PROP PHENOTYPE, DIETARY VARIETY, AND THE RISK OF OBESITY IN WOMEN

by

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ABSTRACT OF THE DISSERTATION

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Exposure to a variety of high-fat/high-energy palatable foods can increase energy intake and mediate positive energy balance that can lead to body weight gain and obesity. Preference for high-fat/high-energy foods has been associated with varied sensitivity to the bitter taste of PROP (6-n-propylthiouracil). Experimental data on the impact of dietary variety on energy intake in PROP taster groups are mainly limited to food intake in single meals. To address this question over the near-term, this study investigated the influence of eating in a buffet setting on daily energy and macronutrient intake as a function of PROP taster status. This study also investigated regulation of energy intake and caloric compensation at a buffet meal after exposure to a high-energy/high-fat soup preload.

Our results showed that, as expected, energy intake was higher for all taster groups in the buffet meals relative to fixed-item meals. In addition, non-tasters (NT) and medium-tasters (MT) consumed an average of 246 kcal more than super-tasters (ST) in the buffet condition. Across all days of the study, NT consumed more cakes and added fats while ST consumed more fruits and vegetables. These findings suggest that exposure

to high variety meals promotes increased energy intake of NT compared to ST and might contribute to group differenced in energy balance over time.

In another study, we demonstrated that after the soup preload, energy intake of NT was higher than ST but did not differ from that of MT. NT also consumed more fat from the test meal than MT and ST. Caloric compensation at the lunch meal in response to the energy content of the high-fat/high-energy soup preload varied among taster groups. NT undercompensated and over-ate at the buffet lunch while MT and ST overcompensated and ate less at lunch after the soup preload. These small discrepancies in short-term energy compensation may play a role in positive energy balance and increased adiposity in women with the PROP non-taster phenotype. The classification of women by PROP status may identify women at increased risk for excess weight gain and the future development of obesity.

Dedication

I lovingly dedicate this thesis to my mum and dad, who supported me every step of the way

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Table of Contents

List of Tables

Chapter 2: **Page**

Chapter 3:

List of Figures

Chapter 1: **Page**

Chapter 2:

Chapter 3:

List of Abbreviations

BMI	Body mass index
BUFF	Buffet condition
CHO	Carbohydrate
En	Energy
(F)	Fullness rating
FIXED	Fixed-item condition
(H)	Hunger rating
(M)	Desire to have a meal rating
MT	Medium-taster
NT	Non-taster
Pro	Protein
PTC	Phenylthiocarbamide
PROP	6-n-propylthiouracil
(S)	Desire to have a snack rating
ST	Super-taster

CHAPTER 1

Literature Review

I.1 *Obesity as a major health risk factor*

Obesity has emerged as a global public health concern in industrialized and developing countries affecting both adults and children. Obesity is a result of prolonged energy imbalance, individual lifestyle, environmental and genetic factors that affects more than 400 million people worldwide, a number that is estimated to increase to 700 million by 2015 (WHO.int). In the U.S. alone, 68% of the population is either overweight (defined as having a BMI of 25-29.9) or obese (defined as having a BMI of ≥30) (Flegal et al 2010, Ogden et al 2007). Being overweight or obese exacerbates many health problems by increasing the risk of metabolic complications such as coronary heart disease, congestive heart failure, stroke, hypertension, type 2 diabetes and certain cancers. Despite public awareness of obesity, its prevalence has increased significantly in the past 25 years (Kopelman 2000). Also, obesity is one of the major causes of morbidity and mortality in the U.S. that generates significant health care associated costs and economic consequences (Finkelstein et al 2004), thus, preventive measures need to be taken in order to lower the incidence of obesity in the general population and to better control metabolic disorders.

I.2 *Dietary factors and the development of obesity*

Obesity is a complex disease, and its prevalence is mediated by a wide variety of genetic, physiological, social and environmental factors (Friel et al 2007, Kopelman 2000). The onset of weight gain and its progression to obesity is usually characterized by an energy imbalance that is a consequence of increased energy intake and decreased

energy expenditure (Jebb 1997). An increasing number of studies have investigated different aspects of energy intake, suggesting that increased portion size (Levitsky and Youn 2004), energy density (Bell and Rolls 2001), food palatability (de Castro et al 2000), and food variety (Rolls et al 1981) each play a role in increased food intake. The interactions between the eating environment and the physical environment have contributed to major changes in dietary and physical activity patterns and body composition over the past several decades. In today's society, a wide range of high-fat, high-sugar, palatable foods are readily available and accessible to the general population that provide more opportunities for the consumption of excess quantities of food. The abundance of fast food establishments and restaurants (Jeffery et al 2006) and the increase in the number and size of high-fat, energy-rich, sugar-dense foods and beverages consumed away from home have also increased. The frequency of consuming food away from home (restaurant food) is positively associated with increased body fatness in adults (McCrory et al 1999a). Even small (~50 kcal/day) increases in energy intake relative to energy needs results in positive energy balance that coupled with low levels of physical activity will contribute to excess body weight gain (Hill et al 2008).

1.3 *Dietary variety and the obesity epidemic*

Dietary variety is considered a prominent contributing factor to eating behaviors and increased levels of energy intake. Even though exposure to a variety of food choices may enhance the likelihood of selecting a more nutritionally adequate diet (Foote et al 2004), over the long term, it can lead to increased food intake and weight gain (Hetherington et al 2006). Today, the variety we get from food comes for the most part

from the new high-energy processed foods that have been introduced into the U.S. market rather than from the low-energy but nutritionally-rich fruits and vegetables.

The effect of food variety on increased energy intake within and between meals compared to a single meal has been observed in both animals and human subjects (Louis-Sylvestre et al 1984, Rolls et al 1983). McCrory and colleagues demonstrated that in adult men and women, dietary variety of sweets, snacks, condiments, entrées and carbohydrates are correlated with higher body fatness whereas a negative association was reported between body fatness and the variety of fruits and vegetables (McCrory et al 1999b). This variety within a single meal can potentially enhance food intake by 14-25%. (McCrory et al 2002, Norton et al 2006) and delay satiation (Hetherington et al 2006). Rolls and associates showed that when subjects were served a varied meal (four-course lunch with varying food in each course), energy intake increased 60% compared to when the subjects were given the same food in each course. The pleasantness of foods eaten decreased while pleasantness of taste of foods that had not been eaten did not change. (Rolls et al 1984).

1.4 *Taste perception and food intake*

Taste is one of the main contributing factors to the selection and perceived palatability of foods and has been extensively investigated to understand factors influencing food choices and eating habits. Perception of sweet, salty, and umami tastes positively influence food palatability whereas increased bitter and sour tastes reduce palatability and pleasantness of foods (Nasser 2001). Highly palatable foods are usually

energy-dense foods compared to less palatable foods that are low in energy density. Since individuals tend to eat a constant amount/volume of food (Bell and Rolls 2001), the higher the energy density of the food consumed, the larger the caloric input that in the long run can lead to excess energy intake. Several short-term studies have shown that energy intake increases as food palatability increases (Guy-Grand et al 1994). On the other hand, evaluating the effect of food palatability on food intake by measuring subjective appetite sensations (hunger/fullness levels) has yielded conflicting results. After consuming a palatable food, subjects have reported more hunger and less fullness compared to a less palatable food (Hill et al 1984). However, some studies have reported the opposite findings or no difference in hunger and fullness ratings of highly palatable meals compared to less palatable meals (Bobroff and Kissileff 1986, De Graaf et al 1999, Guy-Grand et al 1994, Warwick et al 1993). Whether food palatability affects short-term food intake is not well understood.

I.5 *Bitter tasting compounds PROP and PTC*

The ability to perceive the bitter taste of the synthetic compound PTC (phenylthiocarbamide) was discovered in the early 1930s by Arthur Fox. While pouring some powdered PTC into a bottle, some of the powder flew into the air. A colleague working nearby complained that the dust tasted bitter but Fox did not taste anything at all. Intrigued by how the chemical tasted differently to them, they tasted the powder again and realized that they differed in their taste sensitivity to PTC. Later, Fox asked his family and friends to taste the powder and determine how it tasted. He found that

variation in taste sensitivity was common, with some individuals finding the compound to be intensely bitter (tasters) while others rated it as mildly bitter (non-tasters). Tasters were able to taste the compound at very low concentrations while non-tasters were unable to taste the compound except at very high concentrations. Further, Fox showed that sensitivity to PTC was correlated with sensitivity to other compounds that contain the N-C=S (thiourea) moiety such as PROP (6-n-propylthiouracil) (Fox 1932, Guo and Reed 2001, Wooding 2006). PROP is a compound that is chemically related to PTC and characterized by the presence of the N-C=S group. The High correlation between sensitivity to PTC and PROP among individuals and the fact that PROP is odorless and less toxic than PTC has allowed PROP to take the place of PTC in subsequent research (Lawless 1980). Over the years, variation in PROP sensitivity and the relative simplicity of PROP phenotyping has resulted in a large number of studies on PROP sensitivity in human populations.

I.6 *Genetics of PROP tasting*

Sensitivity to PTC and PROP varies among individuals and is a heritable trait (Bartoshuk et al 1994, Tepper 2008) that follows Mendelian principles (Guo and Reed 2001, Olson et al 1989). Taste sensitivity and taste blindness to bitter-tasting PROP/PTC is evident in all populations with varying frequencies and is to some degree affected by gender and age. Women are in general more sensitive to PROP whereas men are more likely to fall in the non-taster category (Bartoshuk et al 1994, Whissell-Buechy 1990). About 30% of the adult Caucasian population is genetically taste blind to PROP

(recognized as non-taster) while 70% of the population falls in the taster category. The frequency of non-tasters varies in different ethnic populations where around 10%-12% of people in China and Japan and 40% of Indians are classified as non-tasters (Guo and Reed 2001). Bartoshuk and colleagues further separated the taster group into medium-tasters (MT) who rate the bitter taste of PTC/PROP as moderate and super-tasters (ST) who perceive PTC/PROP as extremely bitter (Bartoshuk et al 1994).

An important gene that belongs to the TAS2R receptor gene family for bitter taste, TAS2R38, is located on chromosome 7q (Drayna et al 2003, Kim et al 2003) and controls the expression of PTC taster phenotype. Genetic polymorphisms of the TAS2R38 gene locus account for marked differences in bitter taste perception in the general population (Guo and Reed 2001). It was first believed that a simple Mendelian pattern of inheritance with a single polymorphism was responsible for these differences, however, it is now understood that three nucleotide polymorphisms result in three amino acid substitutions (A49P,V262A, and I296V) and give rise to two common PAV (taster) and AVI (non-taster) forms (Bufe et al 2005, Guo and Reed 2001, Kim et al 2003).These haplotypes are also found in AAI and AAV forms which are less common than the AVI and PAV forms. In addition, they are found in two rare forms of PVI and PAI (Kim et al 2003, Mennella et al 2011, Wang et al 2007). Based on the expression of PAV and AVI alleles, PROP-sensitive individuals who have at least one dominant allele (PAV/PAV or PAV/AVI) can be categorized as tasters with varying degrees of sensitivity while individuals who are insensitive to PROP are homozygous recessive for the AVI/AVI allele and are considered non-tasters. Also, a change in PROP sensitivity as a factor of age is more commonly seen in individuals with the heterozygous PAV/AVI haplotype

(Mennella et al 2010). However, the expression of PROP/PTC phenotype may be more complex and allele frequencies may vary by race and ethnicity.

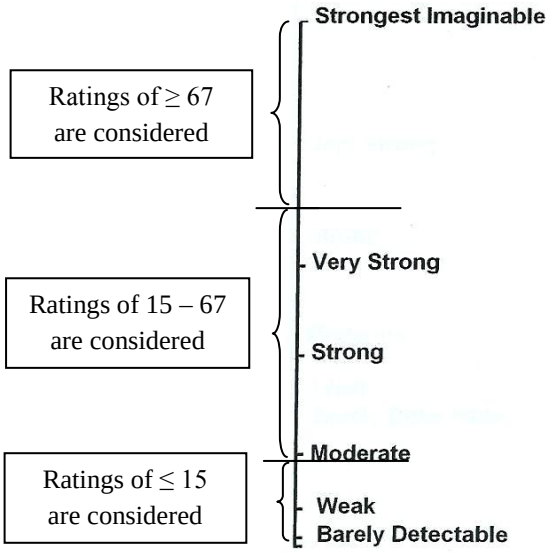
The heightened perception of bitterness by PROP tasters could in part be associated with variations in human taste bud and fungiform papillae density on the anterior tongue. The density of fungiform papillae, innervated structures on the tongue that house taste cells and mechanoreceptors, differs among the three taster groups and is highest in PROP super-tasters (Bartoshuk et al 1994, Hayes et al 2008, Miller and Reedy 1990). Higher density of fungiform papillae and taste buds on the tongue augments sensitivity to oral textures and irritations (Bartoshuk et al 1994, Tepper and Nurse 1997) and directly correlates with the perceived sweetness of sucrose and saltiness of NaCl (Miller and Reedy 1990).

I.7 *PROP classification by filter paper method*

Several methods can be employed to classify individuals by their PROP taster status, however, these methods have their limitations. Threshold (Bartoshuk et al 1994), and suprathreshold (Lawless 1980) methods involve the presentation of varying concentrations of PROP and NaCl solutions and can be used to classify adults and children. The threshold method identifies the lowest detectable concentration of a stimulus whereas the suprathreshold method uses concentrations higher than that used in the threshold method. These methods classify PROP taster groups into taster and non-taster groups. However, these procedures, specifically threshold methods, are unable to further separate the taster group into medium and supertasters. The use of filter paper

methods (Lawless 1980) have been criticized due to reporting a high rate of false positive and false negative responses that do not agree well with threshold methods. In another method, individuals are screened and classified into three non-taster, medium-taster, and super-taster groups based on their responsiveness to PROP using a filter paper method developed by Zhao and colleagues (Zhao et al 2003). PROP and NaCl intensity ratings are recorded on a 100mm, semi-logarithmic Labeled Magnitude Scale (LMS) (Green et al 1996). The scale is anchored at each end with descriptors "barely detectable" and "strongest imaginable" (Figure 1.1). Taste sensitivity to NaCl is also measured mutually with PROP sensitivity and is used as a reference standard since taste sensitivity of this compound does not vary as a function of PROP taste status (Tepper et al 2001, Zhao et al 2003). Non-tasters give higher intensity ratings to NaCl compared to PROP. Intensity ratings of NaCl and PROP are similar for medium tasters, and super-tasters give higher intensity ratings to PROP than to NaCl (Bartoshuk et al 1994, Drewnowski et al 2000). NaCl also allows to better classify subjects who gave borderline ratings to PROP.

Figure 1.1 LMS scale for determining PROP and NaCl intensity ratings



I.8 *PROP status and food preferences*

Bitter taste perception of PROP is an important determinant of food acceptance in the population and is associated with food preferences, food selection, and food likes and dislikes (Drewnowski et al 2001a, Kaminski et al 2000). Several studies have shown that PROP tasters are more sensitive to other bitter and sweet compounds (Drewnowski et al 1997, Drewnowski et al 2001a). Studies also show that super-taster women tend to have a decreased preference for the bitterness of cruciferous vegetables such as broccoli and Brussels sprouts compared to non-tasters (Drewnowski et al 1999, Kaminski et al 2000). Tasters also gave low acceptance ratings to black coffee (Drewnowski et al 1999), perceived beer as more bitter (Intranuovo and Powers 1998) and sensed more irritation from alcohol (Duffy et al 2004) and capsaicin (Prutkin et al 1999).

The influence of PROP sensitivity on bitter food selection and intake has been reported in children where PROP tasters disliked the taste of raw broccoli (Bell and Tepper 2006, Flegal et al 2010) and spinach (Turnbull and Matisoo-Smith 2002) compared to non-tasters. Non-taster children also had a higher overall intake of bitter vegetables (raw broccoli, cucumber, and black olives) compared to taster children (Bell and Tepper 2006) in a short-term food selection study. Therefore, PROP taster status can be adopted as an index or general measure of bitterness perception and as a predictor of dietary preferences.

In addition to investigating the relationship between PROP taster status and bitter taste perception, several studies have examined the effect of genetically mediated sensitivity to PROP on fat discrimination and liking. Super-tasters are able to better discriminate the fat content and creaminess of milk compared to non-tasters but their

liking ratings for creaminess was not different from non-tasters (Duffy et al 2004, Kirkmeyer and Tepper 2005). In another study, Tepper and Nurse showed that medium and super-tasters exhibit an increased ability to discriminate high-fat (40%) salad dressing from a low-fat (10%) dressing. Non-tasters were not able to differentiate the two samples but preferred the high fat sample more (Tepper and Nurse 1997).

However, not all findings support a relationship between sensitivity to PROP and food intake, food preferences, and dietary patterns. Studies have shown that PROP taster status moderately influences food choices and consumption of cruciferous vegetables in elderly women (Jerzsa-Latta et al 1990, Niewind et al 1988). Reporting on data collected by food frequency questionnaire, Yackinous and Guinard showed that intake of bitter fruits, vegetables, and beverages did not differ among the PROP taster groups except for higher intake of green salad in non-tasters (Yackinous and Guinard 2002). Another study reported that PROP taster status had no effect on preferences for sweet or high-fat foods. It also showed no relationship between PROP taster status, food intake, and macronutrient composition of the diet (Drewnowski et al 2007). No relationship between PROP taster status and sensitivity to a number of other bitter compounds (Delwiche et al 2001), plant-based bitter compounds (Guinard et al 1994, Schifferstein and Frijters 1991), bitter salts (Delwiche et al 2001, Schifferstein and Frijters 1991) have been reported.

Even though some studies do not agree, the general conclusion from these findings suggest that PROP tasters have a better ability to discriminate a wide variety of oral stimuli such as sweetness, irritation, creaminess and fattiness of different foods as a result of increased intensity perceptions (Duffy et al 2004, Kirkmeyer and Tepper 2003,

Looy and Weingarten 1992, Tepper and Nurse 1997) and are therefore less accepting of bitter, spicy and fatty foods compared to non-tasters (Tepper 1998, Ullrich et al 2004).

I.9 *PROP status and body weight*

Studies have shown that in general, PROP non-tasters show higher preference for added fats as well as higher intake of dietary fats than super-tasters (Hayes and Duffy 2007, Keller et al 2002, Tepper and Nurse 1998). Based on these findings, it has been hypothesized that body mass index (BMI) is inversely associated with PROP taster status where non-tasters weigh more and have higher BMIs than super-tasters.

Several studies have investigated the relationship between PROP taster status and body weight with conflicting findings. The negative association of PROP taster status and body weight is more evident in women (Duffy et al 2004, Tepper and Nurse 1998, Ullrich et al 2004). Goldstein and colleagues showed that PROP sensitivity was inversely associated with BMI where super-taster women had a healthy BMI of 23.5 kg/m^2 and non-taster women had a BMI of ~30kg/m^2, an excess of 6 BMI units compared to super tasters (Goldstein et al 2005). These findings confirm an earlier study by Tepper and Ullrich that reported a negative association between PROP status and BMI in middle-aged women (Tepper and Ullrich 2002). Tepper and colleagues also investigated the relationship between PROP non-taster phenotype and higher BMI and waste circumference (WC) in 540 healthy male and female residents of the genetically isolated village of Carlantino in southern Italy. BMI and WC varied by PROP taster status among females (n=278) where non-tasters had significantly higher BMI and WC compared to

medium and super tasters. There was no relationship between PROP taster status and either BMI or WC in male subjects (n=173) (Tepper et al 2008). The results of this study are in accordance with findings of a recent study that compared BMI differences across PROP taster groups in 75 male and female Italian volunteers. Results showed that while the mean BMI was in the normal weight range for all 3 taster groups, BMI was higher in non-tasters relative to super-tasters (Padiglia et al 2010).

In contrast, Drewnowski and colleagues did not find a relationship between PROP taster status and BMI in adult men or women aged 18-70 years (Drewnowski et al 2001b), no differences in BMI values or energy intakes were found among healthy male and female college-aged taster groups (Yackinous and Guinard 2002). It has been hypothesized that the differences between studies may be attributable to other factors that affect eating behavior and BMI, such as dietary restraint (Tepper and Ullrich 2002). Differences in PROP screening methods or reliability of such methods can also result in different findings.

Several studies have explored the relationship between variation in BMI and TAS2R38 genotypes. No evidence for an association between BMI and polymorphisms in the bitter taste receptor gene TAS2R38 were found among elderly female participants of the British women's heart and health study (Timpson et al 2005). Tepper and colleagues reported that polymorphisms in the TAS2R38 locus were not associated with BMI and WC in male and female subjects (Tepper et al 2008). These findings were further validated by a follow up study with a larger study population by Sausenthaler and colleagues where no association was established between TAS2R38 haplotypes and BMI (Sausenthaler et al 2009). These data suggest that PROP phenotype is associated with

differences in BMI in women, but variation in the underlying gene (TAS2R38 alleles) is not. Anatomical differences associated with the phenotype (number of taste buds and density of nerve input) may explain this discrepancy. Also, TAS2R38 gene encodes for a bitter taste receptor that determines whether or not a person can taste the bitter substance PTC whereas the PROP phenotype, encompassed varying degrees of PROP responsiveness (non-taster, medium-taster, and super-taster).

I.10 *PROP status and energy intake*

As discussed earlier, one of the mediators of food selection is genetic variation in PROP taste perception. Non-tasters have a higher affinity for high-fat and energy dense foods that could contribute to higher energy intakes in these individuals. Conversely, super tasters consume less carbohydrates and fat than non-tasters. Goldstein and colleagues showed that in pre-adolescent children, energy intakes were negatively associated with PROP taster status (Goldstein et al 2007). However, these findings are derived from data obtained from food frequency questionnaires (FFQ) or 24h food intake diaries (Yackinous and Guinard 2002) and not direct measurements of food and energy intake in a controlled laboratory setting. The limitations to these data collection methods are that food frequency questionnaires and diet recalls do not accurately reflect actual and reliable estimates of intake and have the tendency to overestimate or underestimate energy and food intake (Hunter et al 1988, Jackson et al , Shu et al 2004).

To date, most of the studies have relied on data collected by FFQ and diet recalls. To our knowledge, there are only two studies that have looked at energy intake in PROP

taster groups in a controlled laboratory environment. In a laboratory based feeding study, Tepper and colleagues investigated the effect of PROP sensitivity (non-taster and super-taster status) on energy intake from a fixed-item lunch versus three buffet lunches (Tepper et al 2011). Energy intake of the three PROP taster groups did not differ in the fixed-item lunch. The average food intake from the buffet lunches showed that non-tasters consumed an excess of ~357 kcal compared to the fixed-item lunch (88% increase) while super-tasters consumed ~198 kcal more than the fixed-item lunch (38% more). Thus, as expected, non-tasters consumed more energy in a buffet lunch setting than super-tasters. No differences in macronutrient intake were seen among the taster group (Tepper et al 2011). Thus, the study did not support the hypothesis that differences in fat intake contribute to higher energy intake of non-tasters during buffet feeding. This could be due to the fact that small differences in fat intake may be difficult to detect with a single meal.

On the other hand, Kamphuis and Westertrep-Plantenga showed a reverse effect where PROP tasters consumed comparatively more fat and less carbohydrate from an *ad libitum* high-fat/high-carbohydrate lunch than PROP non-tasters. However, they observed no differences in energy intake between the taster groups (Kamphuis and Westerterp-Plantenga 2003). Macronutrient selection and food and energy intake of taster groups did not differ when they were offered, *ad libitum*, either a high fat (low CHO) lunch or a high carbohydrate (low fat) lunch (Kamphuis and Westerterp-Plantenga 2003).

The above mentioned studies examined food intake over a short term (single meal). No studies have addressed energy intakes of PROP taster groups over a longer period of time to better understand the effect of repeated exposure to high variety meals

on energy intake and regulation. The first part of this thesis investigates the consequences of buffet feeding on daily energy intake over a longer period of time (multiple meals and days) and also determines if higher energy intake in the buffet condition is due to greater consumption of fat or reflects higher intakes from a range of food types.

Moreover, all studies to date investigating energy intake of PROP taster groups have focused on the food intake side of the energy balance equation. The second part of this thesis investigated potential differences in energy compensation as a possible explanation for higher body weights among PROP non-taster women. This demonstration would be consistent with the view that higher body weight in non-taster women represents the combined influences of greater energy and fat intake and weaker compensatory responses to fat in these women.

1.11 OBJECTIVES AND HYPOTHESES

Chapter 2: *Food Intake and Dietary Selection during Buffet Feeding in Women Classified by 6-n-Propylthiouracil (PROP) Taster Phenotype*

Objective: Determine the influence of eating in a buffet setting on daily energy intake, diet composition and selection of specific foods as a function of PROP taster status

Hypothesis1: Non-taster women will consume more daily energy from buffet meals than super-taster women.

Hypothesis 2: Non-taster women will consume more high-fat foods and more calories from fat than super-taster women.

Chapter 3: *Consumption of High Fat Soup Preload Leads to Differences in Short-Term Energy adjustment in PROP Non-Taster Women Compared to Super-Taster Women*

Objective: Determine the effect of a high-fat preload on short-term energy intake, macronutrient selection and intake of specific foods as a function of PROP taster status.

Hypothesis 1: Non-taster women will compensate less well for the calories in the preload than super-taster women

Hypothesis 2: Non-taster women will consume more fat from the buffet lunch that super-taster women

Hypothesis 3:

a) Non-taster women will give higher hedonic ratings to the preloads and the buffet foods

b) Non-taster women will experience less fullness after the preloads than the super-taster women

c) Changes in food palatability ratings or appetitive responses for the preload will be associated with less precise compensation in non-taster women relative to super-taster women

CHAPTER 2

Food Intake and Dietary Selection During Buffet Feeding in Women Classified by 6-n-Propylthiouracil (PROP) Taster Phenotype

2.1 Abstract

Taste blindness to PROP is a common phenotype that has been associated with increased adiposity in women, and might be linked to increased energy and fat intake. Since exposure to a variety of energy-dense foods is known to promote excess energy intake, we investigated if PROP non-taster (NT) women would consume more fat and/or energy in a buffet setting than medium taster (MT) or super-taster (ST) women. Subjects were 75 (n=25 in each taster group) non-diet restrained, lean (BMI= 21.5 ± 0.6), women (26.1 ± 1.3 yr). Subjects ate lunch and dinner in the laboratory for 3 consecutive fixed-item (FIXED) days and 3 consecutive buffet (BUFF) days; there was a 4-d washout between conditions. During FIXED they consumed *ad-libitum* meals; during BUFF they selected from a variety of foods. A standard 300 kcal breakfast was consumed during both conditions. As expected, all groups consumed more energy (kcal/day) and fat (% En) during BUFF than FIXED ($p<0.0001$). However carbohydrate intake (% En) was higher in the FIXED week relative to BUFF week ($p\leq0.002$). During BUFF week, energy intake (kcal/d) in NT (2149 ± 49) and MT (2209 ± 48) was higher than ST (1933 ± 50). ST consumed more protein (% En) in both FIXED and BUFF ($p\leq$ 0.01-0.04) conditions compared to NT and MT. There were no significant differences in energy intake of taster groups during lunch meals in either study condition. However, NT and MT consumed more energy ($p\leq$ 0.01) and fat ($p\leq$ 0.01-0.0003) compared to ST in the buffet dinner meals. Across all days of the study, ST consumed more fruits and vegetables than NT ($p\leq0.003$-0.0001) whereas NT consumed more cakes, and added fats ($p\leq0.003$-0.0002). These data suggest that in a buffet setting, NT women consume more energy than ST which might contribute to group differences in energy balance over time.

2.2 Introduction

Obesity has emerged as a global public health concern in industrialized and developing countries affecting both adults and children. In the US alone,68% of the population is either overweight (defined as having a BMI of 25-29.9) or obese (defined as having a BMI of ≥30) (Flegal et al 2010, Ogden et al 2007). Being overweight or obese exacerbates many health problems by increasing the risk of metabolic complications such as coronary heart disease, congestive heart failure, stroke, hypertension, type 2 diabetes and certain cancers. Despite public awareness of obesity, its prevalence has increased significantly in the past 25 years (Kopelman 2000).

The onset of weight gain and its progression to obesity is usually characterized by an energy imbalance that is a consequence of increased energy intake and decreased energy expenditure (Jebb 1997). An increasing number of studies have investigated different aspects of energy intake, suggesting increase in portion size (Levitsky and Youn 2004), energy density (Bell and Rolls 2001), food palatability (de Castro et al 2000), and food variety (Norton et al 2006, Rolls et al 1981) to positively correlate with increased food intake. Dietary variety is a prominent contributing factor to eating behaviors and increased levels of energy intake that over time can lead to increased food intake and weight gain (Hetherington et al 2006). Food variety within a single meal can potentially enhance food intake by 14-25% (McCrory et al 2002, Norton et al 2006) and delay satiation (Hetherington et al 2006). Importantly, dietary variety in everyday food selection has been shown to increase energy intake and body weight (McCrory et al 1999b).

Individual differences in food preferences contribute to dietary habits and could alter a person's vulnerability to dietary variety. These individual differences could be due to genetic variations in taste perception across individuals. Sensitivity to bitter tasting compounds PTC (phenylthiocarbamide) and PROP (6-n-propylthiouracil) that contain the functional N-C=S (thiourea) moiety varies among individuals, and is a heritable trait (Bartoshuk et al 1994, Tepper 2008). Taste sensitivity and taste blindness to PROP/PTC is evident in all populations with varying frequencies and is to some degree affected by gender and age. Individuals can be separated into taster (perceive PROP/PTC as bitter) and non-taster (do not perceive PROP/PTC as bitter) groups. Women are in general more sensitive to PROP and men are more likely to fall in the non-taster category (Bartoshuk et al 1994, Whissell-Buechy 1990). Bartoshuk and colleagues further separated the taster group into medium-tasters (MT); individuals who rate the bitter taste of PTC/PROP as moderate and super-tasters (ST); individuals who perceive PTC/PROP as extremely bitter after rating the bitterness of PROP against the saltiness of NaCl (Bartoshuk et al 1994).

Bitter taste perception of PROP is an important determinant of food acceptance in the population, and can shape food preferences, food selection, food likes and dislikes and influence dietary behaviors (Drewnowski et al 2001a, Kaminski et al 2000). Several studies have shown that PROP tasters are more sensitive to other bitter compounds and to sweet compounds (Drewnowski et al 1997, Ly and Drewnowski 2001). Super-taster women compared to non-tasters have a decreased preference for the bitterness of cruciferous vegetables (Drewnowski et al 1999), perceive beer as more bitter (Intranuovo and Powers 1998) and sense more oral irritation and greater bitterness from alcohol (Duffy et al 2004, Lanier et al 2005). PROP sensitivity is also associated with lower

liking of naringin in grapefruit juice, sweet and high fat foods, and green tea (Akella et al 1997, Drewnowski et al 1997) and decreased preference for green vegetables (Brussels sprouts, broccoli, and spinach) (Kaminski et al 2000). The effect of PROP sensitivity can also be seen in children where PROP tasters liked the taste of raw broccoli (Bell and Tepper 2006, Keller et al 2002), and spinach (Turnbull and Matisoo-Smith 2002) less than non-tasters.

In addition to investigating the relationship between PROP taster status and bitter taste perception, several studies have examined the effect of genetically mediated sensitivity to PROP on fat discrimination and liking. Super-tasters were able to better discriminate the fat content and creaminess of milk compared to non-tasters but their liking ratings for creaminess was not different from non-tasters (Hayes and Duffy 2007, Kirkmeyer and Tepper 2003, Tepper and Nurse 1997, Tepper and Nurse 1998). Tepper and Nurse have shown that medium and super-tasters exhibited an increased ability to discriminate high-fat (40%) salad dressing from a low-fat (10%) dressing. Non-tasters were not able to differentiate the two samples but preferred the high fat sample (Tepper and Nurse 1997). Keller and colleagues showed that pre-school non-taster girls gave higher acceptance ratings to full-fat milk than taster girls and boys. Based on data collected from FFQ, the authors also showed that non-taster children's daily intake of discretionary fats (salad dressing, mayonnaise, and butter/margarine) was two-three times higher than tasters (Keller et al 2002).

These findings suggest that PROP tasters have a better ability to discriminate a wide variety of oral stimuli such as sweetness, irritation, creaminess and fattiness of different foods as a result of increased intensity perceptions (Duffy et al 2004, Kirkmeyer

and Tepper 2003, Looy and Weingarten 1992, Tepper and Nurse 1997) and they may be less accepting of bitter, spicy and fatty foods compared to non-tasters (Duffy and Bartoshuk 2000, Tepper 1998, Ullrich et al 2004).

The effect of PROP sensitivity on food selection and higher energy intake is the subject of ongoing debate. Based on self reported dietary intake data, some studies failed to report a link between PROP insensitivity and increased intake of high-fat foods (Drewnowski et al 2007) or consumption of fruits, vegetables, and beverages (Yackinous and Guinard 2002). Lack of reliability of self-reported intakes might be one of the reasons for these conflicting findings. Therefore, studies to directly measure energy intake in general, and fat intake in particular in PROP taster groups are warranted.

Variation in PROP taste perception can also impact body composition and BMI. Studies have looked at the association of PROP taster status and body weight in both men and women but most data point to women as the group most influenced by this phenotype. Goldstein and colleagues showed that PROP sensitivity was inversely associated with BMI where super-tasters had a healthy BMI of 23.5 kg/m^2 and non-taster women had a BMI of ~30kg/m^2 (Goldstein et al 2005). A recent study compared BMI differences across PROP taster groups in 75 male and female Italian volunteers. Results showed that while the mean BMI was in the normal weight range for all 3 taster groups, BMI varied with taster status where PROP non-tasters had higher BMIs and supertasters had lower BMI (Padiglia et al 2010). Several studies had also reported an inverse association between PROP status and BMI in low-diet restraint (Tepper et al 2008), middle-aged women (Tepper and Ullrich 2002). However, some studies have failed to establish a relationship between PROP taster status and BMI in adult men or women

(Drewnowski et al 2007, Kaminski et al 2000, Yackinous and Guinard 2002). There were also no significant differences for BMI and energy intakes among healthy male and female college-aged PROP taster groups (Yackinous and Guinard 2002).

Tepper and colleagues had hypothesized that high preferences for fatty foods by non-tasters women may explain, to some extent, why they are heavier (higher BMI) than super-taster women (Tepper et al 2008). To explore differences in energy intake in response to dietary variety, a laboratory based study compared mean energy intake at three buffet lunches to energy intake from a fixed-item control lunch in PROP non-taster and super-taster women. Results showed that as expected, both non-tasters and super-tasters consumed more energy from the buffet lunches compared to control lunch. However, non-tasters consumed 357±64 kcal (88%) more and super-tasters consumed 198±71 (38%) more than the control lunch (Tepper et al 2011). Although non-tasters were expected to consume more fat , the study failed to support this conclusion. Also, the study could not identify the specific food groups (snacks, beverages) that contributed to the higher energy intakes of non-tasters in the buffet condition. These differences may be small and difficult to detect within a single meal.

The objective of the current study was to investigate the influence of 3-days of eating in a buffet setting on daily energy intake, diet composition, and selection of specific foods as a function of PROP taster status. We hypothesized that non-taster women will consume more daily energy from buffet meals than super-taster women. We also hypothesized that non-taster women will consume more high-fat foods and more calories from fat than super-taster women.

2.3 Methods

2.3.1 Subjects

Participants were recruited from Rutgers campus and the local community. Subjects were healthy women, 18-45 yr of age with a BMI of (18-25) kg/m^2. The ability to taste PROP is inversely associated with body weight therefore, weight stable subjects who had no weight fluctuations of >2kg in the 3 months prior to the study were selected. Potential subjects were asked to complete three questionnaires; A general questionnaire to collect demographic data, the Eating Attitude Test (EAT) to detect atypical attitudes toward food (Garner and Garfinkel 1979), and the Three Factor Eating Questionnaire to measure dietary restraint, disinhibition and perceived hunger (Stunkard and Messick 1985). Subjects were excluded if they were restrained eaters (defined as a score of >11); pregnant or lactating, or if they had chronic disease (i.e. diabetes or kidney disease) and were on medication that could affect taste, food intake, and appetite. Also excluded from the study were subjects who had major food allergies (i.e. wheat, dairy, nuts) or were engaged in organized sports or physical activity of more than 3-5 h/wk. Subjects' body weight (kg) was measured using an electronic scale to the nearest 0.2 kg and height (cm) was measured using a stadiometer to the nearest 0.2 cm. Measures were taken over lightweight clothing and without shoes in the sensory lab. 78 subjects participated in this study. Three subjects were excluded from the study for not adhering to the study protocol. Subject characteristics are given in Table 2.1. The experimental protocol was approved by the Rutgers University Institutional Review Board. All subjects gave written informed consent to participate in the study and received financial compensation for their participation.

2.3.2 PROP Screening and Taster Status

Participants were screened and classified into three non-taster (NT), medium-taster (MT), and super-taster (ST) groups based on their sensitivity to PROP using a filter paper method developed by Zhao and colleagues (Zhao et al 2003). Subjects were asked to place filter paper disks impregnated with 50mM PROP (6-n-propylthiouracil, Sigma-Aldrich, St. Louis, MO) or 1M NaCl(VWR Scientific, Bridgeport, NJ) on the tip of their tongues and rate the intensity of the perceived taste by drawing a line across a 100mm, semi-logarithmic Labeled Magnitude Scale (LMS) (Green et al 1996). The scale is anchored at each end with descriptors "barely detectable" and "strongest imaginable". This is a valid method relative to established classification methods with high test-retest reliability (Tepper and Friedman 1991, Zhao et al 2003). NaCl is used as a reference standard since taste sensitivity of this compound doesn't vary as a function of PROP taste status (Tepper et al 2001, Zhao et al 2003).

For the PROP screening, subjects rinsed with water, placed the NaCl-impregnated disk on the tip of the tongue until it was wet, and rated the intensity of the taste on the LMS. Subjects rinsed again and repeated the procedure for the PROP-impregnated disk. The disks were identified with random code numbers to avoid subject bias. Subjects were classified into three groups based on cutoff scores for PROP intensity that were derived empirically in our previous study (Zhao et al 2003). The cutoff score for NT and ST was <15 and >67 respectively. Subjects who rated PROP intensity between 16 and 66 points on the LMS were classified as MT. The rational for using NaCl as a reference standard is due to the fact that ratings of PROP are generally much lower than NaCl for non-tasters

while super-tasters give much higher PROP ratings compared to NaCl. Thus, NaCl rating is used to classify subjects who gave borderline ratings to PROP.

2.3.3 Test Foods

Fixed item meals: Subjects were offered a choice of two main course entrées (with relatively similar macronutrient composition) for lunch and dinner each day (Table 2.1). They also had access to a salad bar, fresh fruits, desserts (choice of two types) and choice of beverage. Subjects could consume as much or as little of the food as they wanted but they could only go back for additional helpings of the items they selected for that meal. A choice of main course and dessert was offered in order to guard against the possibility of subjects not eating a food they disliked if only one food choice was offered.

Buffet meals: Similar to fixed item meals, subjects had free access to a variety of foods, a salad bar as well as a variety of desserts, fruits, and beverages, and could eat as much or as little as they desired. However, unlike fixed item meals, they could personalize and modify their entrées and could select more than one type of dessert or beverage.. For each lunch and dinner meal, different entrées were served in order to provide variety across the buffet meals and to avoid monotony (Table 2.1). A list of serving sizes and macronutrient composition of all foods is provided in Appendix 2. All items were either pre-weighed at the time of serving and offered in standard USDA portion sizes (US Department of Agriculture 2008) or were commercially packaged and served in their original containers (i.e. chips, beverages). Labels indicating the name of the foods were displayed with every food item.

Table 2.1 Lunch and dinner menus during 6 day buffet study

	Fixed-Item Meals			**Buffet Meals**		
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
Breakfast	Standard 300 Kcal breakfast (fruit yogurt, coffee/tea, toast, orange juice)					
Lunch	Tuna salad or Grilled cheese sandwich; Brownie; Assortment of regular or diet soft drinks and water	Beef Taco or Manicotti w/tomato sauce, Ice cream sandwich; Assortment of regular or diet soft drinks and water	Macaroni & cheese or Spinach Loraine quiche; Cookies; Assortment of regular or diet soft drinks and water	Sandwich bar: breads/rolls; sandwich meats (turkey, ham, roast beef, salami), cheeses; side salads (potato, macaroni); condiments (mustard, mayo, etc.); chips; cakes/cookies; Assortment of regular or diet soft drinks and water		
	Fruits (Apple, Orange, Grape, Banana) Raw vegetables (lettuce, green peppers, red peppers, onion, carrot, cucumber, grape tomatoes, celery), Choice of ranch or blue cheese dressing					
Dinner	Meat lasagna or Spaghetti with meatballs and broccoli; custard pie or Éclair;	Chicken parmesan or Fried chicken potato wedges; broccoli or carrots; apple pie or peach pie	Beef broccoli with white rice or Chicken broccoli with white rice; Chocolate cake or Pound cake	Ground beef tacos or chicken tacos with yellow rice, black or refried beans, cheese, salsa; cookie bar	Rotisserie chicken or chicken tenders with mashed potatoes, oven fries, dipping sauces; pastry bar	Pasta with Alfredo/ meat/ marinara sauce and garlic bread; make-your-own ice cream sundaes
	Mixed green salad: (lettuce, green/red peppers, onion, carrot, cucumber, grape tomatoes, celery, mushroom) with choice of dressing (red wine vinaigrette, balsamic vinaigrette, Italian, creamy ranch, blue cheese), Fruits (Apple, Orange, Grape, Banana, Honeydew, Cantaloupe), Assortment of regular or diet soft drinks and water					

2.3.4 Study Procedure

A two-week, 3-day/week feeding study was conducted in the laboratory. Subjects consumed all their meals (lunches and dinners) on three consecutive FIXED and BUFF weekdays in the laboratory in individual booths. The FIXED and BUFF conditions were separated by at least 4 washout days (Figure 2.1).

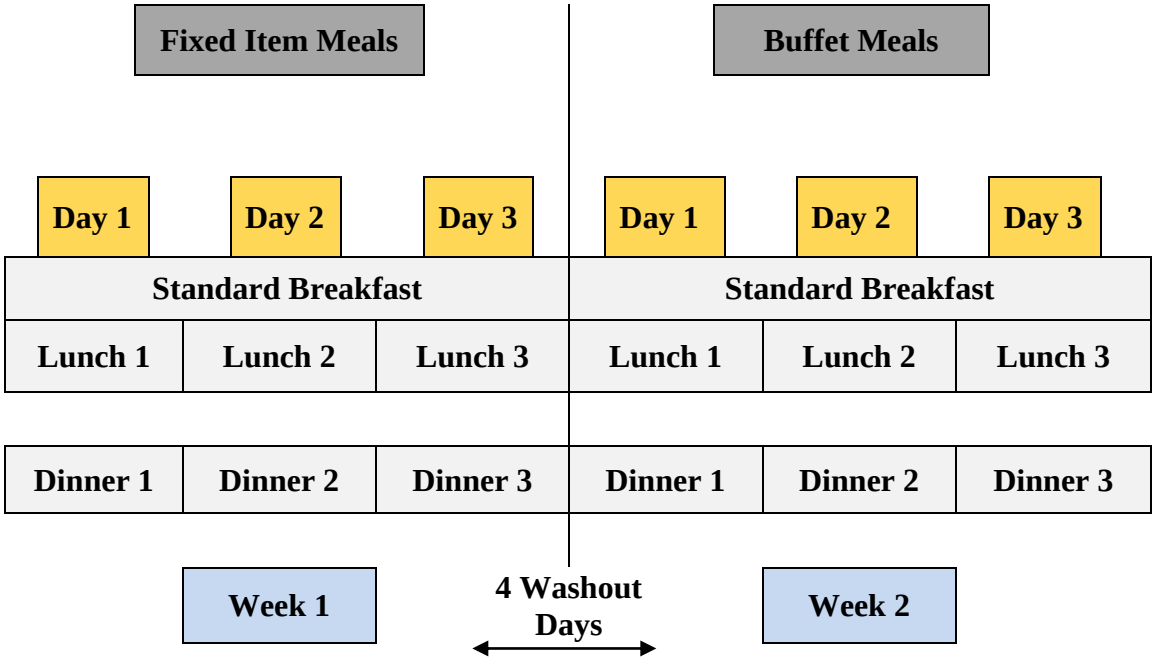
Subjects were provided with a standard 300kcal breakfast to consume each day of the study at home, at least 3 hours prior to coming to the laboratory for lunch. The foods were presented on a table adjacent to the eating area. Subjects placed their selections on their food trays and were free to return for more food as many times as the wanted. They consumed their meals in individual testing booths in the laboratory. While in the booths, subjects were free to read or listen to music but were prohibited from interacting with each other. At the end of each meal, empty packages were counted and plate waste was collected and weighed (to the nearest 0.2g). Food intake was measured by subtracting uneaten food from the starting weight of each package.

During the 6 study days, subjects were asked to only eat the foods provided to them by the researchers, therefore, they were given snacks and beverages to take with them to consume during the times that they were not in the lab. Subjects were instructed to record non-provided food items that were consumed in meal logs provided by the researchers on the day prior to the start of the study. They were also asked to record any provided food items that were consumed outside of the laboratory.

The sample size for this study was based on previous research with similar subject population and test meal (Goldstein et al 2007). Based on the power analysis, a sample size of 25 subjects per group would allow the detection of a 250 kcal (1046 kJ) or more

difference in energy intake among all three taster groups at a significance level of 0.05 and a power of 80%.

Figure 2.1 Outline of buffet study experiment



2.3.5 Data Analysis

Nutritional information: Nutrient intakes were analyzed using NDSR software (Nutrition Data System for Research (version2010; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). The software calculated the energy content and nutrient composition of the foods consumed based on the weight of each item. The major outcome variables in the study were: energy intake (kcal); percent energy from fat,

carbohydrate, and protein; selection of foods from major food groups (fruits, vegetables...) and subgroups (sweets, sweetened beverages...). Major food groups were constructed based on USDA food groups (fruits, vegetables, meats, dairy, and grains). Food subgroups were developed to reflect intake of PROP taster groups.

Statistical analysis: Daily energy and macronutrient intakes were measured and analyzed for each day of the study (6-day study, 3 days per FIXED and BUFF conditions). Preliminary analyses (General Linear Model procedure) showed no trends or significant differences in energy intake or macronutrient selection across days. Therefore, a nested analysis of covariance (ANCOVA) model was used to assess the daily intakes within each study condition where daily intake was nested with study conditions (FIXED and BUFF). Data were analyzed to examine differences in energy intake and macronutrient selection as a function of condition, taster status, and condition-taster interaction. Analysis of covariance (ANCOVA) was used to further analyze the data by looking at each study condition separately.

Data (presented as mean values ± SEM) were analyzed using SAS for Windows (version 9.2 SAS institute, Cary, NC). Analysis of co-variance (ANCOVA) was conducted to account for differences in body composition (body weight) among subjects. Post-hoc comparisons were conducted using Tukey's test where appropriate. Repeated measures ANOVA was employed to look at differences in hunger and fullness ratings as a function of taster status and study condition. Spearman correlation analysis was conducted to evaluate the association between hunger/fullness ratings (before and after meals) and energy intake at lunch and dinner. Statistical significance was set at $p \leq 0.05$ for all tests.

2.4 Results

2.4.1 Subject characteristics

After pre-screening for weight, restrained eating score, and PROP taster status, 75 volunteers (n=25 per taster group) with a mean ± SE age of 26.2 ± 1.3 years participated in the study. Forty seven percent of the subjects were Caucasian, 11 % were Hispanic, 29% were Asian, and 4% were Indian. Subject characteristics are shown in Table 2.2 There were no significant differences in age, weight, height or restraint score as a function of taster status.

Table 2.2 Subject Characteristics. [1] Values are number of participants. [2] Values are mean ± SEM

	Subject Demographics			
	Non-Taster (n=25)	**Medium-Taster (n=25)**	**Super-Taster (n=25)**	**All Taster Groups (n=75)**
Ethnicity:[1]				
Caucasian	16	10	9	35
African-American	0	0	0	0
Hispanic	4	3	1	8
Asian	3	12	14	29
Other	2	1	0	3
Age (yr)[2]	24.7 ± 1.2	26.4 ± 1.1	27.1 ± 1.5	26.1 ± 1.3
Weight (Kg)	59.6 ± 1.9	54.6 ± 1.6	54.3 ± 1.8	56.2 ± 1.8
Height (m)	1.6 ± 0.01	1.6 ± 0.01	1.6 ± 0.01	1.6 ± 0.01
BMI (kg/m^2)	22.0 ± 0.6	21.3 ± 0.5	21.3 ± 0.6	21.5 ± 0.6
Restraint Score	6.6 ± 0.7	7.1 ± 0.3	7.1 ± 0.6	7.3 ± 0.5

2.4.2 Mean energy and macronutrient intake in FIXED and BUFF conditions

For all subjects, mean energy (kcal/day) intake varied across study conditions [F (2,431) = 40.8; p≤ 0.0001]. As expected, energy intake was significantly higher during BUFF condition (2098 ± 28) compared to FIXED condition (1844 ±28) (Table 2.3). When exposed to a buffet style eating environment, energy intake increased by 254 Kcal. All subjects consumed more fat (% En) [F (2,431) = 15.31; p≤ 0.0001] and less carbohydrate (% En) [F (2,431) =9.3; p≤ 0.002] in the BUFF condition compared to the FIXED condition. However, no significant difference in protein intake was observed. The intake of saturated fat [F (2,431) = 9.9; p≤ 0.0001] was also higher in the BUFF condition relative to FIXED condition.

Table 2.3 Energy intake and macronutrient selection for all subjects by study condition. [1]Values are means ± SEM averaged across all 6-days of the study. [2] Means in the same row with different superscripts (a,b) are significantly different (p ≤ 0.05)

	Fixed-Item Meal[1]	**Buffet Meal**
Energy Intake (Kcal)[2]	1844 ± 28 [a]	2098 ± 28 [b]
Fat Intake (% En)	32.6 ± 0.4 [a]	34.5 ± 0.4 [b]
Saturated Fat (% En)	11.3 ± 0.2	11.8 ± 0.3
Saturated Fat (g)	23.2 ± 0.6 [a]	27.9 ± 0.6 [b]
Monounsaturated Fat (g)	24.5 ± 0.6 [a]	29.5 ± 0.7 [b]
Polyunsaturated Fat (g)	14 ± 0.4 [a]	18.3 ± 0.6 [b]
Cholesterol (mg)	216.5 ± 5.2	206.1 ± 5.2
CHO Intake (% En)	52.7 ± 0.4 [a]	50.9 ± 0.4 [b]
Pro Intake (% En)	16.5 ± 0.2	16 ± 0.2

Additionally, since Asian subjects comprised the majority of the medium-taster and super-taster groups, in order to control for this, we analyzed the data without the data obtained from Asian women. Energy and macronutrient intake varied among taster groups [F (2,253) = 2.9-34.5, $p \leq$ 0.001-0.05]. NT and MT consumed significantly more energy and fat in the BUFF condition than the FIXED condition ($p \leq$ 0.01-0.05). There were no differences in fat and carbohydrate intake among groups.

2.4.3 Effect of PROP taster status on mean energy and macronutrient intake in FIXED and BUFF conditions

We had hypothesized that energy intakes of non-tasters would be higher in a buffet setting compared to energy intakes of super-tasters. Results showed that in the BUFF condition, energy intake of NT and MT (2149 ± 49 Kcal/d and 2209 ± 48 Kcal/d respectively) were significantly higher than that of ST (1933 ± 50 kcal/d) ($p \leq$ 0.006-0.0003) subsequent to taster x condition interaction [F (2,215) = 7.3; $p \leq$ 0.001]. NT and MT consumed an average of 246 kcal/d more than ST. There were no differences in energy intake of taster groups during the FIXED condition (Table 2.4). Although percent fat intake did not vary by taster status in the BUFF condition, gram intakes of saturated fat and cholesterol [F (2,215) = 3.9-5.9; $p \leq$ 0.02-0.003] varied among the groups and were higher for NT and MT compared to ST ($p \leq$ 0.0.01-0.06). Higher protein intake was a consistent feature of the intake pattern of ST in this study. ST consumed significantly more protein (% En) than NT and MT in both the FIXED and BUFF conditions [F (2,215) = 3.4-3.8; $p \leq$ 0.03-0.04] subsequent to the taster main effect ($p \leq$ 0.01-0.04) (Table 2.4).

Table 2.4 Daily energy and macronutrient intake of PROP taster groups in FIXED and BUFF conditions. [1]Values are means ± SEM. [2] For each condition, means with different superscripts (a,b) are significantly different ($p \leq 0.05$)

	Fixed-Item Meal[1]			**Buffet Meal**		
	NT	**MT**	**ST**	**NT**	**MT**	**ST**
Energy Intake [2] (Kcal)	1807 ± 45	1930 ± 43	1795 ±45	2149± 49[a]	2209± 48[a]	1933± 50[b]
Fat Intake (% En)	33.3 ± 0.6	32.9 ± 0.6	32 ± 0.6	35.3 ± 0.7	35.4 ± 0.6	33.3 ± 0.7
Saturated Fat (% En)	11.7 ± 0.4	11.3 ± 0.3	10.9 ± 0.4	12.7 ± 0.4	11.9 ± 0.5	10.6 ± 0.6
Saturated Fat (g)	24.2 ± 1	23.9 ± 0.9	21.5 ± 0.9	30.9± 1.5[a]	29.2± 1.3[a]	23.7 ± 2[b]
Monounsaturated Fat (g)	24.4 ± 1	25.8 ± 1.2	23.2 ± 1	30.6 ± 1.5	30.9 ± 1	26.8 ± 1.9
Polyunsaturated Fat (g)	15 ± 0.8	14.4 ± 0.7	12.5 ± 0.9	18.4 ± 1.4	19.2 ± 1.2	17.3 ±1.5
Cholesterol (mg)	204.8±8.9[a]	236.2±8.6[b]	207.3±8.9[a]	217±9.2[a]	220.2±15[a]	179±12.3[b]
CHO Intake (% En)	52.4 ± 0.8	52.5 ± 0.7	53.2 ± 0.8	50.2 ± 0.8	50.6 ± 0.7	51.7 ± 0.8
Pro Intake (% En)	16.0 ± 0.3[a]	16.3± 0.3[a]	17.2 ±0.3[b]	15.9± 0.3[a]	15.5± 0.3[a]	16.7± 0.3[b]

2.4.4 Mean energy and macronutrient intake of PROP taster groups across all study days

Across all days of the study, intakes of energy (kcal/d), fat (% En), saturated fat (g) and protein (%En) varied for taster groups [$F(2,431) = 3\text{-}40.8$, $p \leq 0.08\text{-}0.0001$)]. NT

and MT consumed significantly more energy p≤ 0.02-0.0001) compared to ST. Mean energy intake of NT and MT was about 164 kcal higher than ST. Fat and saturated fat intake of NT and MT was also higher than ST (p≤ 0.0001-0.007). Protein intake of ST was significantly higher than NT and MT (p≤ 0.001-0.004). Carbohydrate intake did not differ across the taster groups (Table 2.5).

Table 2.5 Daily energy and macronutrient intakes of PROP taster groups from all study days. [1]Values are means ± SEM. [2] Means in the same row with different superscripts (a,b) are significantly different ($p \leq 0.05$)

	PROP Taster Status[1]		
	NT	**MT**	**ST**
Energy Intake (Kcal)[2]	1978 ± 35[a]	2070 ± 34[a]	1864 ± 35[b]
Fat Intake (% En)	34.3 ± 0.5[a]	34.2 ± 0.5[a]	32.5 ± 0.5[b]
Saturated Fat (% En)	12.2 ± 0.3	11.6 ± 0.3	10.7 ± 0.3
Saturated Fat(g)	27.6 ± 0.8 [a]	26.6 ± 0.7 [a]	22.6 ± 0.8 [b]
Monounsaturated Fat (g)	27.5 ± 0.7 [ab]	28.3 ± 0.8 [a]	25 ± 0.9 [b]
Polyunsaturated Fat (g)	16.7 ± 0.6 [a]	16.8 ± 0.6 [a]	14.9 ± 0.7 [b]
Cholesterol (mg)	213.2 ± 6.9 [ab]	227 ± 7.7 [a]	192.2 ± 7 [b]
CHO Intake (% En)	51.3 ± 0.5	51.6 ± 0.5	52.5 ± 0.5
Pro Intake (% En)	16 ± 0.2[a]	16 ± 0.2[a]	17 ± 0.2[b]

2.4.5 Effect of PROP taster status on meal and snack intakes during FIXED and BUFF conditions

The next stage of analysis examined how taster status influenced energy and macronutrient intake of lunch and dinner under the two study conditions (Table 2.6).

FIXED condition: Fat and carbohydrate intakes varied by taster group during lunch meals [$F(2,215) = 3.5\text{-}4.6$, $p \leq 0.01\text{-}0.03$]. Fat intake of NT was significantly higher than ST ($p \leq 0.01$). However, NT consumed less carbohydrate than ST ($p \leq 0.003$). There were no differences in energy and protein intake of taster groups during FIXED lunch meals.

During dinner meals, protein intake of ST was significantly higher than NT and MT ($p \leq 0.01\text{-}0.03$) subsequent to taster effect [$F(2,215) = 3.4$, $p \leq 0.03$]. No other differences in macronutrient intake were observed (Table 2.6).

Energy intake of snacks varied among taster groups [$F(2,215) = 5$, $p \leq 0.008$]. NT and MT consumed significantly more energy than ST ($p \leq 0.005\text{-}0.008$). No other differences in macronutrient intake among taster groups were found

BUFF condition: There were no differences in energy or macronutrient intake among taster groups from lunch meals (Table 2.6).

During dinner meals, energy, fat, and protein intake varied among taster groups [$F(2,215) = 4.1\text{-}6.9$, $p \leq 0.01\text{-}0.001$]. NT and MT consumed more energy ($p \leq 0.01$) and fat ($p \leq 0.01\text{-}0.0003$) compared to ST. However, protein intake of ST was significantly higher than NT ($p \leq 0.005$) but not different than MT.

Energy intake from snacks varied as a result of taster status [$F(2,203) = 3.4$, $p \leq 0.04$] where NT and MT consumed significantly more energy than ST ($p \leq 0.01$). No other differences in macronutrient intake were observed (Table 2.6).

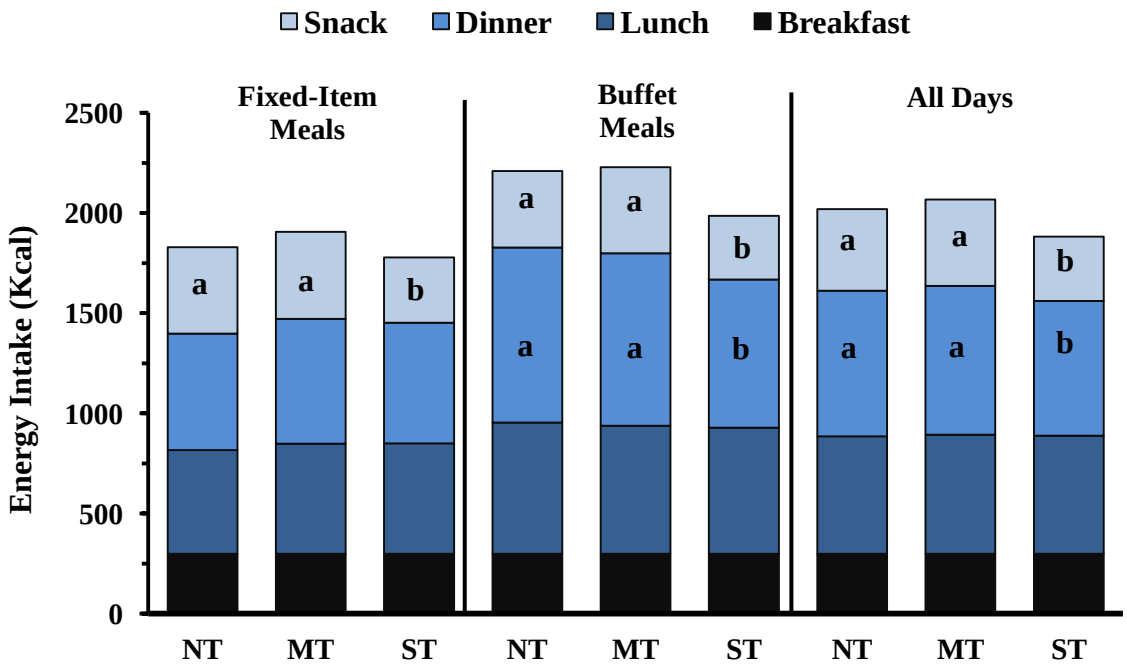
Table 2.6 Energy and macronutrient intake during FIXED and BUFF conditions as a function of PROP taster status. [1]Values are means ± SEM. [2] For each condition, means with different superscripts (a,b) are significantly different ($p \leq 0.05$)

		Fixed-Item Meals[1]			Buffet Meals		
		NT	**MT**	**ST**	**NT**	**MT**	**ST**
Lunch	**Energy Intake (Kcal)[2]**	519 ± 23	547 ± 22	550 ± 23	667 ± 25	631 ± 24	623 ± 25
	Fat Intake (% En)	46.7±1.1^{a}	44.1±1.1^{ab}	42.8±1.1^{b}	42.4 ±1.2	44.1± 1.2	40.1± 1.2
	CHO Intake (% En)	38.3±1.3^{a}	42.1±1.3^{ab}	43.9±1.3^{b}	38.9 ±1.3	38.6± 1.2	42.1 ± 1.3
	Pro Intake (% En)	1.36 ±0.5	15.4 ±0.5	15.2±0.5	19.4 ±0.6	18.2 ±0.6	19.1 ±0.6
Dinner	**Energy Intake (Kcal)**	578 ± 27	623 ± 26	604 ± 28	876 ± 34^{a}	862 ± 33^{a}	738± 35^{b}
	Fat Intake (% En)	36.6±0.8	37.1 ± 0.7	35.1 ± 0.8	40.7±0.9^{a}	39.1±0.9^{a}	35.8±0.9^{b}
	CHO Intake (% En)	39.7±1.1	38.9±1.1	38.5±1.1	42.9±1.1	42.8 ± 1	44.1 ± 1.1
	Pro Intake (% En)	24.9±0.7^{a}	24.8 ± 0.7^{a}	27.3±0.7^{b}	17.8± 0.6^{a}	18.7±0.6^{ab}	20.3± 0.6^{b}
Snacks	**Energy Intake (Kcal)**	431±27^{a}	434±27^{a}	325±28^{b}	382±31^{a}	430±30^{a}	318±32^{b}
	Fat Intake (% En)	27.1±1.9	24.3±1.8	26.3±1.9	24.6±2.1	26.8±2.1	28.5±2.1
	CHO Intake (% En)	69.2±2.5	71.7±2.5	68.8±2.6	69.5±2.9	67.9±2.9	60.3±2.9
	Pro Intake (% En)	6.6±0.5	6.8±0.5	6.6±0.5	6.2±0.5	7.1±0.5	6.6±0.6

2.4.6 Contribution of breakfast, lunch, dinner, and snacks to daily energy intake (kcal/d) of PROP taster groups during FIXED and BUFF meals

The contribution of meals and snacks to daily energy intakes are depicted in Figure 2.2. In the FIXED condition, NT, and MT consumed more energy from snacks than ST ($p \leq 0.005$-0.008), however, this increased energy intake did not significantly influence daily energy intake of taster groups. In the BUFF condition, NT and ST consumed more energy from dinner meals ($p \leq 0.01$) and snacks ($p \leq 0.01$). Across all days of the study, energy intakes of NT and MT from dinner meals ($p \leq 0.02$) and snacks ($p \leq 0.0002$-0.004) were significantly higher than that of ST. Overall, Higher energy intake of NT and MT from BUFF dinner meals and snacks contributed to the higher daily energy intake of NT and MT compared to ST.

Figure 2.2 Contribution of meals and snacks (consumed outside the lab) to daily energy intakes of taster groups during each condition of the study. For each condition, different superscripts (a,b) are significantly different ($p \leq 0.05$)



2.4.7 Mean intake of food groups (servings/d) as a function of PROP taster status

Foods offered to subjects during FIXED and BUFF conditions were initially classified into 5 major groups (based on USDA food groups). These major food groups include: fruits, vegetables, meats, dairy, and grains. Food groups were further divided into subgroups to reflect intake of PROP taster groups. There were no differences in food group intake (servings/d) for taster groups in FIXED and BUFF conditions subsequent to taster x condition interaction. However, when intake data were collapsed for all days of the study, we saw differences as a function of taster status. Intakes of fruits, vegetables, dark green vegetables, unsweetened dairy, grains, cakes, and added fats varied among groups [$F(2,427) = 3.8\text{-}9.9$, $p \leq 0.001\text{-}0.03$].

NT consumed fewer servings of fruits ($p \leq 0.003\text{-}0.0001$) and vegetables ($p \leq 0.0001\text{-}0.0003$) compared to MT and ST. Intakes of cakes ($p \leq 0.0003\text{-}0.003$), and added fats ($p \leq 0.0002$) were higher in NT and MT compared to ST (Table 2.7).

Table 2.7. Food group intake (servings/d) of PROP taster groups across all days of the study. [1]Values are mean ± SEM. [2]Means of each food group with different superscripts (a,b) denotes significant difference ($p \leq 0.003$) after adjusting with Bonferroni correction

	PROP Taster Status[1]		
	NT	**MT**	**ST**
Fruits[2]	3 ± 0.1 [a]	3.7 ± 0.1 [b]	3.5 ± 0.1 [b]
Vegetables	3.7 ± 0.1 [a]	4.4 ± 0.1 [b]	4.5± 0.1 [b]
Dark green vegetables	1.8 ± 0.1	2.1 ± 0.1	2.2 ± 0.1
Meats	4.5 ± 0.2	5 ± 0.2	4.7 ± 0.2
Dairy	1.9 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Sweetened dairy	0.8 ± 0.03	0.8 ± 0.03	0.9 ± 0.03
Unsweetened dairy	1 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
Grains	6.7 ± 0.2	6.7 ± 0.2	6 ± 0.2
Carbohydrates	4.5 ± 0.1	4.5 ± 0.1	4.3 ± 0.1
Cakes	0.9 ± 0.1 [a]	1 ± 0.1 [a]	0.5 ± 0.1 [b]
Sweet snacks	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
Salty snacks	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Added fats	3.8 ± 0.2 [a]	4.1 ± 0.2 [a]	3 ± 0.2 [b]
Added sugars + candy+ chocolate	1.1 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
Beverages	4.6 ± 0.3	4.9 ± 0.2	5 ± 0.3
Sweetened beverages	1.4 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
Unsweetened beverages	3.2 ± 0.2	3.4 ± 0.2	3.8 ± 0.2
Condiments	1 ± 0.1	0.8 ± 0.1	0.9 ± 0.1

2.5 Discussion

The primary objective of this study was to determine if non-taster women would consume more daily energy and fat than super-taster women when eating in a buffet setting. Results showed that during the buffet condition, daily energy intakes were higher in both non-tasters and medium-tasters relative to super-tasters, and both groups consumed more grams of fat per day (but not more fat as %-En/day) than super-tasters. These data support our hypothesis that NT and MT women are more susceptible to eating in a buffet setting which could constitute one mechanism for increased energy intake and body weight gain. High variety eating environment is in part responsible for influencing increased food intake which is one underlying cause of increased body weight.

Examination of meals and snacks revealed which eating occasions contributed to differences in daily energy intake during BUFF feeding. Results showed that during the buffet condition, NT and MT consumed more energy and % fat during dinner and more energy from snacks compared to ST. Buffet lunches did not contribute to higher intakes of energy or fat in the NT and MT groups. Since dinner is typically the largest and most varied meal of the day, it is likely to have the greatest impact on daily intakes of energy and fat.

Two dietary patterns persisted across both conditions of our experiment. First, NT and MT consumed more energy from snacks during each study condition and across all days of the study than did ST women. These data suggest that increased snacking may be a characteristic feature of the eating patterns of NT and MT women, regardless of the diet condition they were under. Second, in contrast, ST women consumed more protein (%-En) during both conditions and across all days of the study than did NT and MT. Since

protein is considered more satiating than the other macronutrients (Astrup 2005), it is possible that this dietary pattern contributed to lower daily energy intakes in ST relative to the other groups. Future work should address the role of protein in the hunger and satiety responses of women classified by PROP status.

We also attempted to identify food groups that might have contributed to differences in energy intakes among taster groups. However, since the mean number of servings consumed during each 3-day phase of the experiment was relatively low, differences between groups were difficult to detect. When these data were collapsed across all days of the study, NT and MT consumed more cakes, and added fats, while MT and ST consumed more serving of fruits and vegetables. Since bakery products and added fats are more calorically dense than fruits and vegetables, these data are consistent with the energy differences we observed between groups.

Our study also resolves an important inconsistency in the literature on the role of PROP status in eating behavior and obesity. According to current hypotheses, non-tasters have higher preferences and intakes of bitter-tasting fruits and vegetables (grapefruit, spinach, olives, cucumber, and broccoli and kale), they also consume more bitter-tasting vegetables, fatty foods and energy (full-fat milk, meats, and cheeses) (Bell and Tepper 2006, Dinehart et al 2006, Drewnowski et al 1997, Keller et al 2002, Kirkmeyer and Tepper 2003, Turnbull and Matisoo-Smith 2002). However, diets associated with obesity are typically higher in fat and energy, but lower in fruits and vegetables. Thus, the hypothesized diet patterns of non-tasters would not be expected to lead to overweight and obesity. Nevertheless, our study showed that when given a choice, non-tasters forgo fruits and vegetables in favor of more energy-dense, high-fat foods. Thus, it is plausible that

chronic exposure to a variety of energy-dense foods in the everyday eating environment coupled with a sedentary lifestyle could lead to higher weight in non-taster women as we have observed in our previous work (Goldstein et al 2005, Tepper and Ullrich 2002, Tepper et al 2009).

The present results agree with and extend the results of our previous study in which young women with similar characteristics consumed buffet lunches in the laboratory (Tepper et al 2011). In that study, PROP non-taster and super-taster women were fed buffet lunches or an *ad-libitum* control lunch. Non-tasters consumed 357 kcal more during the buffet lunches than the control lunch (88% more), whereas super-tasters consumed 198 kcal more during the buffet lunches relative to the control (38% more). Our earlier study failed to show differences in fat selection between taster groups, however, these differences might have been undetectable in the timeframe of single meals.

In another study with a similar time-frame (3-day periods), Stubbs and colleagues examined the effect of food variety (5, 10, or 15 different foods per day) on energy intake in men living in a residential feeding laboratory. Results showed that the high-variety condition led to a 15% rise in daily energy intake relative to the low-variety condition (Stubbs et al 2001). Our study showed a similar increase (13.8%) in daily energy intake in young women exposed to buffet feeding regimen. However, when the data were examined by PROP taster group, NT and MT increased their daily intakes by 22.2% and 19.4%, respectively, relative to ST who increased their daily intake by only 10.4%. Thus, characterizing women by their PROP taster status exposed individual differences in dietary susceptibility to variety that would not have been otherwise observed.

Our study has several strengths and limitations. The strengths of our study include evaluating energy and macronutrient intake of PTOP taster groups in a controlled laboratory setting without the need to rely on self-reported food intakes and dietary recalls. The design of the study also provided all taster groups with the same food choices for lunch, dinner and snacks and therefore, represents a more controlled environment to assess dietary intakes of the subjects. Conducting the buffet food intake study in the laboratory could also be one of the limitations of our study. Even though it provides a better environment to monitor food intake, it does not represent the environment where individuals would normally consume their meals. The majority of our subjects were college-aged healthy lean women who do not entirely represent the community. Finally, Asian women were more highly represented in the MT and ST groups in our study, which could have influenced our results. To guard against this possibility, we also eliminated all Asian women from the cohort and reanalyzed the data. Except for a loss of statistical power associated with smaller sample size, the results were similar with or without these women. Thus, ethnic differences in PROP tasting and eating habits did not alter our findings.

Future studies should include overweight and obese women to determine if the relationships observed here, are relevant to the general female population. PROP status is an easy-to-measure trait (Zhao et al 2003) that could have utility in the clinical setting for identifying women at risk for increased food intake and body weight.

CHAPTER 3

Consumption of High Fat Soup Preload Leads to Differences in Short-Term Energy Adjustment in PROP Non-Taster Women Compared to Super-Taster Women

3.1 Abstract

Taste blindness to the bitter compound PROP (6-n-propylthiouracil) is considered a genetic marker for food selection and adiposity. We have previously shown that PROP non-taster (NT) women have higher BMIs and consume more fat and energy than either medium-taster (MT) or super-taster (ST) women. These data imply that differences in dietary selection underlie the body weight differences among PROP taster groups. However, no studies have investigated caloric compensation in women classified by PROP status. We investigated if NT women would compensate less accurately for the calories in a high-fat soup preload in a subsequent test meal compared to MT and ST women. Energy intake from a test meal was measured in 75 healthy non-diet-restrained, lean women (BMI= 21.8 ± 0.3) 30 min after the ingestion of a high-fat soup preload (0.8 Kcal/g; 50% calories from fat), calculated to represent 10% of resting energy expenditure for each subject, or the same volume of water. Subjects ate a standard 300 Kcal breakfast (3 hrs prior to preload) and an *ad-libitum* buffet lunch in the lab on two occasions separated by 6 washout days. There were no differences in energy or macronutrient intake across the taster groups in the water condition. In the soup preload condition, NT consumed more energy than ST (690 ± 48 Kcal/d and 542 ± 45 Kcal/d in NT vs. ST respectively; $p \leq 0.03$). NT women also consumed more fat ($p \leq 0.02$-0.004) from the test meal than MT and ST. Caloric compensation at the lunch meal in response to the energy content of the high-fat/high-energy soup preload varied among taster groups. Non-tasters undercompensated and over-ate at the buffet lunch compared to MT and ST ($p \leq 0.01$-0.002). On the other hand, MT and ST overcompensated and ate less at lunch after the soup preload. Small discrepancies in short-term energy compensation may play a role in positive energy balance and increased adiposity in women with the PROP non-taster phenotype. The classification of women by PROP status may identify women at increased risk for excess body weight and the future development of obesity.

3.2 Introduction

The ability to taste bitter tasting compounds PROP (6-n-propylthiouracil) and PTC (phenylthiocarbamide) that contain a thiourea moiety (N-C=S) is genetically determined (Bartoshuk et al 1994, Tepper 2008) and follows Mendelian principles (Guo and Reed 2001, Olson et al 1989). Taste sensitivity and taste blindness to PROP and PTC exists in all populations with varying frequencies, and is partially influenced by age and gender. Women are in general more sensitive to PROP than men. Individuals who can taste the bitterness of PROP/PTC are classified as tasters, and individuals who are taste blind to PROP/PTC are classified as non-tasters (Bartoshuk et al 1994, Whissell-Buechy 1990). About 30% of the adult Caucasian population is genetically taste blind to PROP while 70% of the population falls in the taster category. The frequency of non-tasters varies in different ethnic populations where around 10%-12% of people in China and Japan and 40% of Indians are classified as non-tasters (Guo and Reed 2001). PROP taster group can be further separated into medium-tasters (MT), who rate the bitter taste of PTC/PROP as moderate, and super-tasters (ST),who perceive PTC/PROP as extremely bitter after rating the bitterness of PROP against the saltiness of NaCl (Bartoshuk et al 1994).

Variation in taste responsiveness to PROP is thought to play a role in food preferences and eating behaviors. Several studies have established an association between PROP responsiveness and enhanced sensitivity to other bitter compounds. PROP tasters perceived more bitterness from bitter vegetables (asparagus, Brussels sprouts, and kale) (Dinehart et al 2006) and beverages (beer, whiskey, espresso and unsweetened grapefruit juice) (Lanier et al 2005). Kaminski and colleagues showed that PROP tasters rated

Brussels sprouts as more bitter than non-tasters. They also showed that the bitter perception of test foods was directly associated with decreased pleasantness and acceptability of those foods (Kaminski et al 2000). It has also been reported that PROP tasters consume fewer vegetables (Dinehart et al 2006) and added fats (Keller et al 2002) while non-tasters prefer high-fat salad dressings and full-fat milk and experience less oral sensation from fats (Hayes and Duffy 2007, Kirkmeyer and Tepper 2003, Tepper and Nurse 1997). Even though some studies support lack of an association between PROP taste perception and preference and intake of bitter or high-fat foods (Drewnowski et al 2007, Yackinous and Guinard 2002), bitter taste perception of PROP is considered an important determinant of food acceptance in the population and plays an important role in shaping food preferences, food selection, and food likes and dislikes (Drewnowski et al 2001a, Kaminski et al 2000).

Several studies have shown that PROP non-tasters, especially non-taster women, have higher BMI than super-taster women (Goldstein et al 2005, Keller and Tepper 2004, Padiglia et al 2010). Greater liking for a variety of foods, including dietary fats may contribute to higher energy intakes in non-taster women and higher body weights. These data support the idea that differences in body weight between non-taster and super-taster groups may be mediated by excess energy intakes driven by food palatability. A study recently conducted in our laboratory reported that eating in a high variety buffet setting stimulates energy intake in NT compared to ST. Results showed that energy intake during buffet lunches compared to a fixed-item lunch increased by 88% in NT and 38% in ST (Tepper et al 2011). These findings match the results of the second chapter of this thesis where energy intake of all subjects was higher in the BUFF condition compared to the

FIXED condition specifically, NT and MT who consumed more energy than ST in the BUFF condition.

To date, virtually all of the literature on PROP tasting and body weight has focused on food palatability as the driver of increased energy intake in non-tasters, ignoring the influence of energy regulation on food intake. Only one study has considered the possibility that the ability to regulate food intake in response to ingested calories may vary across taster groups, and the results of this study were equivocal. Results showed that medium and super-tasters consumed more fat and less carbohydrate than non-tasters, but there was no difference in energy intake of PROP taster groups after consuming three separate *ad libitum* lunches; high-fat, high-carbohydrate, or mixed meal (Kamphuis and Westerterp-Plantenga 2003). The results of the mentioned study were not clear and failed to show evidence of altered regulation in any particular PROP taster group.

Numerous studies have examined short-term compensatory eating in response to a preload to assess short-term regulation of food intake (Akhavan et al 2010, Cecil et al 2005, Flood and Rolls 2007, Spill et al 2011). Generally, semi-solid and liquid foods such as soup, yogurt, and beverages have been used as preloads since these foods provide an easy medium for manipulation of their macronutrient composition. Also, these foods can be consumed quickly and fill the stomach uniformly because they do not involve any chewing.

Consumption of a low energy dense soup as a preload in a variety of forms (broth and vegetables served separately, puréed, chunky-pureed, and chunky), has been shown to reduce energy intake in a subsequent meal (~20%) while reducing hunger and increasing fullness sensation (Flood and Rolls 2007). It has also been shown that the

satiating effect of equal weights of a soup preload is higher than solid foods such as crackers and cheese (Kissileff 1984, Rolls et al 1990). After consuming preloads high in energy content, individuals compensate well and adjust their food intake during an *ad-libitum* meal in response to variations in the energy density of the preload. However, the compensation level is different after fat and carbohydrate preloads. High fat preloads suppress energy intake at lunch less than high carbohydrate preloads (Rolls et al 1994, Rolls and Hammer 1995). The low compensatory influence of fats could be due to their low satiating effect compared to carbohydrates (Gerstein et al 2004).

The present study was designed to examine differences in lunch meal compensation in response to a high-fat soup preload among lean young women classified by PROP taster status. Although the literature as a whole does not support macronutrient-specific compensation in short-term caloric challenges (Foltin et al 1992, Hulshof et al 1995), we chose to administer a high-fat preload based on our prior observations that non-taster women spontaneously consumed more fat relative to super-taster women when allowed to self-select their food in a buffet setting. We therefore hypothesized that non-taster women will consume more energy after a high-fat preload compared to super-tasters. We also hypothesized that changes in food palatability ratings or appetitive responses for the preload will be associated with less precise compensation in non-taster women relative to super-taster women.

3.3 Methods

3.3.1 Subjects

Female participants were recruited from The Rutgers University campus and the local community. Subjects were healthy women, 18-45 yr of age with BMI of (18-25) kg/m2. Subjects were weight stable and had no weight changes of >2kg in the 3 months leading to the study. Potential subjects were asked to complete three questionnaires; A general questionnaire to collect demographic data, the Eating Attitude Test (EAT) to detect atypical attitudes toward food (Garner and Garfinkel 1979), and the Three Factor Eating Questionnaire to measure dietary restraint, disinhibition and perceived hunger (Stunkard and Messick 1985). Subjects were excluded if they were restrained eaters (defined as a score of >11); pregnant or lactating, or if they had chronic disease (i.e. Diabetes or kidney disease) and were on medication that could adversely affect taste, food intake, and appetite. Also excluded from the study were subjects who had major food allergies (i.e. wheat, dairy, nuts) or were engaged in organized sports or physical activity of more than 3-5 h/wk. Subjects' body weight (kg) was measured using an electronic scale to the nearest 0.2 kg and height (cm) was measured using a stadiometer to the nearest 0.2 cm. Measures were taken over lightweight clothing and without shoes in the sensory lab. 75 subjects participated in this study. Subject characteristics are given in Table 3.3. The experimental protocol was approved by the Rutgers University Institutional Review Board, and all subjects gave written informed consent to participate in the study and received financial compensation for their participation.

3.3.2 PROP Screening and Taster Status

Participants were screened and classified into three non-taster (NT), medium-taster (MT), and super-taster (ST) groups based on their sensitivity to PROP using a filter paper method developed by Zhao and colleagues (Zhao et al 2003). Subjects were asked to place filter paper disks impregnated with either 50mM PROP (6-n-propylthiouracil, Sigma-Aldrich, St. Louis, MO) solution or 1 M NaCl (VWR Scientific, Bridgeport, NJ) on the tip of their tongues and rate the intensity of the perceived taste by drawing a line across a 100mm, semi-logarithmic Labeled Magnitude Scale (LMS) (Green et al 1996). The scale is anchored at each end with descriptors "barely detectable" and "strongest imaginable". This is a valid method relative to established classification methods with high test-retest reliability (Tepper et al 2001). NaCl is used as a reference standard since taste sensitivity of this compound does not vary as a function of PROP taste status (Tepper et al 2001, Zhao et al 2003). NT give much lower ratings to PROP than to NaCl and ST give much higher ratings to PROP than to NaCl. Thus, NaCl rating is used to classify subjects who give borderline ratings to PROP.

For the PROP screening, subjects rinsed with water, placed the NaCl-impregnated disk on the tip of the tongue until it was wet, and rated the intensity of the taste on the LMS. Subjects rinsed again and repeated the procedure for the PROP-impregnated disk. The disks were identified with random code numbers to avoid subject bias. Subjects were classified into three groups based on cutoff scores for PROP intensity that were derived empirically in our previous study (Zhao et al 2003). The cutoff score for NT and ST was <15 and >67 respectively. Subjects who did not meet either of these criteria were classified as medium tasters.

3.3.3 Test Foods

Preload: A high fat soup (210 calories/252g, 110 calories from fat) was selected as the preload since its fluid nature provided an easy medium to test and manipulate compared to a solid food (Table 3.1). The soup (Progresso Traditional Potato, Broccoli & Cheese Chowder) provided 0.8 kcal/g with 50 (% En) fat, 38.4 (% En) carbohydrate, and 12.3 (% En) protein. Water was chosen as the control. It is necessary to have a water control to match the gastric destination of the soup preload. The amount (g) of soup served was calculated to deliver 10% of each subject's resting energy expenditure (REE) based on each subject's weight, height, and age using the Harris-Benedict equation (Female: (665.1 + 9.6 * weight) + (1.8 * height) - (4.7 * age). The average serving size based on this calculation was 148g, which provided 109 calories. The amount of water served in the water condition matched the volume of soup. Prior to serving, the soup preload was homogenized by a blender to provide a smooth creamy consistency.

Table 3.1 Nutrition profile (energy and macronutrient composition) of soup preload

	Serving Size = 252 g
Total fat	**12g**
Saturated Fat	3.5g
Trans Fat	0.5g
Polyunsaturated Fat	4.5g
Monounsaturated Fat	2g
Cholesterol	**15g**
Sodium	**860mg**
Total Cholesterol	**20g**
Dietary Fiber	2g
Sugars	2g
Protein	**5g**

Breakfast and Lunch meal: A standard 300kcal breakfast (orange juice, fruit yogurt, slice of toast, and a cup of coffee/tea) was provided to subjects to be consumed in the laboratory. Subjects were instructed to consume the entire breakfast. Lunch was a buffet-style, self selection meal that allowed *ad-libitum* consumption of a variety of foods. The lunch menu included an assortment of breads, chips, sliced turkey breast, ham, salami, American cheese as well as access to a salad bar and a variety of desserts and beverages (Table 3.2). All items were either pre-weighed prior to serving and offered in standard USDA portion sizes (US Department of Agriculture, 2008) or were commercially packaged and served in their original containers (i.e., chips, beverages). Labels indicating the name of the foods were displayed with every food item.

Table 3.2. Lunch menu

	Buffet Meal
Lunch	Deli meats (turkey, roast beef, salami, ham) Cheeses (American, Provolone, Swiss) Bread (wheat, white, sandwich roll, pita pockets) Variety of potato chips
Salad bar	Mixed green salad Raw vegetables (green peppers, red peppers, red onion, carrot, cucumber, grape tomatoes, celery) Choice of dressing (creamy ranch, blue cheese)
Dessert	Chocolate brownie, Lemon meringue pie, Chocolate chip and sugar cookies
Fruits	Apple, Orange, Grapes, Banana
Beverage	Assortment of regular or diet soft drinks, regular and diet iced-tea and water
Condiments	Mayonnaise, mustard, creamy ranch, blue cheese dressing

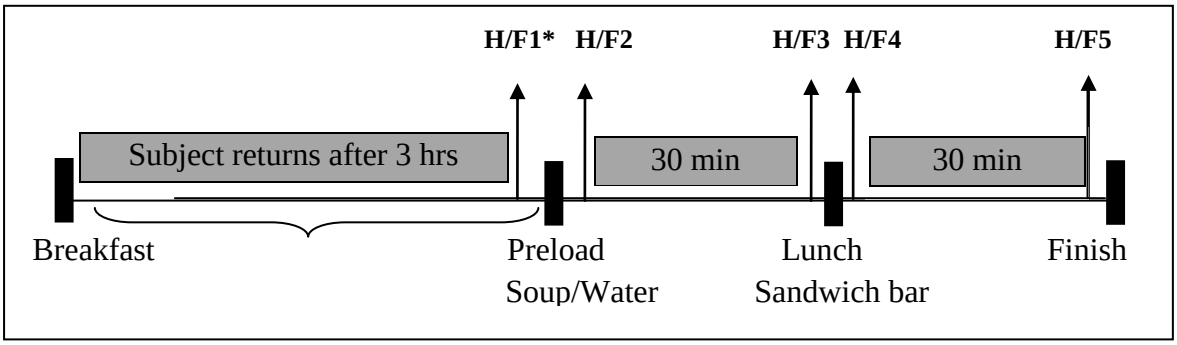
3.3.4 Procedure

The study was conducted in the laboratory in two sessions with 6 washout days in between sessions. Subjects were randomized so that half of the subjects got soup first and the other half got water first. The second week of the study, subjects got the opposite preloads of week one. During the study days, subjects consumed breakfast in the laboratory. There was a three hour gap between breakfast and preload (soup or water) where subjects were free to leave the lab and engage in normal routine activity, but were instructed not to eat or drink anything (except plain water). They returned to the laboratory to consume the preload and lunch (Figure 3.1).

Subjects were asked to rate their hunger and fullness levels five times during the session; directly before and directly after the preload, 30 minutes after the preload (i.e., directly before lunch); and 30 min after lunch. Subjects used 150mm visual analog scales (VAS) to record their ratings. This 30 minute wait has shown to deliver the most precise compensation to the energy content of a preload meal (Rolls and McDermott 1991). Subjects could eat as much or as little of the food they had chosen and could go back for additional helpings.

All foods and beverages were weighed (0.2 g) prior to serving, and reweighed after the end of lunch to determine the amount consumed. For the duration of each session, subjects were seated in individual booths and were allowed to conduct light activity (reading, surfing the web) while waiting for 30 minutes.

Figure 3.1: Outline of the preload experiment. *H/F= Hunger/Fullness ratings



3.3.5 Caloric compensation calculation

Percent caloric compensation for PROP taster groups in water and soup preload conditions was calculated using the following equation:

$$\textbf{\% Compensation} = \frac{\text{Energy intake in water preload}}{\text{(Kcal from soup + Energy intake in soup preload)}}$$

Values of more than 100% indicate over-compensation (i.e. under-eating) and compensation values of less than 100% represent under-compensation (i.e. over-eating) (Rolls et al 1999).

3.3.6 Data Analysis

Nutritional information: Food consumption data were collected after each meal. Briefly, after the completion of each meal, the leftovers were weighed and nutrient

intakes were evaluated using NDSR software (Nutrition Data System for Research (version 2010); Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). The software calculated the energy content and nutrient composition of the foods consumed based on the weight of each item consumed. The major outcome variables of the study were; energy intake (kcal), percent energy from fat, carbohydrate, and protein.

Statistical analysis: Energy and macronutrient intakes of PROP taster groups in the water preload condition were measured and compared to that of the soup preload condition. A repeated measures analysis of variance (ANOVA) model was used with energy, fat, carbohydrate, and protein as the repeated variables. Data were analyzed to measure differences in energy and macronutrient intake as a function of preload condition, taster status, and preload condition-taster interaction. Analysis of variance (ANOVA) was used to evaluate differences in energy compensation among groups in the soup preload condition. Post-hoc comparisons were conducted using Tukey's test where appropriate. Repeated measures ANOVA was employed to examine hunger and fullness ratings as a function of taster status and preload condition. Spearman correlation analysis was conducted to evaluate the association between hunger/fullness ratings (before/after preload and before/after meals) and energy intake at lunch for each preload condition.

Data (presented as mean values ± SEM) were analyzed using SAS for Windows (version 9.2 SAS institute, Cary, NC). Statistical significance was set at $p \leq 0.05$ for all tests.

3.4 Results

3.4.1 Subject characteristics

After pre-screening for weight, restrained eating score, and PROP taster status, 20 non-tasters, 32 medium-tasters, and 23 super-tasters with a mean ± SE age of 24.3 ± 1.1 years and mean BMI of 21.9 ± 0.5 participated in the study. Fifty nine percent of the subjects were Caucasian, 1% were percent African-American, 8% were Hispanic, 24% were Asian, and 8% were Indian. Subject characteristics are shown in Table 3.3. There were no significant differences or trends for any of the variables as a function of taster status.

Table 3.3 Subject characteristics. [1] Values are number of participants. [2] Values are mean ± SEM

	Subject Demographics			
	Non-Taster (n=20)	**Medium-Taster (n=32)**	**Super-Taster (n=23)**	**All Taster Groups (n=75)**
Ethnicity:[1]				
Caucasian	13	16	15	44
African-American	1	0	0	1
Hispanic	2	3	1	6
Asian	2	10	6	18
Other	2	3	1	6
Age (yr)[2]	23.8 ± 1.1	24 ± 0.7	25.1 ± 1.5	24.3 ± 1.1
Weight (Kg)	60 ± 1.8	57.1 ± 1.6	55.1 ± 1.7	57.4 ± 1.7
Height (m)	1.6 ± 0.02	1.6 ± 0.02	1.6 ± 0.02	1.6 ± 0.01
BMI (kg/m^2)	22.3 ± 0.5	22.4 ± 0.6	21.1 ± 0.5	21.9 ± 0.5
Restraint Score	6 ± 0.7	7.2 ± 0.5	6.9 ± 0.6	6.7 ± 0.6

3.4.2 Energy and macronutrient intake as a function of PROP taster status in water and soup preload conditions

Energy and macronutrient intakes of taster groups were examined at lunch in water and preload conditions. Values in table 3.4 are intakes from the buffet lunch only and exclude energy (kcal) and macronutrient intakes from the soup preload. Results showed that energy intake varied across preload conditions [$F(1,144) = 17.2$, $p \leq 0.001$]. As expected, for all subjects, energy intake after the soup preload was lower than after water preload ($p \leq 0.02$-0.001).

Also, as expected, there were no differences in energy or macronutrient intake among the taster groups in the water (control) (Table 3.4).

In the soup condition, energy intake varied among taster groups [$F(2,144) = 33$, $p \leq 0.4$]. NT consumed significantly more energy than ST ($p \leq 0.03$) but not more than MT. NT also consumed more fat (%En) than ST ($p \leq 0.02$) and MT ($p \leq 0.004$). We also saw a directional trend for MT and ST to consume more carbohydrate than NT ($p=0.06$). There were no differences in protein intake among taster groups.

In general, percent difference in energy intake of preload conditions varied across taster groups [$F(2,72) = 4.7$, $p \leq 0.01$]. After the soup preload, ST reduced their meal energy intake (28%) more than did NT (8.3%) ($p \leq 0.004$). Energy intake of MT (22%) was also significantly lower than NT ($p \leq 0.03$) but did not differ from that of ST.

We also examined total energy intake (soup + buffet lunch) as a function of taster status and preload condition. Total energy intake of all subjects was higher in the soup condition compared to water condition [$F(1,72) = 4.9$, $p \leq 0.03$]. There was also a taster by condition effect [$F(1,72) = 4.2$, $p \leq 0.02$] in the soup condition, where NT consumed

significantly more total energy (792.2 ± 48) than ST (641.8 ± 45) ($p \leq 0.02$) but not more than MT (687 ± 39).

Table 3.4 Energy and macronutrient intake of PROP taster groups at lunch in water and soup preload conditions. [1]Values are mean ± SEM. [2] In each preload condition, means with different superscripts (a,b) are significantly different ($p \leq 0.05$)

	Water Preload[1]			**Soup Preload**		
	NT	**MT**	**ST**	**NT**	**MT**	**ST**
Energy Intake (Kcal) [2]	752 ± 38	757 ± 37	750 ± 54	690±48 [a]	586±39[ab]	542±45[b]
Energy density	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Fat Intake (% En)	42.6± 2.2	38.1±1.9	37.1±2.9	46.4±2.4[a]	36.1±1.9[b]	38.1±2.3[b]
CHO Intake (% En)	41.2± 1.9	45.7± 2.1	44.8± 2.9	42.2±2.1	49.1±1.7	46.6±2.6
Pro Intake (% En)	17.4± 0.7	17.1± 0.8	16.9± 0.9	16.1±0.7	17.2±0.6	17.3±1.1

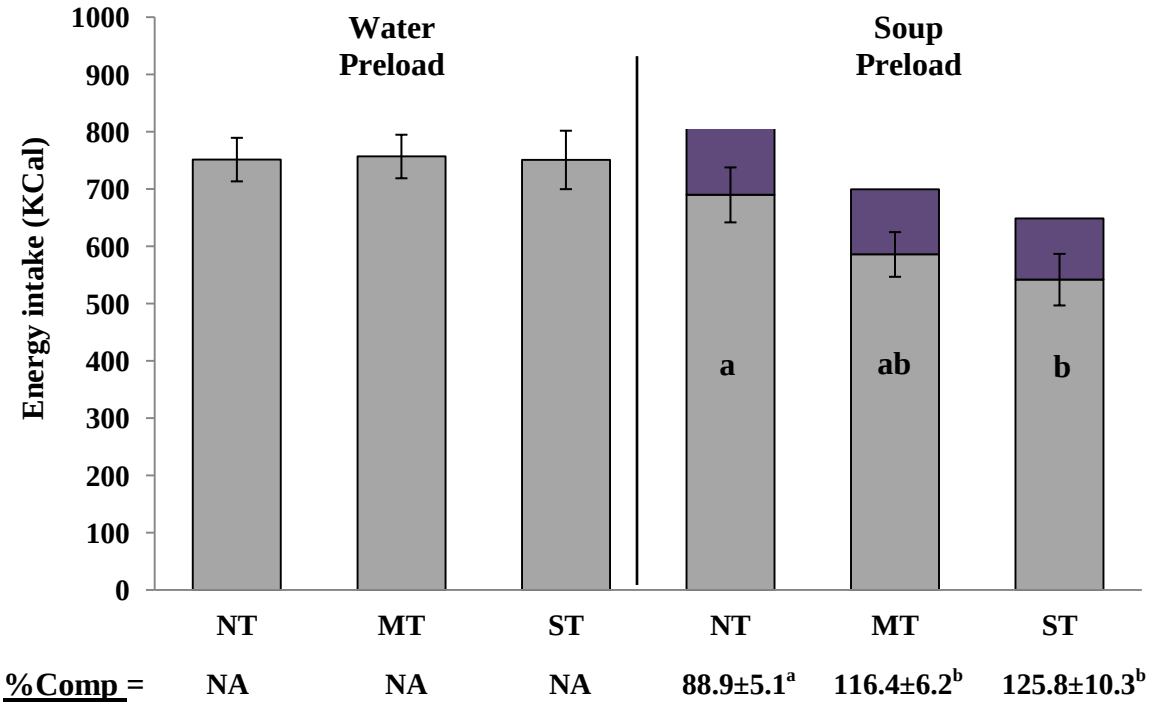
3.4.3 Caloric compensation as a function of PROP taster status after soup preload:

Percent caloric compensation for PROP taster groups in the water and soup preload conditions was calculated as previously mentioned. Values of more than 100%

indicate over-compensation (i.e. under-eating) and compensation values of less than 100% represent under-compensation (i.e. overeating).

Taster groups varied in their caloric compensation after the high-fat soup preload compared to the water preload [F (2,72) = 5.5, p≤ 0.005]. Compensation for the energy intake of soup preload during the buffet lunch in NT (88.9% ± 5.1) was significantly different than MT (116.4% ± 6.2) ($p \leq 0.01$) and ST (125.8% ± 10.3) ($p \leq 0.002$). NT undercompensated and over-ate at the buffet meal while MT and ST overcompensated and underrate at lunch in response to the soup preload (Figure 3.2).

Figure 3.2 [1]Mean energy intake at lunch and percentage compensation (% Comp) in water and soup preload conditions. Solid purple bars represent energy from soup preload. Solid gray bars represent energy intake at lunch for PROP taster groups. [2] In the soup condition, different superscripts (a,b) denote significant difference ($p \leq 0.05$)



3.4.4 Hunger and fullness ratings as a function of PROP taster status and preload condition

Hunger and fullness ratings were examined as a function of preload condition and taster status. Hunger and fullness ratings were collected immediately before and after preloads (H1/F1 and H2/F2 respectively), before lunch (H3/F3), after lunch (H4/F4), and 30 min after the subjects had finished their meal (H5/F5) (Figure 3.1).

Hunger ratings: Hunger ratings varied by preload condition [$F(1,72) = 6.3\text{-}20.9$, $p \leq 0.01\text{-}0.0001$] (Figure 3.3A). There were no differences in the initial hunger ratings (H1) in either preload condition. Subjects felt hungrier (H2) after the water preload than after the soup preload ($p \leq 0.01$). This pattern continued where they gave higher pre-meal ratings in the water condition than the soup condition. There were no differences in the immediately after-meal (H4) or 30 min after-meal (H5) ratings. There were also no significant differences in hunger ratings as a function of taster status (Figure 3.3B).

Fullness ratings: Fullness ratings varied as a function of preload condition [$F(1,72) = 14.4\text{-}34.1$, $p \leq 0.0001\text{-}0.0003$] (Figure 3.4A). There were no significant differences in initial fullness ratings (F1) in either condition. Fullness ratings after consuming the preload (F2) were significantly different where subjects gave higher fullness ratings after the soup preload than the water preload ($p \leq 0.0003$). Fullness ratings dropped prior to the meal, however, subjects felt less full in the water condition than the soup condition ($p \leq 0.0001$). There were no differences in the after-meal (F4) or 30 min after-meal (F5) ratings. There were no significant differences in hunger ratings as a function of taster status (Figure 3.4B).

Although not significant, there was a directional trend (p=0.07) in the post preload hunger and fullness ratings where ST felt less hungry and more full than MT and ST.

Figure 3.3 Mean ratings of hunger sensation over time as a function of (A) preload condition and (B) PROP taster status

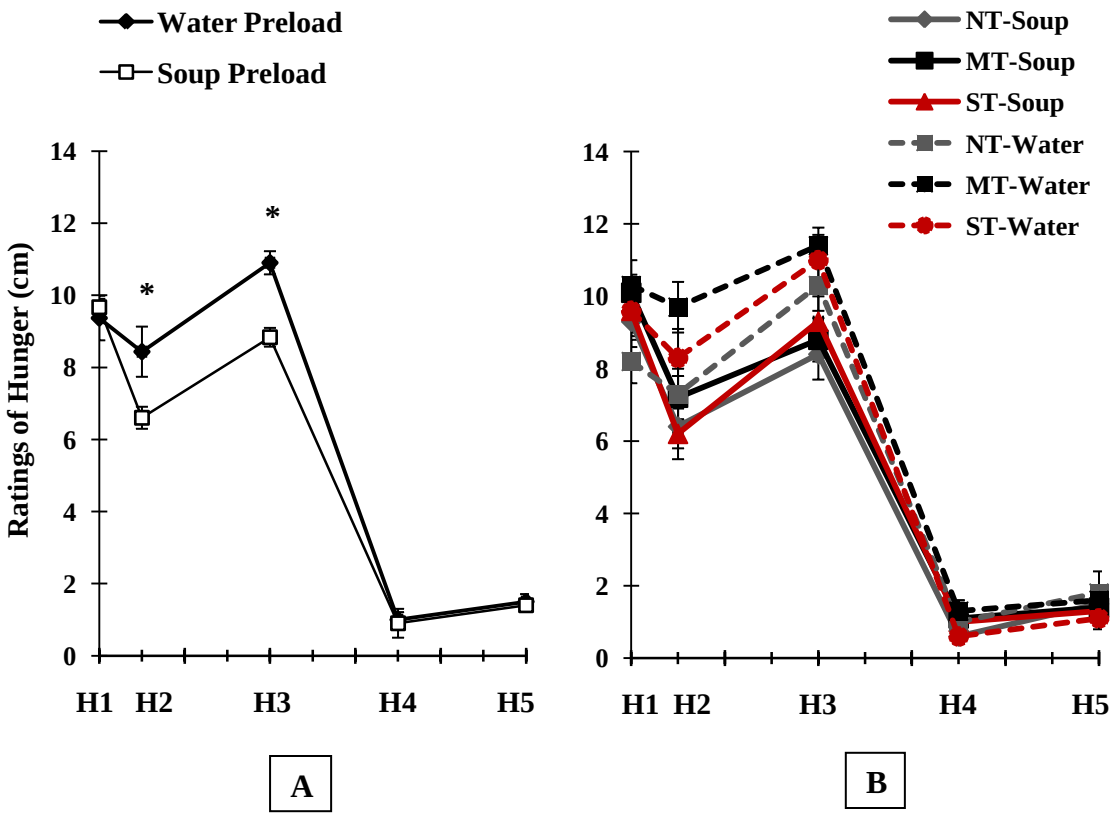
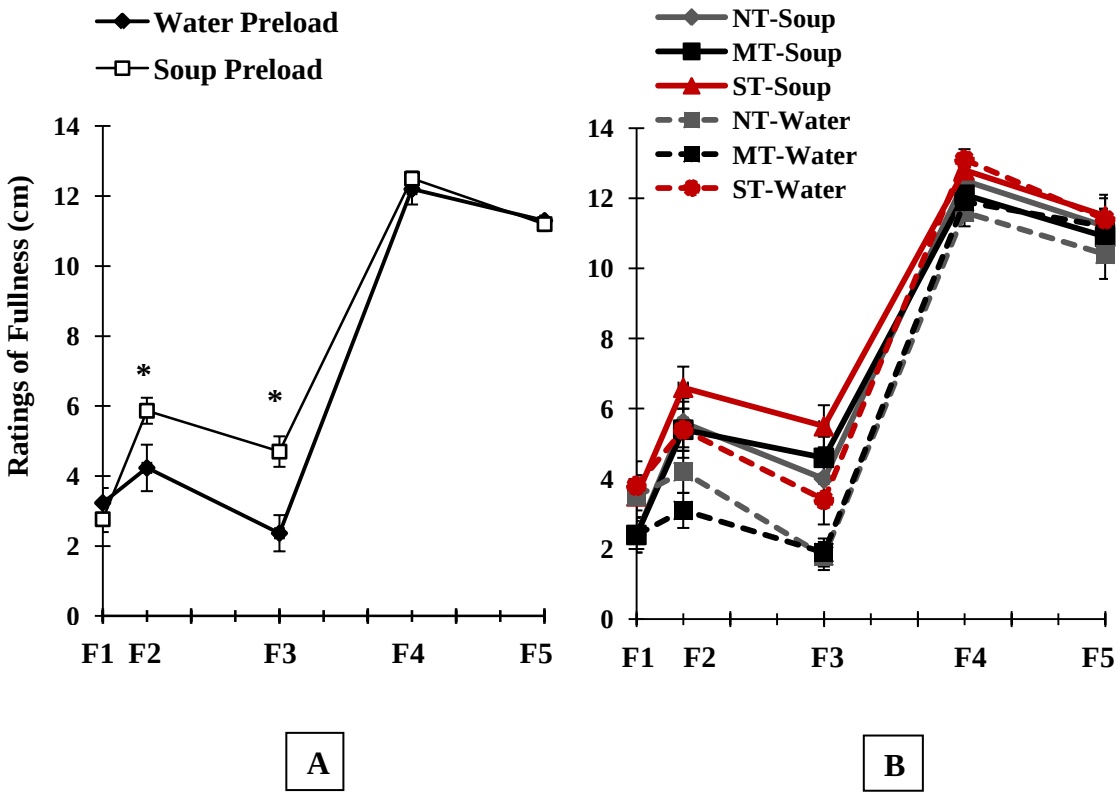


Figure 3.4 Mean ratings of fullness sensation over time as a function of (A) preload condition and (B) PROP taster status



3.5 Discussion

The purpose of this study was to investigate the influence of PROP phenotype on short-term energy regulation from a buffet test meal after the consumption of either a high-fat/high-energy soup preload or water. If non-taster status is associated with reduced ability to regulate calories, as we expected, then non-taster women would consume more fat and energy after the soup preload than MT and ST women, and caloric compensation would be less precise in NT women as well. Our results showed that relative to water, the soup preload led to a smaller reduction in *ad libitum* lunch energy intake in NT women (8.3%) as compared to MT and ST (21.6% and 28%, respectively). In addition, NT consumed more energy and fat (% En) after the soup than did MT or ST. Overall, subjects did not show precise compensation at lunch for the calories of the high-fat soup preload. NT women undercompensated in response to the high-fat soup challenge and over-ate at the lunch meal. On the other hand, MT and ST women overcompensated and under-ate as the result of the high-fat soup preload. NT may not have perceived the soup as a high-fat preload and therefore, did not decrease their subsequent energy intake as much as the MT and ST. To our knowledge, this is the first study to demonstrate differences in short-term caloric compensation among individuals varying in PROP taste responsiveness.

We chose to investigate a liquid (soup) preload, using water as a non-caloric, control for gastric distention. Our design most closely matches studies that have used yogurt preloads due to the similarity in physical form between yogurt and soup. One study (Zandstra et al 2000) showed that a high-fat/high-energy yogurt led to lower energy intake in subsequent meal relative to no preload. A second study (Rolls et al 1991)

showed accurate compensation for energy dense, high-fat and high-CHO yogurts in a subsequent self-selection meal. Thus, our findings are in general agreement with these previous works. Since we did not investigate compensation for the other macronutrients (carbohydrate and protein), our conclusions are limited to fat. This issue needs to be addressed in future studies in light of other data showing that high-fat yogurts suppressed lunch intake less than high-CHO yogurts (Rolls et al 1994). Longer-term studies, i.e., those measuring intake over the rest of the day or over multiple days have generally demonstrated a weaker satiety response to fats (Caputo and Mattes 1992, Stubbs and Harbron 1996) and considerable individual variation in the ability to compensate for ingested calories, including calories from fat (Caputo and Mattes 1992)

Hunger and fullness ratings changed in a predictable way across each preload condition, decreasing after preloads and lunch and increasing after meals. Also subjects felt more full and less hungry after the soup preload compared with water, and remained so prior to the meal. We saw a non-significant trend for super-tasters to feel more full and less hungry after the preloads compared to NT and MT. However, differences in hunger/fullness ratings did not explain the differences in energy intake among the groups after soup. We also correlated the appetite ratings with energy consumption from the meals to determine how strongly appetite ratings and intake were related (data not shown). However, none of the correlations were high and predictive of subsequent intake. Subjective ratings of appetite are considered imperfect measures of satiety and prospective consumption. It is possible that the differences between groups were too small to capture in a single session, and multiple sessions in each condition are necessary to illustrate these effects. An alternate explanation is that non-tasters exhibited passive

overconsumption of fat and calories based on habit and experience. Both of these possibilities deserve further consideration.

Our study has several strengths and weaknesses. This study is unique since no other studies have used a dietary preload challenge to assess compensatory eating and macronutrient selection among PROP taster groups. Also, this study investigated compensatory energy intake from a high variety buffet lunch in a controlled setting and not single-dish lunches in taster groups (Kamphuis and Westerterp-Plantenga 2003). One of the limitations of our study was the fact that this study consisted of only one day of preload challenge and one day of control. As stated previously, extending this study over multiple days for each preload condition may allow us to better capture differences in compensatory ability and appetite among the groups.

In summary, this was the first study to show that normal-weight, non-taster women consume more fat and energy following a short-term diet challenge than MT or ST. These findings complement our previous findings showing that NT women consume more energy and select more high-fat foods when offered a variety of foods (chapter 2 of thesis). Together these findings define a profile of NT women characterized by a greater attraction to high-fat/energy dense foods and reduced ability to calorically compensate for such foods. This profile may explain previous observations showing that NT women maintain higher body weights than ST women (Goldstein et al 2005, Ullrich et al 2004). Classifying women by PROP phenotype may help us to identify women susceptible to dietary-induced obesity and may be useful in developing weight-management and obesity treatment interventions.

CHAPTER 4

Conclusions and Future Directions

The incidence of obesity has steadily increased in the past thirty years where more than 65% of the U.S population is either overweight or obese. Increased energy intake is one of the factors that can result in a positive energy balance. Since taste is one of the key factors that influences food preferences and food intake, we investigated how variations in PROP taste perception in women might affect an individual's food preferences and food intake.

We had hypothesized that exposure to buffet meals would result in increased energy intakes of all subjects. We also hypothesized that non-tasters were more susceptible to over-eating in a buffet setting even after consuming a high-fat preload, and would drive most of their increased energy intake from higher fat consumption.

Collectively, the results of our studies presented here have expanded our knowledge of the influence of PROP phenotype on energy intake and macronutrient selection. When exposed to high variety buffet meals compared to fixed-item meals, total energy intake of NT was significantly higher than MT and ST. This difference was partly associated with higher intakes of fat (% En) and saturated fat (g) in NT as well as higher energy intakes from snacks and dinner meals. Similarly, energy intake of NT was significantly higher than ST after the soup preload compared to the water preload. Subjects did not show precise regulation of short-term energy intake and did not accurately compensate for the calories of the high-fat soup at the subsequent buffet lunch. NT undercompensated and over-ate at lunch whereas MT and ST women overcompensated and under ate as the result of the high-fat preload. The results of the preload study did not provide strong evidence for disruptions in the subjective experience

of satiety in NT. However, studies have not examined satiety signals in PROP-classified subjects.

We can expand our understanding of short-term energy regulation in taster groups by investigating additional factors that might contribute to this process.

Conducting the preload study, preferably over the course of multiple meals and days, with varying energy and macronutrient content of preloads can help us determine whether non-tasters are able to alter their energy intake as a result of the macronutrient composition of different preloads. Considering that the source of fat (Long chain or medium chain triglyceride) may be relevant in energy intake and caloric compensation since medium chain triglycerides (MTG) are more satiating than long chain triglycerides (LTG) (St-Onge and Jones 2002). Examining how these fats influence satiation and energy intake in different taster groups might provide additional clues about the mechanisms involved.

Several studies have suggested that food intake is in part mediated by a number of gut hormones (Havel 2001, Orr and Davy 2005). Therefore, collecting blood samples at multiple time intervals during the preload study can help us determine if there is an association between gut hormones and energy regulation in taster groups.

Ghrelin is a fast acting circulating hormone that is secreted from the stomach and promotes food intake by stimulating NPY (neuropeptide Y) and AgRP (agouti-related protein) in the hypothalamus (Gil-Campos et al 2006, Klok et al 2007). Measuring Ghrelin levels prior to and after a preload and a subsequent meal may allow for new insights into understanding food intake and regulation in taster groups.

Cholecystokinin (CCK), a gastrointestinal peptide, influences short-term energy intake by inhibiting food intake (mediating meal termination by suppressing hunger before a meal). CCK plasma levels are fat-stimulated satiety factors that play a role in short-term fat regulation (Greenberg et al 1992, Little et al 2008). Since NT consumed more fat than MT and ST from the buffet meals, investigating CCK levels in the non-taster group might provide a better understanding of fat regulation in these individuals.

Another hormone of interest is leptin which secreted predominantly from the adipocyte tissue and has been mentioned in the literature to influence long-term regulation of energy intake by suppressing food intake (Klok et al 2007, Montague et al 1997). It would be of interest to look at serum level concentrations of leptin and their effect on food intake in PROP taster groups.

The two studies conducted here open up many possibilities for studying food selection and regulatory mechanisms and will help us understand individual differences in response to diet. In the long run, this will help us understand dietary and genetic factors that contribute to increased body weight and the development of obesity.

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APPENDIX 1

Consent Forms and Questionnaires

CONSENT FORM

Bitter Taste Phenotype, Diet Variety and Obesity in Women – Screening

Principal Investigator: Beverly J. Tepper, Ph.D.
Sensory Evaluation Laboratory (Room 211)
Department of Food Science, Rutgers University
65 Dudley Road, New Brunswick, NJ 08901
(732) 932-9611 x 221
email: tepper@aesop.rutgers.edu

PURPOSE: Genetic differences in taste are believed to play an important role in food selection. The overall goal of this project is to better understand how genes that control food preferences differ among people and to link these differences with diet and health factors. In order to participate in this research, I must complete a screening procedure to see if I quality for this study.

PROCEDURES: I will be asked to taste filter paper disks that may or may not have a taste to me. The ability to taste one of these substances (called PROP) is a genetic trait. I also will be asked to complete brief questionnaires about my health and eating habits. These activities will take ~10 min for me to complete. I will be notified whether or not I qualify for the main study.

RISKS/BENEFITS: The activities I will be participating in pose no forseeable risks to my health. Although I will receive no direct benefits from participating in this study, this research will benefit society by providing a better understanding of the relationship between taste and diet.

COMPENSATION: No monetary compensation will be provided to me for participating in the screening procedure.

MY RIGHTS AS A RESEARCH SUBJECT/CONFIDENTIALITY: My participation in this screening is completely voluntary and I have the right to withdraw at any time without explanation or penalty. The information collected in this experiment will be kept strictly confidential, my identity protected by a code number, and all data kept in a locked filing cabinet or on a pass-word protected computer. Only research staff involved in this study will have access to these files.

AGREEMENT: I have read the above description. All my questions have been answered to my satisfaction and I agree voluntarily to participate. I understand that I have the right to leave the experiment at any time without penalty. I also understand that Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research. If I have questions about this research, I can contact the Principal Investigator at the number listed above or the Rutgers University Institutional Review Board for the Protection of Human Subjects, Office of Research and Sponsored Programs, 3 Rutgers Plaza, New Brunswick, NJ 08901-8559. Tel: 732-932-0150 ext. 2104 or Email: humansubjects@orsp.rutgers.edu

_____	_____
Name of participant (print)	Date
_____	_____
Signature of Participant	Signature of Investigator

I have received a copy of this statement for my records _____

(initials)

This informed consent form was approved by the Rutgers Institutional Review Board for the Protection of Human Subjects on 7/21/2008; approval of this form expires on 7/21/2009.

Bitter Taste Phenotype, Diet Variety and Obesity in Women

Genetic Testing

Cells will be collected by gently brushing the inside of the cheek with a soft brush. There is no discomfort from this procedure. The genetic material you provide will allow us to determine whether you are positive or negative for a gene that controls bitter taste sensitivity. This information will help us confirm the results of our behavioral tests and better understand the inheritance of this gene. The genetic material you provide will be used solely for this purpose and will not be sold or donated to a third party for unrelated purposes. If you agree to participate in this procedure please sign and date below. If you decline to participate in this procedure you can still participate in the main study.

_____ _____

Signature of participant Date

CONSENT FORM

Bitter Taste Phenotype, Diet Variety and Obesity in Women – Food Selection Study

Principal Investigator: Beverly J. Tepper, Ph.D.
Sensory Evaluation Laboratory (Room 211)
Department of Food Science, Rutgers University
65 Dudley Road, New Brunswick, NJ 08901
(732) 932-9611 x 221
email: tepper@aesop.rutgers.edu

PURPOSE: This study will examine the relationship between genetic taste sensitivity to PROP and selection of foods. I am invited to participate in this research because I have already been screened for PROP taster status and qualify for this study.

PROCEDURES: This study will take approximately 2 weeks of my time to complete. For 3 consecutive days during week 1 and 3 consecutive days during week 2, all of my meals and snacks will be provided to me. I will be asked to consume lunch and dinner in the Sensory Evaluation Laboratory during each test day. I can choose as much or as little to eat from the foods offered. All foods are commercially available items. A sample menu of the foods I will be offered is attached. I should only eat the foods provided by the study coordinator during test days. I will also wear an activity monitor around my waist for 5 consecutive days to measure my physical activity. There is no discomfort to wearing the monitor. My height and weight will be measured and my body composition will be measured using a method called biological impedance analysis (BIA). I will be asked to stand on a digital scale and a small electric current will be passed through my body. My weight will be measured and a computer in the scale will estimate how much fat is in my body. This is a safe and painless procedure that is commonly used in hospitals, gyms, and health centers. I will also complete questionnaires about my eating habits, hunger/fullness and food attitudes.

RISKS/BENEFITS: The activities I will be participating in pose no forseeable risks to my health. Although I will receive no direct benefits from participating in this study, this research will benefit society by providing a better understanding of the relationship between taste and diet.

COMPENSATION: At the completion of the study I will receive a single payment of $ 100. If I withdraw from the study prior to its completion, my payment will be pro-rated $ 10 for each session completed.

MY RIGHTS AS A RESEARCH SUBJECT/CONFIDENTIALITY: My participation in this study is completely voluntary and I have the right to withdraw at any time without explanation or penalty. The information collected in this experiment will be kept strictly confidential, my identity protected by a code number, and all data kept in a locked filing cabinet or on a pass-word protected computer. Only research staff involved in this study will have access to these files.

AGREEMENT: I have read the above description. All my questions have been answered to my satisfaction and I agree voluntarily to participate. I understand that I have the right to leave the experiment at any time without penalty. I also understand that Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research. If I have questions about this research, I can contact the Principal Investigator at the number listed above or the Rutgers University Institutional Review Board for the Protection of Human Subjects, Office of Research and Sponsored Programs, 3 Rutgers Plaza, New Brunswick, NJ 08901-8559. Tel: 732-932-0150 ext. 2104 or Email: humansubjects@orsp.rutgers.edu

_____ _____

Name of participant (print) Date

_____ _____

Signature of Participant Signature of Investigator

I have received a copy of this statement for my records _____

(initials)

This informed consent form was approved by the Rutgers Institutional Review Board for the Protection of Human Subjects on _____; approval of this form expires on _____.

CONSENT FORM

Bitter Taste Phenotype, Diet Variety & Obesity in Women
Energy Metabolism and Lunch Meal Study

Principal Investigators: Beverly J. Tepper, Ph.D.
Sensory Evaluation Laboratory (Room 211)
Department of Food Science, Rutgers University
65 Dudley Road, New Brunswick, NJ 08901
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Daniel J. Hoffman, Ph.D.
Department of Nutritional Sciences
Thompson Hall, 26 Nichol Ave
Rutgers University
New Brunswick, NJ 08901
(732) 932-6568
email: dhoffman@aesop.rutgers.edu

PURPOSE: Genetic differences in taste are believed to play an important role in food selection. The purpose of this study is to determine if these same genes also contribute to differences in metabolic rate and short term food intake. I am invited to participate in this study because I have already been screened for PROP taster status and qualify for this study.

PROCEDURES: The study will take a total of 4 days to complete over a 2-week period.

Day 1:
The study coordinator will provide all my food (meals, snacks and beverages) for Day 1. I should only consume the foods provided. This is to ensure that each subject eats a similar diet on the day prior to testing. The foods consist of oatmeal and fruit for breakfast and microwave meals containing pasta, vegetables and chicken. SlimFast shake will balance any caloric differences for lunch and dinner. I can pick up the foods at the Sensory Evaluation Lab in the Food Science Building on the afternoon prior to Day 1. I will be asked to limit physical activity to no more than 30 minutes of moderate exercise (walking, slow jogging, etc.) on the diet day.

Day 2:
Day 2 is the test day. The evening prior to this test, I will be asked to not eat or drink anything (except plain water) from 8:00 pm until I complete the metabolic test the next morning. The following morning, I will come to the metabolic lab in Thompson Hall for this testing. First I will be asked to void into a disposable container for routine urine analysis. My resting energy metabolism will be measured using indirect calorimetry.

I will be asked to rest on a bed for thirty minutes during which time a clear plastic hood will be placed over my head to measure the amount of oxygen inhaled and carbon dioxide exhaled. I will be able to breathe normally during the test. The test can be stopped at any time by lifting the hood off my head. My height and weight will be measured and my body composition will be measured using a method called biological impedance analysis (BIA). I will be asked to stand on a digital scale and a small electric current will be passed through my body. My weight will be measured and a computer in the scale will estimate how much fat is in my body. This is a safe and painless procedure that is commonly used in hospitals, gyms, and health centers.

Following these measurements, I will eat a breakfast consisting of juice, yogurt, toast, and black coffee or tea. After breakfast, I am free to leave the lab to engage in my usual activities. However, I cannot eat or drink anything (except plain water) for the next 3 hrs. I will then come to the Sensory Evaluation Lab to consume a test meal and then lunch. I will be offered a variety of foods for the lunch meal and I can consume as much or a little of the foods as I wish. All foods are commercially available products. I will also complete questionnaires about the test meal and lunch.

Day 3 and 4: The same procedures are repeated during the second week of the study.

RISKS/BENEFITS: I will not experience any physical discomfort from participating in this research and none of the activities I will be participating in pose any foreseeable risks to my health. Although l will receive no direct benefits from participating in this study, this research will benefit society by providing a better understanding of the role taste genetics in energy metabolism and food selection.

COMPENSATION: At the completion of the study I will receive a single payment of $ 100. If I withdraw from the study after the first test day, I will be paid $ 30.

MY RIGHTS AS A RESEARCH SUBJECT/CONFIDENTIALITY: My participation in this study is completely voluntary and I have the right to withdraw at any time without explanation or penalty. The information collected in this experiment will be kept strictly confidential, my identity protected by a code number, and all data kept in a locked filing cabinet or in password protected computer files. Only research staff involved in this study will have access to these files.

AGREEMENT: I have read the above description. All my questions have been answered to my satisfaction and I agree voluntarily to participate. I understand that I have the right to leave the experiment at any time without penalty. I also understand that Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research. If I have questions about this research, I can contact either of the Principal Investigators at the numbers listed above or the Rutgers University Institutional Review Board for the Protection of Human Subjects, Office of Research and Sponsored Programs, 3 Rutgers Plaza, New Brunswick, NJ 08901-8559. Tel: 732-932-0150 ext. 2104 or Email: humansubjects@orsp.rutgers.edu

Subject initials: _____

_____ _____

Name of participant (print) Date

_____ _____

Signature of Participant Signature of Investigator

I have received a copy of this statement for my records _____

(initials)

This informed consent form was approved by the Rutgers Institutional Review Board for the Protection of Human Subjects on _____; approval of this form expires on _____.

I.D. _____
Date: _____

Demographic and Health Information

Instructions

Please answer these questions about you <u>to the best of your knowledge</u> and make sure you answer every question. Thank you for your time.

A. GENERAL INFORMATION ABOUT YOU

Please provide the following information:

1. Name:

2. Date of birth: ☐☐ month ☐☐ day ☐☐☐☐ year

3. Age: _____

4. Gender: ☐ 1 male ☐ 2 female

5. Contact Telephone Number:

6. Email Address:

7. Home Address:

8. Occupation:

9. Were you born in the United States? **Yes** ☐ 1 **No** ☐ 2

If "No," Please write in the country in which you were born: _____

10. To which of the following races do you consider yourself to belong? (You may choose all that apply)

☐ 1 Black or African-American
☐ 2 White
☐ 3 American Indian or Alaska native
☐ 4 Asian or Pacific islander
☐ 5 Hispanic or Latino
☐ 6 Other *(please specify):*

Continued on next page

11. In addition, which of the following groups describes your ethnicity? (You may choose all that apply)

- ☐ 1 African ***(please specify):*** _____
- ☐ 2 West Indian / Caribbean ***(please specify)***: _____
- ☐ 3 Mexican / Mexican-American/ Chicano
- ☐ 4 Puerto Rican
- ☐ 5 Cuban
- ☐ 6 Central American
- ☐ 7 Other Latino/Hispanic **(*please specify):*** _____
- ☐ 8 Asian Indian
- ☐ 9 Japanese
- ☐ 10 Chinese
- ☐ 11 Korean
- ☐ 12 Filippino
- ☐ 13 Vietnamese
- ☐ 14 Other Asian, **(*please specify):*** _____
- ☐ 15 Native Hawaiian
- ☐ 16 Guamanian or Chamorro
- ☐ 17 Samoan
- ☐ 18 Tongan
- ☐ 19 Other, **(*please specify):*** _____
- ☐ 20 None of the above

B. HEALTH INFORMATION

12. Do you have a history of or are currently being treated for any of the following medical conditions? (Please check all that apply)

- ☐ 1 Diabetes (Type I or Type II)
- ☐ 2 Heart problems
- ☐ 3 Blood problems (haemophilia)
- ☐ 4 Kidney problems
- ☐ 5 Hypertension
- ☐ 6 Stroke
- ☐ 7 PKU (phenylketonuria)
- ☐ 8 Otiitis Media (chronic ear infection, especially as a young child)
- ☐ 9 Severe hayfever or allergies
- ☐ 10 Asthma
- ☐ 11 Cancer
- ☐ 12 Sinusitis

Continued on next page

13. Are you currently pregnant or nursing? (please check one)

☐ 1 YES ☐ 2 NO

14. Have you had a cold/flu or ear infection in the past 2 weeks? (Please check one)

☐ 1 YES ☐ 2 NO

If yes, please describe:

_____

15. What, if any, prescription medications are you currently taking (including birth control) and how often?

_____

16. Have you been to the dentist in the past 2 weeks? (Please check one)

☐ 1 YES ☐ 2 NO

17. Have you had hay fever/ nasal allergies in the past two weeks? (Please check one)

☐ 1 YES ☐ 2 NO

18. Do you dislike or avoid eating certain foods? (Please check one)

☐ 1 YES ☐ 2 NO

If yes, please describe:

_____

19. Do you have any food allergies? (Please check one)

☐ 1 YES ☐ 2 NO

If yes, please describe: _____

20. How often do you try unfamiliar foods?

☐ 1 Never ☐ 2 Rarely ☐ 3 Sometimes ☐ 4 Often ☐ 5 Very Often

Continued on next page

21. Have you taken multi-vitamins or vitamin A, C, or E supplements in the past month?

☐ 1 YES ☐ 2 NO

22. On average, how many hours do you sleep per night? _____

23. Are you currently dieting to lose weight? (Please check one)

☐ 1 YES ☐ 2 NO

24. *How many times have you been on a diet to lose weight over the past six months?*_____

25. Have you unintentionally gained or lost more than five pounds in the past six months? (Please check one)

☐ 1 YES ☐ 2 NO

26. What is your current height?

☐ FT. ☐ IN. **OR** ☐ M.

27. What is your current weight?

☐ LBS. **OR** ☐ KG

28. What is the highest weight you have ever been?

☐ LBS. **OR** ☐ KG

29. What is the lowest weight you have ever been?

☐ LBS. **OR** ☐ KG

30. Do you currently smoke? (Please check one)

☐ 1 YES ☐ 2 NO

If yes, please specify cigarettes, cigar, or pipe: _____

Continued on next page

31. If you smoke, how many:

cigarettes per day? _____
cigars per day? _____
pipes per day? _____

32. Have you smoked in the past?

☐ 1 YES ☐ 2 NO

If yes, how many years ago did you quit? _____

C. OTHER INFORMATION

Please answer the following questions about your family.

33. What is the highest education level you have finished? (Please "X" only one answer)

☐ 1 6th grade or less
☐ 2 8th grade or less
☐ 3 Attended some High School
☐ 4 High School Graduate or GED
☐ 5 Technical School
☐ 6 Some College
☐ 7 College Graduate
☐ 8 Post Graduate Study

34. What was the approximate total income, before taxes, of your household last year? Please include wages, salaries, social security, interest, child support, public assistance, unemployment compensation, rent from property and all other income. (Please "X" only one answer)

☐ 1 Less than $5,000
☐ 2 $5,000 - $9,999
☐ 3 $10,000 - $19,999
☐ 4 $20,000 - $29,999
☐ 5 $30,000 - $39,999
☐ 6 $40,000 - $49,999
☐ 7 $50,000 - $59,999
☐ 8 $60,000 - $69,999
☐ 9 $70,000 - $79,999
☐ 10 $80,000 - $89,999
☐ 11 $90,000 - $99,999
☐ 12 Over $100,000

Thank you. You are done with this form. Please return this form to the test administrator.

Eating Attitudes Test

Age:_____ **Sex: _____** **Height: _____**

Current weight: _____ **Highest weight (excluding pregnancy): _____**

Lowest adult weight: _____ **Ideal weight: _____**

Please choose one response for the following statements by marking a check in the corresponding box. Please only choose one answer.

1. Am terrified about being overweight	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
2. I Avoid eating when I'm hungry.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
3. I Find myself preoccupied with food	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
4. I Have gone on eating binges where I feel that I may not be able to stop.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
5. I Cut my food into small pieces.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
6. I Aware of the calorie content of foods that I eat.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
7. I Particularly avoid food with high carbohydrate content (i.e. bread, rice, potatoes, etc.)	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
8. I Feel that others would prefer if I ate more.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
9. I vomit after I have eaten.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
10. I Feel extremely guilty after eating.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
11. I Am preoccupied with a desire to be thinner.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
12. I Think about burning up calories when I exercise.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
13. Other people think I am too thin.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐

14. I Am preoccupied with the thought of having fat on my body.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
15. I Take longer than others to eat my meals.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
16. I Avoid foods with sugar in them.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
17. I Eat diet foods.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
18. I Feel that food controls my life	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
19. I Display self-control around food	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
20. I Feel that others pressure me to eat	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
21. I Give too much time and thought to food.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
22. I Feel uncomfortable after eating sweets.	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
23. I Engage in dieting behavior	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
24. I Like my stomach to be empty	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
25. I Have the impulse to vomit after meals	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐
26. I Enjoy trying new rich foods	Always ☐	Usually ☐	Often ☐	Sometimes ☐	Rarely ☐	Never ☐

Behavioral Questions:		
Gone on eating binges where you feel that you may not be able to stop? (Eating much more than most people would eat under the same circumstances) If you answered yes, how often during the worst week:	Yes ☐	No ☐
Ever made yourself sick (vomited) to control your weight or shape? If you answered yes, how often during the worst week: _____	Yes ☐	No ☐
Ever used laxatives, diet pills, or diuretics (water pills) to control your weight or shape? If you answered yes, how often during the worst week: _____	Yes ☐	No ☐
Ever been treated for an eating disorder? When: _____	Yes ☐	No ☐

Name: _____

Date: _____

THREE FACTOR EATING QUESTIONNAIRE

Directions: Part 1 contains a number of statements. Each statement should be answered TRUE or FALSE. Read each statement and decide how you feel about it. If you agree, answer TRUE by checking the box next to T, if you disagree, answer FALSE by checking the box next to F. If a question does not seem to apply to you exactly, answer as best as you can. **PLEASE ANSWER ALL QUESTIONS.**

1. When I smell a steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	□ T	□ F
2. I usually eat too much at social occasions, like parties and picnics.	□ T	□ F
3. I am usually so hungry that I eat more than three times a day.	□ T	□ F
4. When I have eaten my quota of calories, I am usually good about not eating any more.	□ T	□ F
5. Dieting is so hard for me because I just get too hungry.	□ T	□ F
6. I deliberately take small helpings as a means of controlling my weight.	□ T	□ F
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	□ T	□ F
8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat	□ T	□ F
9. When I feel anxious, I find myself eating.	□ T	□ F
10. Life is too short to worry about dieting.	□ T	□ F
11. Since my weight goes up and down, I have gone on reducing diets more than once.	□ T	□ F

12. I often feel so hungry that I just have to eat something.	□ T	□ F
13. When I am with someone who is overeating, I usually overeat too.	□ T	□ F
14. I have a pretty good idea of the number of calories in common foods.	□ T	□ F
15. Sometimes when I start eating, I just can't seem to stop.	□ T	□ F
16. It is not difficult for me to leave something on my plate.	□ T	□ F
17. At certain times of the day, I get hungry because I have gotten used to eating then.	□ T	□ F
18. While on a diet, if I eat a food that is not allowed, I consciously eat less for a period of time to make up for it.	□ T	□ F
19. Being with someone who is eating, often makes me hungry enough to eat also.	□ T	□ F
20. When I feel blue, I often overeat	□ T	□ F
21. I enjoy eating too much to spoil it by counting calories or watching my weight.	□ T	□ F
22. When I see a real delicacy, I often get so hungry that I have to eat right away.	□ T	□ F
23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.	□ T	□ F
24. I get so hungry that my stomach often seems like a bottomless pit.	□ T	□ F
25. My weight has hardly changed at all in the last ten years.	□ T	□ F
26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.	□ T	□ F
27. When I feel lonely, I console myself by eating.	□ T	□ F
28. I consciously hold back at meals in order not to gain weight.	□ T	□ F
29. I sometimes get very hungry late in the evening or at night.	□ T	□ F

30. I eat anything I want, any time I want.	□ T	□ F
31. Without even thinking about it, I take a long time to eat.	□ T	□ F
32. I count calories as a conscious means of controlling my weight.	□ T	□ F
33. I do not eat some foods because they make me fat.	□ T	□ F
34. I am always hungry enough to eat at any time.	□ T	□ F
35. I pay a great deal of attention to changes in my figure (body shape).	□ T	□ F
36. While on a diet, if I eat a food that is not allowed, I often then splurge (go ahead) and eat other high calorie foods.	□ T	□ F

Directions: For **Part 2,** please answer the following questions by checking the box next to the response that is most appropriate for you. **AGAIN, PLEASE ANSWER EVERY QUESTION** as best as you can.

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

□ Rarely □ Sometimes
□ Usually □ Always

38. Would a weight fluctuation of 5 pounds affect the way you live your life?

□ Not at all □ Slightly
□ Moderately □ Very much

39. How often do you feel hungry?

□ Only at meals □ Sometimes between meals
□ Often between meals □ Almost always

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

□ Never □ Rarely
□ Often □ Always

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

□ Easy □ Slightly
□ Moderately □ Very

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

□ Not at all □ Slightly
□ Moderately □ Extremely

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

□ Almost never □ Seldom
□ Usually □ Almost always

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

□ Unlikely □ Slightly likely
□ Moderately likely □ Very likely

45. Do you eat sensibly in front of others and splurge (readily go ahead) when alone?

□ Never □ Rarely
□ Often □ Always

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

□ Unlikely □ Slightly likely
□ Moderately likely □ Very likely

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

□ Almost never □ Seldom
□ At least once a week □ Almost every day

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

□ Unlikely □ Slightly likely
□ Moderately likely □ Very likely

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

□ Never □ Rarely
□ Sometime □ At least once a week

50. Which of the following statements best describes you?

- □ Eat whatever I want, whenever I want it
- □ Usually eat whatever I want, whenever I want it
- □ Often eat whatever I want, whenever I want it
- □ Often limit food intake, but often "give in"
- □ Usually limit food intake, rarely "give in"
- □ Constantly limit food intake, never "give in"

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

"I start dieting in the morning, but because of any 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
- □ Describes me perfectly

Please take a moment to fill in any questions you may have skipped.

THANK YOU VERY MUCH

I.D. _____

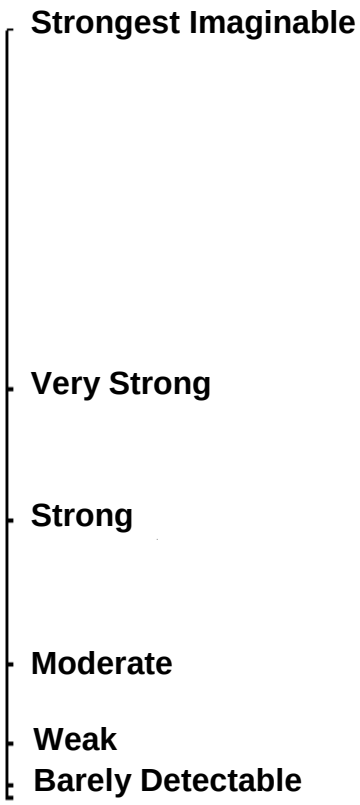
Date: _____

Paper Disc Samples

Instructions:

You will receive two paper discs to taste. Rinse your mouth thoroughly with water before you begin. Place the disc that matches the number below on the tip of the tongue for 30 second or until it is wet. Rate the **intensity of the taste** of the paper disc by drawing a mark on the scale for your answer. You can draw your mark on any place on the scale. For the next sample, go to the next page.

First Sample: **151**

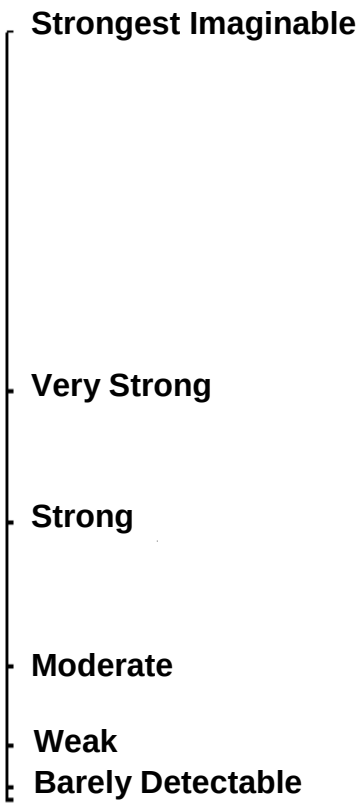


I.D. _____

Date: _____

Please rinse with water and wait for 45 seconds before you begin.

First Sample: **627**



Hunger and Fullness Questionnaire – Before Meal

Please answer the following questions by drawing a single mark on each line for your answer.

1. **How <u>hungry</u> are you right now?**

|----------|
Not at All **Extremely**

2. **How <u>full</u> are you right now?**

|----------|
Not at All **Extremely**

3. **How strong is your desire to eat a <u>meal</u> right now?**

|----------|
None **Very Strong**

4. **How strong is your desire to eat a <u>snack</u> right now?**

|----------|
None **Very Strong**

Comments: _____
_____
_____
_____
_____
_____

Hunger and Fullness Questionnaire – After Meal

Please answer the following questions by drawing a single mark on each line for your answer.

1. **How <u>hungry</u> are you right now?**

|----------|

Not at All **Extremely**

2. **How <u>full</u> are you right now?**

|----------|

Not at All **Extremely**

3. **How strong is your desire to eat a <u>meal</u> right now?**

|----------|

None **Very Strong**

4. **How strong is your desire to eat a <u>snack</u> right now?**

|----------|

None **Very Strong**

5. **How pleasant was the overall <u>taste</u> of your meal?**

|----------|

Not Pleasant **Very Pleasant**

Comments: _____
_____
_____
_____
_____
_____

APPENDIX 2

Additional Tables - Food Selection (Buffet) Study (Chapter 2)

Table A2.1 Energy and macronutrient intake during FIXED and BUFF Lunch and Dinner meals. Values are mean ± SEM. Means with different superscripts (a,b) are significantly different ($p \leq 0.05$)

		Fixed-Item Meals	Buffet Meals
Lunch	**Energy Intake (Kcal)**	538.2 ± 13[a]	640 ± 13[b]
	Fat Intake (% En)	44.5 ± 0.7[a]	42.2 ± 0.7[b]
	CHO Intake (% En)	41.4 ± 0.7	39.9 ± 0.7
	Prot Intake (% En)	15.6 ± 0.3[a]	18.9 ± 0.3[b]
Dinner	**Energy Intake (Kcal)**	602.5 ± 17[a]	824.4 ± 18[b]
	Fat Intake (% En)	36.3 ± 0.5[a]	38.5 ± 0.5[b]
	CHO Intake (% En)	39.1 ± 0.6[a]	43.3 ± 0.6[b]
	Prot Intake (% En)	25.7 ± 0.4[a]	19 ± 0.4[b]
Snacks	**Energy Intake (Kcal)**	396.7 ± 16.5[a]	376.7 ± 16.9[b]
	Fat Intake (% En)	25.9 ± 1.1	26.8 ± 1.1
	CHO Intake (% En)	69.9 ± 1.5	65.9 ± 1.6
	Prot Intake (% En)	6.7 ± 0.3	6.6 ± 0.3

Table A2.2 Energy and macronutrient intake from lunch, dinner, and snacks as a function of taster status.

		PROP Taster Status		
		NT	**MT**	**ST**
Lunch	**Energy Intake (Kcal)**	585 ± 17	593 ± 16	589.4 ± 17
	Fat Intake (% En)	44.5 ± 0.8[a]	44.1 ± 0.8[a]	41.4 ± 0.8[b]
	CHO Intake (% En)	38.6 ± 0.9[a]	40.4 ± 0.9[a]	42.9 ± 0.9[b]
	Prot Intake (% En)	17.8 ± 0.4	16.8 ± 0.4	17.1 ± 0.4
Dinner	**Energy Intake (Kcal)**	727.5 ± 22[a]	742.2 ± 21[a]	670.7 ± 22[b]
	Fat Intake (% En)	38.7 ± 0.6[a]	38 ± 0.6[a]	35.4 ± 0.6[b]
	CHO Intake (% En)	41.3 ± 0.7	40.9 ± 0.7	41.3 ± 0.8
	Prot Intake (% En)	21.4 ± 0.5	21.8 ± 0.5	23.8 ± 0.5
Snacks	**Energy Intake (Kcal)**	406.8 ± 20.7[a]	432.2 ± 20.3[a]	321.2 ± 21.0[b]
	Fat Intake (% En)	25.8 ± 1.4	25.6 ± 1.4	27.4 ± 1.4
	CHO Intake (% En)	69.4 ± 1.9	69.8 ± 1.9	64.6 ± 1.9
	Prot Intake (% En)	6.4 ± 0.4	6.9 ± 0.4	6.6 ± 0.4

Table A2.3 Energy and Macronutrient content of foods served in laboratory meals
Source: USDA National Nutrient Database for standard Reference or manufacturers' nutrition facts label

Food Item	Serving size (g)	Kcal	Protein (g)	Fat (g)	Carbohydrate (g)	Product brand
Fruits						
Apple	182	95	-	-	25	Shoprite
Orange	140	69	1.3	-	18	Shoprite
Grape	100	69	0.7	0.2	18.1	Shoprite
Banana	126	112	0.4	0.4	28.8	Shoprite
Cantaloupe	100	34	0.8	0.2	8.2	Shoprite
Honeydew	100	36	0.5	0.1	9.1	Shoprite
Vegetables						
Pepper	35	7	0.3	0.1	1.6	Shoprite
Onion	20	8	0.2	0.02	1.9	Shoprite
Celery	35	6	0.2	0.06	1.1	Shoprite
Carrot	45	19	0.4	0.1	4.3	Shoprite
Tomato	35	6	0.3	0.1	1.4	Shoprite
Grape tomato	35	6	0.3	0.1	1.3	Shoprite
Mushroom	10	2	0.3	0.03	0.3	Shoprite
Lettuce - Romaine	25	4	0.3	0.04	0.7	Dole
Lettuce- Iceberg	15	2	0.1	0.02	0.5	Shoprite
Desserts						
Chocolate brownie	120	486	5.8	19.6	76.7	Shoprite
Custard pie	104	270	6.1	13.7	31.4	Shoprite
Éclair	55	144	3.5	8.6	13.3	Shoprite
Ice cream sandwich	100	237	4.3	8.6	37.1	Shoprite
Apple pie	85	201	1.6	9.4	28.9	Shoprite
Peach pie	85	190	1.6	8.5	27.9	Shoprite
Cookies	56	255	2.8	11.6	36.5	Shoprite
Chocolate cake	95	352	5.1	14.3	50.7	Shoprite
Pound cake	45	175	2.3	8.1	23.6	Shoprite
Ice-cream sundae	100	207	3.5	11	23.6	Turkey Hill
Whipped cream	20	51	0.6	4.4	2.5	Reddi Wip
Chocolate sauce	30	105	1.4	2.7	18.9	Hershey's
Caramel sauce	30	76	0.5	0.03	19.8	Smucker's
Rainbow sprinkles	20	71	0.3	4.8	10.5	CakeMate
Walnuts-chopped	15	98	2.3	9.8	2.1	Shoprite

Food Item	Serving size (g)	Kcal	Protein (g)	Fat (g)	Carbohydrate (g)	Product brand
Condiments						
Mayonnaise	25	98	0.2	8.4	5.9	Hellman's
Deli mustard	15	10	0.7	0.6	0.8	Shoprite
Honey mustard	25	116	0.2	10.2	5.8	Ken's
Ketchup	17	15	-	-	4	Heinz
BBQ sauce	25	38	-	0.1	9.1	KC Masterpiece
Parmesan cheese	*10*	41.5	3.8	2.7	3.4	Kraft
Sour cream	30	58	0.6	5.9	0.9	Shoprite
Salsa	40	11	0.6	0.1	2.5	Chi Chi's
Butter	10	72	0.1	8.1	0	Shoprite
Margarine	10	72	0.02	8.1	0.1	Shoprite
Dressings						
Balsamic vinaigrette	25	22	0.12	-	4.3	Wishbone
Red Wine vinaigrette	25	5	0.01	-	0.1	Kraft
Italian	25	73	0.1	7.1	2.6	Wishbone
Ranch	25	121	0.3	12.3	1.7	Kraft
Blue cheese	25	119	0.3	12.8	1.2	Kraft
Bread						
Slice	86	205	9.2	1.9	37.8	Shoprite
Pita bread	56	149	5.5	1.5	30.8	Kangaroo
Sandwich roll	42.5	119	3.7	1.9	21.6	Shoprite
White tortilla	70	201	6.1	4.2	34.7	Mission
Garlic bread	45	150	3.6	7.1	17.9	Pepperidge farm
Cheese						
American	30	111	5.4	9.5	1.1	Shoprite
Swiss	48	182	12.9	13.3	2.6	Shoprite
Provolone	46	161	11.8	12.3	0.9	Shoprite
Cheese-Shredded	34	137	8	11.2	-	Shoprite
Deli meats						
Turkey	55	64	12.3	0.5	2.3	Shoprite
Roast beef	60	110	18.8	3.3	-	Shoprite
Ham	50	82	8.3	4.3	1.9	Shoprite
Salami	30	128	6.5	11.1	0.4	Shoprite

Food Item	Serving size (g)	Kcal	Protein (g)	Fat (g)	Carbohydrate (g)	Product brand
Potato salad	*50*	57	0.8	3.1	6.8	Shoprite
Macaroni salad	50	114	7.9	1.7	9.5	Shoprite
Coleslaw	40	61	0.4	3.9	5.9	Shoprite
Black beans	150	198	13.3	0.8	35.6	Goya
Refried beans	150	136	8.1	1.8	22.9	El Paso
Tuna salad	125	234	20.1	11.6	11.8	Bumble Bee
Grilled cheese sandwich	130	436	15.1	24.6	38.6	Shoprite
Meat lasagna	278	353	22.9	13.2	35.8	Stouffer's
Meatballs	240	220	11.6	17.5	10.5	Shoprite
Pasta	200	316	1.9	11.6	61.7	Barilla
Marinara sauce	170	83	2.4	2.5	12.8	Barilla
Meat sauce	180	115	3.6	4.3	14.4	Barilla
Alfredo sauce	160	213	16	5.3	10.7	Barilla
Broccoli - cooked	100	35	2.4	0.4	7.2	Green giant
Carrots - cooked	90	32	0.7	0.2	7.4	Green giant
Taco shell	25.4	119	1.8	5	16	El Paso
Ground beef-taco	170	462	46	30	-	Shoprite
Manicotti	279	395	19.8	15.4	45	Stouffer's
Chicken parmesan	230	490	39	24	33	Shoprite
Fried chicken	240	660	48	42	24	Banquet
Tortilla chips	28	139	2.2	6.6	18.6	Tostitos
Potato chips	28	152	2	10	15	UTZ
Mac & cheese	225	340	14	16	36	Stouffer's
Quiche – Lorraine	135	389	18.3	20.7	34.2	Shoprite
Quiche – Spinach	135	338	12.7	24.5	16.9	Shoprite
Beef + stir fry sauce	140	335	45	16	2.6	Shoprite
Chicken + stir fry sauce	140	307	42.8	12.7	2.4	Shoprite
White rice	125	156	2.8	0.2	34.5	Goya
Yellow rice						Carolina
Chicken tenders	150	440	23.7	26.6	25.5	Tyson
Rotisserie chicken	*150*	390	34.8	27.9	-	Shoprite
Mashed potato	*150*	170	2.9	6.3	25.4	Ore Ida
Potato wedges	85	120	2	3	18	Alexia

Food Item	Serving size (ml)	Kcal	Protein (g)	Fat (g)	Carbohydrate (g)	Product brand
Beverages						
Regular soda	368	140	-	-	36	Coke, sprite
Diet soda	368	-	-	-	-	Coke, Sprite
Sweetened iced tea	368	125	-	-	31	Snapple
Sweetened diet iced tea	368	-	-	-	-	Snapple
Water	250	-	-	-	-	Poland Spring

APPENDIX 3

Additional Figures - Preload Study (Chapter 3)

Figure A3.1 Mean ratings of desire to have a meal over time as a function of preload condition.

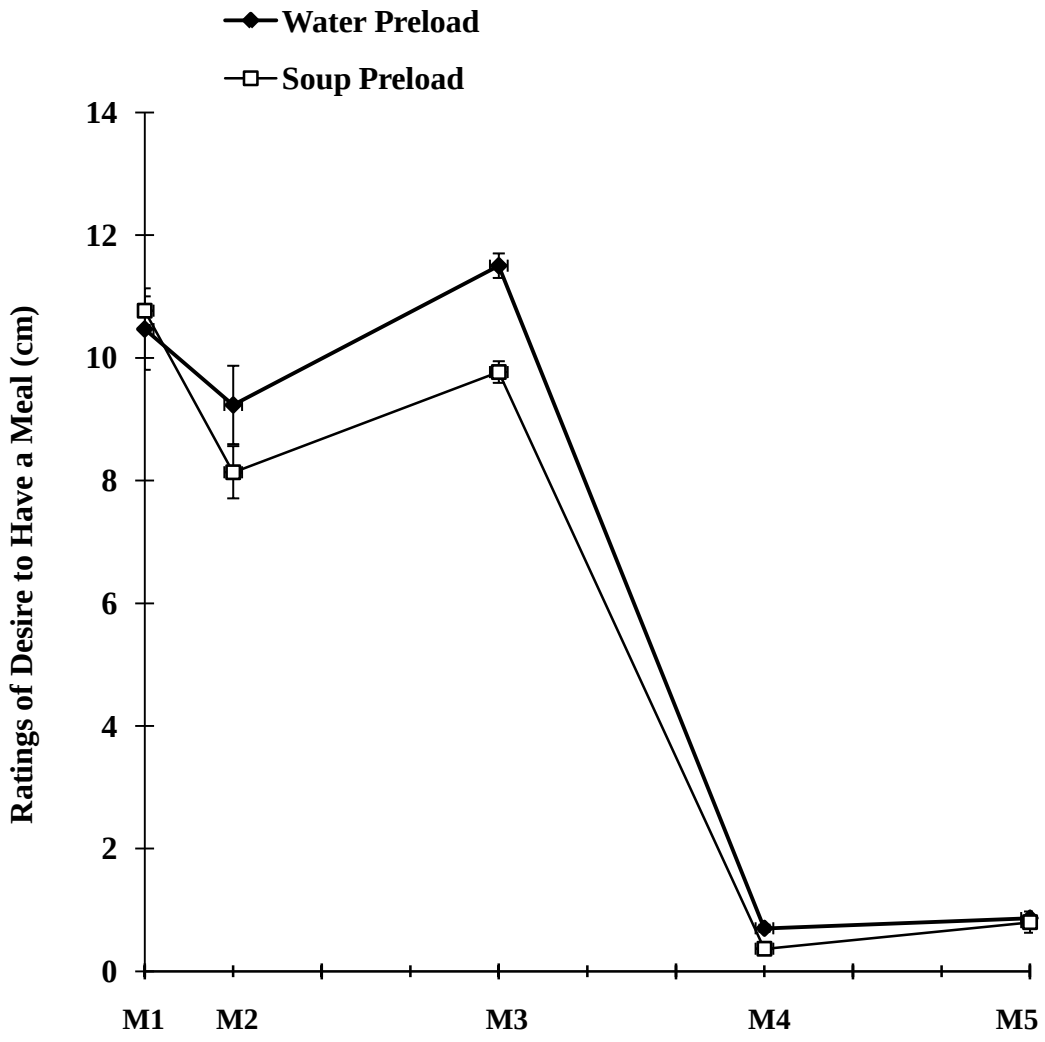


Figure A3.2 Mean ratings of desire to have a meal over time as a function of PROP taster status.

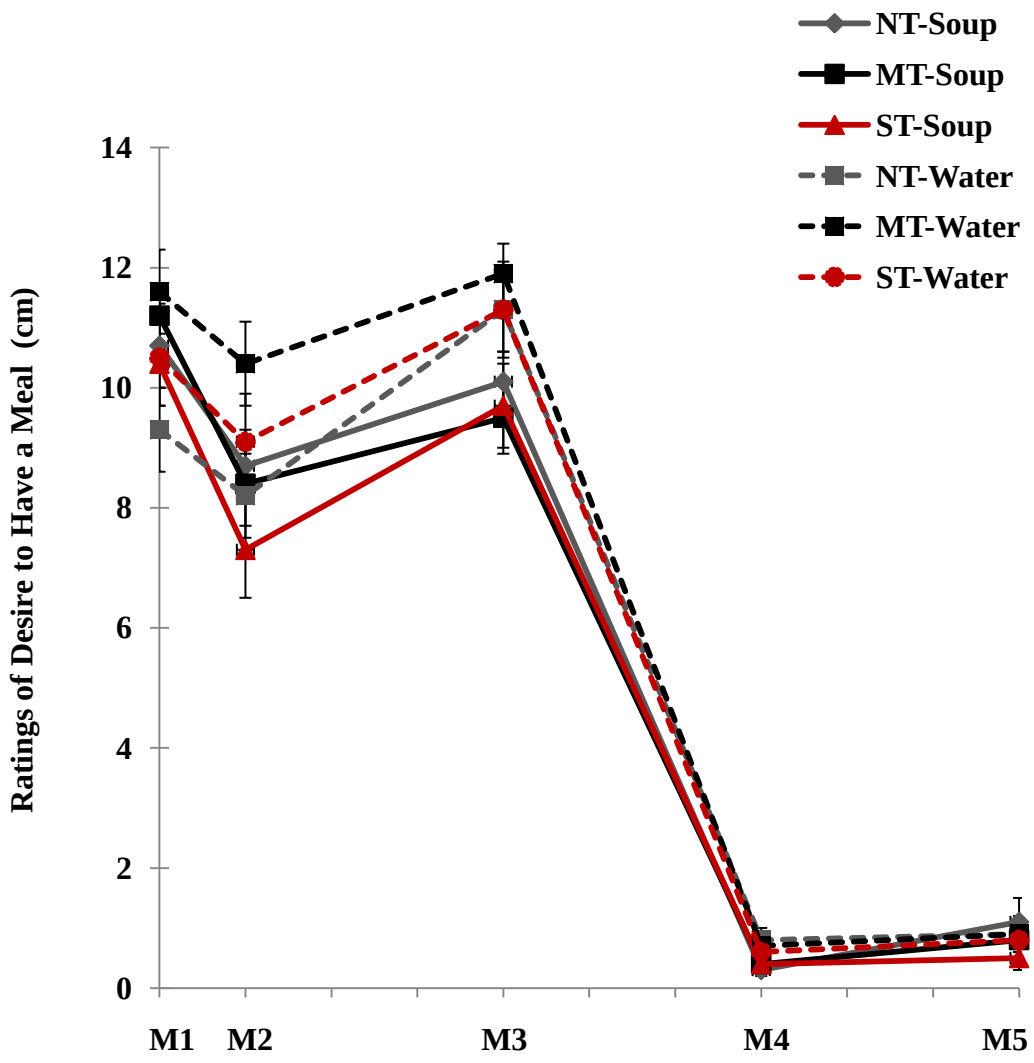


Figure A3.3 Mean ratings of desire to have a snack over time as a function of preload condition.

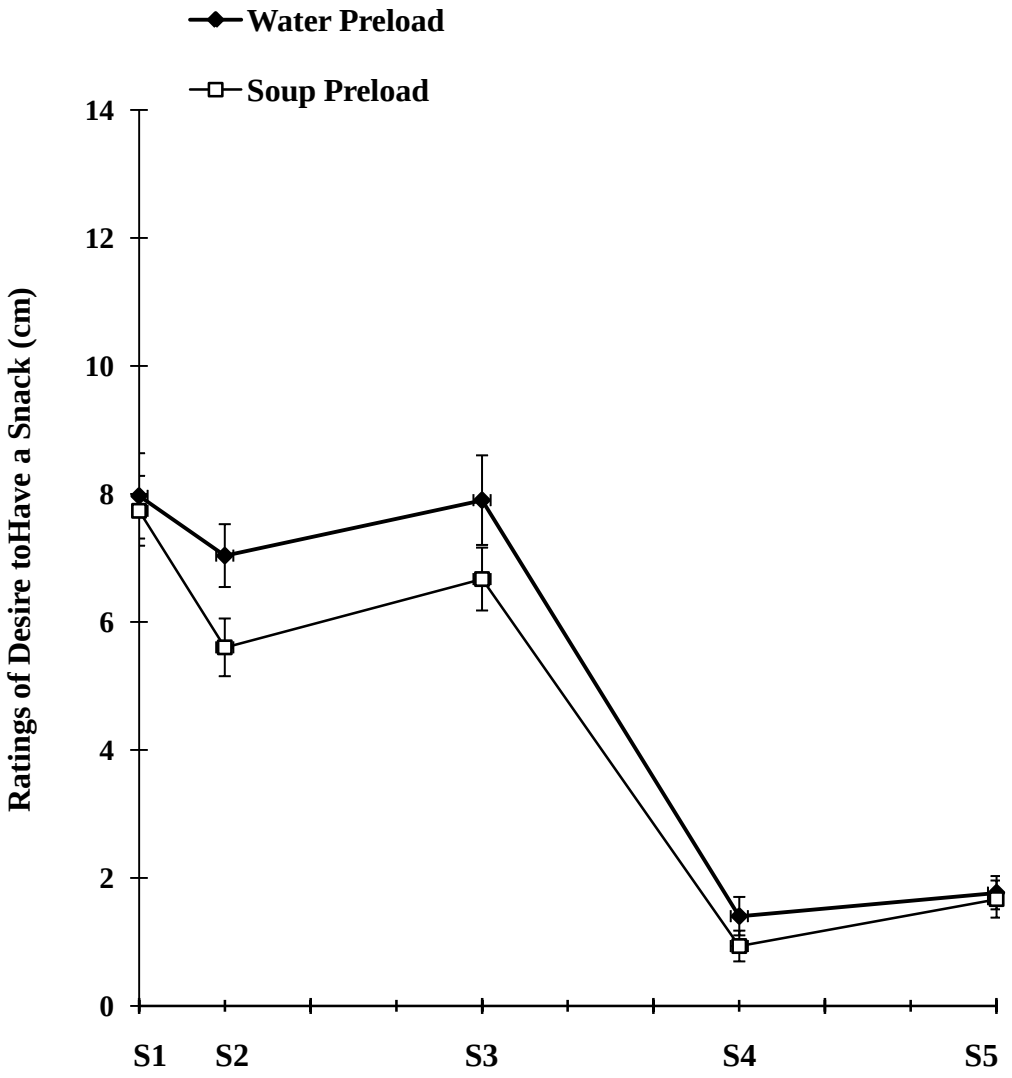
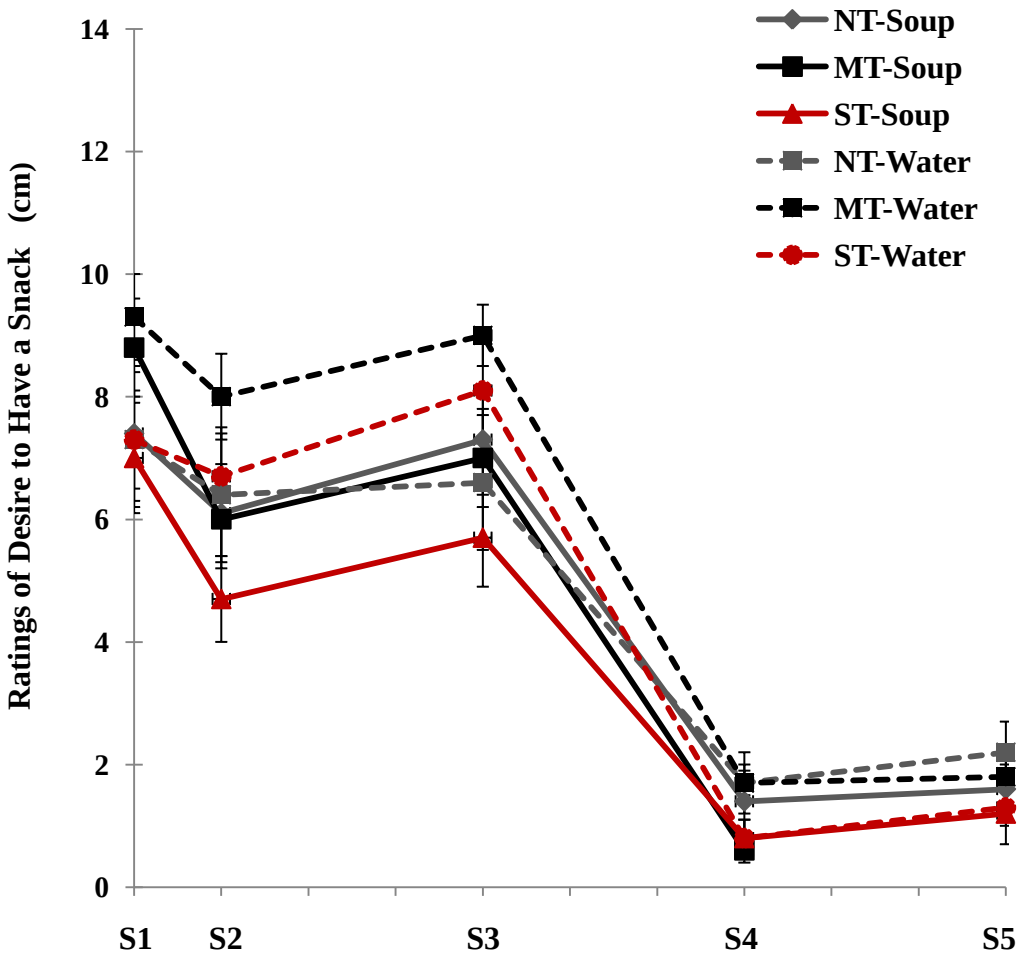


Figure A3.4 Mean ratings of desire to have a snack over time as a function of PROP taster status.



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Shafaie, Y., and M.M. Rafi. 2008. Cinnamon extract modulates the expression of obesity related genes in mouse adipocytes. Institute of Food Technologists (IFT), New York and Central New Jersey Subsection, Regional Meeting, New Brunswick, NJ (Poster)

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Shafaie, Y., and K.R. Matthews. 2005. Role of post-harvest processing practices in contamination of lettuce. In Proceedings International Association for Food Protection, Annual Meeting, Baltimore, MD (Poster)