❑	❑
25. My weight has hardly changed at all in the last 10 years.	❑	❑
26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.	❑	❑
27. When I feel lonely, I console myself by eating.	❑	❑
28. I consciously hold back at meals in order not to gain weight.	❑	❑
29. I sometimes get very hungry late in the evening or at night.	❑	❑
30. I eat anything I want, anytime I want.	❑	❑
31. Without even thinking about it, I take a long time to eat.	❑	❑
32. I count calories as a conscious means of controlling weight.	❑	❑
33. I do not eat some foods because they make me fat.	❑	❑
34. I am always hungry enough to eat at any time.	❑	❑
35. I pay a great deal of attention to changes in my figure.	❑	❑
36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.	❑	❑

PART 2

Please answer the following questions by circling the number which applies to you.

37. How often are you dieting in a conscious effort to control your weight ?

Rarely	Sometimes	Usually	Always
1	2	3	4

38. Would a weight fluctuation of 5lbs (2 kgs) affect the way you live your life ?

Not at all	Slightly	Moderately	Very much
1	2	3	4

39. How often do you feel hungry ?

Only At mealtimes	Sometimes between meals	Often between meals	Almost always
1	2	3	4

40. Do your feelings of guilt about overeating help you control your food intake ?

Never	Rarely	Often	Always
1	2	3	4

41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next 4 hours ?

Easy	Slightly Difficult	Moderately Difficult	Very Difficult
1	2	3	4

42. How conscious are you of what you are eating ?

Not at all	Slightly	Moderately	Extremely
1	2	3	4

43. How frequently do you avoid « stocking up » on tempting foods ?

Almost Never	Seldom	Usually	Almost always
1	2	3	4

44. How likely are to shop for low calorie foods ?

Unlikely	Slightly Unlikely	Moderately likely	Very likely
1	2	3	4

45. Do you eat sensibly in front of others and splurge alone ?

Never	Rarely	Often	Always
1	2	3	4

46. How likely are you to consciously eat slowly in order to cut down on how much you eat ?

Unlikely	Slightly Unlikely	Moderately likely	Very likely
1	2	3	4

47. How frequently do you skip dessert because you are no longer hungry ?

Almost Never	Seldom	At least once per week	Almost every day
1	2	3	4

48. How likely are you to consciously eat less than you want ?

Unlikely	Slightly Unlikely	Moderately likely	Very likely
1	2	3	4

49. Do you go on eating binges though you are not hungry ?

Never	Rarely	Sometimes	At least Once per week
1	2	3	4

50. On a scale of 1 to 5, where :

0 (zero) means no restraint in eating
(eating whatever you want, whenever you want it)

and

5 means total restraint
(constantly limiting food intake and never "giving in"),

What number you give yourself?

- **Eat whatever you want, whenever you want it**
  0

- **Usually eat whatever you want, whenever you want it**
  1

- **Often eat whatever you want, whenever you want it**
  2

- **Often limit food intake, but often « give in »**
  3

- **Usually limit food intake, rarely « give in »**
  4

- **Constantly limiting food intake, never « giving in »**
  5

51. To what extent does this statement describe your eating behavior ?

"I start dieting in the morning, but because of nay number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow"

Not like Me	Little like me	Pretty good description of me	Describes me perfectly
1	2	3	4