Acute and chronic energy deprivation improves smell performance and heightens the rewarding value of food: How modality of deprivation differently impacts olfaction, food reward, appetite, peptide hormones, and energy intake

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I've been in school for 30 straight years. No breaks. People often ask: Aren't you sick of school by now? My answer is a resounding and resolute, NO! And the great facilitator—my Capitaine—has always been there steering a path and managing my scientific career. Alas, I salute you Dr. Éric Doucet. My two internal evaluators, Dr. Pascal Imbeault and Dr. François Haman, have provided me excellent criticism and a tremendous amount of positive and productive feedback. Merci. Dr. Gary Goldfield of the Children's Hospital of Eastern Research Institute has also played a significant role giving me a lot of good feedback on the reinforcing value of food throughout my Ph.D. and pushed me towards a good path in developing into an independent researcher. Ann Benninato and Marie-Éve Riou were each also integral parts in promoting the success of my research at the BMRU/URCM. I also must acknowledge the tremendous help that University of Ottawa Heart Institute molecular genetics professor Dr. Frédérique Tesson offered me. It was 6 months of bench-side science that assured me the complexities of studying any topic on a microscopic scale. Dr. John Blundell and Dr. Graham Finlayson from the Biopsychology Group, Institute of Psychobiological Sciences, University of Leeds were kind enough to let me bring their newly developed tool to measure food reward, and both have been quite helpful with any problems along the way. My family is simply too big to start thanking. All have made me who I am today. My 5 year old boy Matheason and my 3 year old girl Alexandra have given me not only great personal joy and personal fufilment, but unbeknownst to them, they have also been star subjects in my study of feeding and reward. Jean Piaget meets Charles Darwin comes to mind. My wife Chantal has perhaps taken on the hardest role one could ask: sticking with a professional student. Perhaps it is time for me to launch into a science-related carreer and forego any further formal education, no? Here is a quote from the ACKNOWLEDGEMENTS section of my Master's Thesis:

"I have to thank my wife Chantal for very patiently waiting for her husband to finish what seems to be a neverending story on the most grandiose scale. I love you."

And so on.

ABSTRACT

The study of feeding behavior, and in particular the study of subjective hedonic experience and objective measures of motivation, are central to understanding how appetite regulation can be compromised in certain individuals. Furthermore, with an integrated picture of physiological and behavioral changes that can occur as a result of energy deprivation what emerges is a better understanding of how palatable food can disrupt attempts at regulating body weight at lower levels of body energy stores. In Article I, the genetic association study examining a potential role for a dopamine-related polymorphism in weight loss, it was shown that contrary to the main hypothesis there was no association between TaqIA polymorphism and the amount of body weight loss. In Article II, it was shown that palatability and olfaction ratings increased as a result of a 24 hour fast and females demonstrated larger improvements in overall olfactory performance. Initial body weight was positively related to improved odor detection threshold and total odour score (TDI). Using the same population sample as Article II, Article III highlights that higher sensitivity to reward and disinhibition scores correlated with responding for palatable snack food stimuli in the relative-reinforcing value of food (RRV) task, further indicating that RRV has strong ties with impulsivity. There was a demonstrable lack of negative alliesthesia under the fasted condition where, after a 75% increase in *ad libitum* energy intake (EI) relative to the fed condition, this greater amount of food consumed was still rated as being more palatable than the lesser amounts consumed under the fed condition. In Article IV it was shown that an equicaloric (-25%) energy deficit by diet alone was a greater challenge to appetite regulation and resulted in greater compensatory increases in EI than deprivation by exercise alone. Independent of deprivation modality there were significant improvements in odour threshold scores. TDI score increased only under diet alone; furthermore, the noted increase in mean TDI score was positively related to increased *ad libitum* EI. The picture that emerges is that, acutely, a complete fast has more pronounced effects on appetite and *ad libitum* EI than dieting alone, which in turn had greater effects than exercise alone or controls. Also, TDI improved under all three methods of energy deprivation, but moreso under conditions of deprivation by diet alone.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS…… ii

ABSTRACT…… iii

LIST OF TABLES……..vi

LIST OF FIGURES…… vii

PART ONE: EMPIRICAL, THEORETICAL AND METHODOLOGICAL CONSIDERATIONS

CHAPTER

I INTRODUCTION…….…….…….... **2**

Significance of the Study ..…….....…….. 8

Definitions…….....……. 11

II REVIEW OF LITERATURE…… **13**

Episodic Nature of Feeding……..… 17

Reinforcement.....…… 20

Food as Reward.....……. 21

Peripheral Feeding Signals……..… 31

Food Hedonics…….....…… 32

Energy deprivation and Palatability.....…… 37

Summary Points.....…….…. 39

Assumptions, Limitations and Delimitations……..…. 50

Objectives and Hypotheses……..…… 56

III **METHODOLOGY** (remitted to article in Part II)…….. **59**

PART TWO: RESULTS OF THE STUDY AND DISCUSSION

IV **Results and Discussion: Article I.....…… 54**

V **Results and Discussion: Article II.....…… 75**

VI **Results and Discussion: Article III.....…….. 87**

VII **Results and Discussion: Article IV.....…… 111**

PART THREE: CONCLUSIONS AND RECOMMENDATIONS

VIII **CONCLUSIONS AND RECOMMENDATIONS.....…… 150**

PART FOUR: CONTRIBUTION OF COLLABORATORS

VI **STATEMENT OF CONTRIBUTION OF COLLABORATORS… 157**

PART FIVE: REFERENCES AND APPENDICES

REFERENCES……. 161

APPENDICES…….. 175

LIST OF TABLES

Article I

Table 1. Subjects' characteristics by weight and Taq1A genotype…….. 74

Article III

Table 1. Subjects' characteristics under FED and FASTED conditions ….. 107

Table 2. Food Reward: The RRV and LFPQ computer tasks..... ….. 108

Article IV

Table 1. Subjects' characteristics for CON, DER and DEX.....…….. 143

Table 2. Concentrations of fasting plasma ghrelin and leptin..... ….. 144

LIST OF FIGURES

Article II

Figure 1. Olfactory performance under FED and FASTED..... 85

Article III

Figure 1. Protocol for 24 hour fasting.....….. 109

Figure 2. Implicit wanting and explicit liking..... ….. 110

Article IV

Figure 1. Protocol for the cross-over design..... 138

Figure 2. Plasma concentrations of leptin and ghrelin by condition….. 139

Figure 3. Olfaction scores by diet alone or exercise alone..... 140

Figure 4. Food reinforcement and palatability by condition..... …..141

Figure 5. Measures of wanting and liking by condition..... ….. 142

PART ONE: EMPIRICAL, THEORETICAL AND METHODOLOGICAL CONSIDERATIONS

CHAPTER I: INTRODUCTION

Upon closer investigation it will be argued in the following pages that a significant amount of the variation in the individual response to a similar food environment can begin to be explained not only at the gene level, but also by examining differences in the evaluation of food reward. This manuscript follows studies from rodents to primates (including humans)—and in examining the hedonic evaluation of food, nasal chemosensory performance, food reward, appetite and related peptides, and *ad libitum* energy intake (EI), this thesis will attempt to describe some of the intricacies behind one the most integrated of all behaviours, feeding. At the core of each of the four original research papers included in this document will be the analysis of how varying degrees of energy deprivation differently impact all or some of the abovementioned variables.

All else being equal, interventions either decreasing energy intake (EI) or increasing energy expenditure (EE) (or both) should produce a negative energy balance and result in weight loss. Commensurate with sustained energy deprivation—acute or chronic—there are varying, and often graded, responses that the human body experiences: changes in metabolic, autonomic, neuroendocrine, and behavioural factors, all of which define how each individual differently responds to a theoretically similar absolute level of energy deprivation. Thus there needs to be a more integrated approach when attempting to discern the underlying changes that accompany sustained energy deprivation in what is often glazed over teleologically as simply an “energy in” versus “energy out” problem. The pursuit of pleasure—or the hedonics (from the Greek word ‘delight’) of food—is what often guides feeding behaviour. But what belies the physiology and psychobiology of overfeeding or underfeeding remains elusive with plenty of unanswered research questions. It is intuitive to conclude that eating less would by convention increase appetite, and in particular hunger. In fact, several groups have shown that after approximately 3-5 days of complete starvation appetite scores return to baseline levels in the fed state. Or one may intuitively think that an energy deprivation produced by exercise would result in a compensatory increase in EI, but exercise is not only known to produce acute anorexia—“exercise anorexia”—but evidence also suggests that regular exercisers have smaller variations in hunger and

fullness. Following diet induced weight loss, an increase in orexigenic drive in the fasted state has been reported (Doucet, et al., 2000; Keim, et al., 1998) and similar findings have been shown with aerobic exercise (King, et al., 2009; Whybrow, et al., 2008). Other groups have found no change in appetite or EI within hours after exercise (King, et al., 1994; King, et al., 1996) or on the day after a single session of vigorous aerobic exercise (King, Miyashita, et al., 2010; King, et al., 1997).

The identification and action of the orexigenic and anorexigenic petide hormones, ghrelin and leptin, respectively, have been well described (for review see (Doucet, et al., 2007) but much of the conclusive original research in appetite and feeding has been in animals and performed by the administration of pharmacological doses of these peptides, either peripherally (intravenously) or centrally (intracerebroventricularly). Furthermore, though there are convincing data to show that ghrelin levels rise pre-prandially (Cummings, et al., 2001), are positively correlated with EI (Erdmann, et al., 2004), and suppressed proportional to amount of food ingested (Callahan, et al., 2004), controversy still remains regarding how this hunger signal responds to energy deprivation by dieting or by exercise (Stensel, 2010) and whether such changes are associated with measurable psychobiological responses. Regarding leptin, the response to energy deprivation occurs rapidly and disproportionately with losses of fat mass. For example, despite a loss of only 2.6% of initial body weight, mean levels of plasma leptin decreased by 62 ± 25% in just 3 days (Weigle, et al., 1997). Although the role of leptin as an adipostat has subjugated much attention, recent animal findings suggest that leptin is also involved in the signaling of food reward (Figlewicz, et al., 2007; Fulton, et al., 2004; Fulton, et al., 2000) and may have a role in olfaction (Getchell, et al., 2006; Martin, et al., 2009). Furthermore, evidence suggests that an increased hunger-state can increase food palatability and changes in peripheral leptin can account for heightened hedonics. For example, leptin-deficient individuals report higher liking rating for food, and leptin replacement therapy has been shown to normalize liking ratings even before weight loss was achieved (Farooqi, et al., 2007). It has yet to be established, however, if a reduction in circulating leptin as seen with negative energy balance can impact food reward or olfaction in humans.

Olfaction plays an integral role in locating food sources, determining which food items are acceptable to consume, and largely contributes to the sense of taste. Although research has suggested a role for energy deprivation in the modulation of smell function (Albrecht, et al., 2009; Goetzl, et al., 1947), it remains to be determined how this may impact feeding and overall body weight status. For example, using the well validated Sniffin' Sticks (Hummel, et al., 2007) to measure odour detection threshold, odour discrimination, and odour identification it was shown that individuals demonstrated higher (better) scores after 12 hours (Stafford, et al., 2011) and 24 hours (Cameron, et al., 2012b) fasting versus in the fed state, but there was not a relationship with increased *ad libitum* energy intake (Cameron, et al., 2012b). Demonstrating these weren't likely examples of type II error, the test-retest reliability of Sniffin' Sticks was measured in a separate study in subjects tested at 0, 35, and 105 minutes, and approximately one month later, and no significant difference was found in threshold scores (Albrecht, et al., 2009). It remains to be determined, however, with respect to olfaction, what role is played by the underlying metabolic changes that occur with energy deprivation, and whether measured changes in smell function can impact feeding and appetite differently when a deprivation is induced by dieting or by exercise.

According to the "incentive salience hypothesis" (Berridge, et al., 2009), there is a change at the neurological level that describes perceptual (*e.g.* cognitive) and motivational (*e.g.* unconscious) components that accompany the shift from a stimulus being neutral to something that is attractive and can energize and motivate behavior. One method that has been designed to examine explicit "liking"/ "wanting" and implicit "wanting" of food is the recent development of a forced choice computer-based procedure (Leeds Food Preference Questionnaire; LFPQ) (Finlayson, et al., 2007b). This task presents photographic food stimuli of twenty different items varying along two dimensions—fat (high or low) and taste (savoury or sweet). Implicit "wanting" is measured by the speed with which one stimulus is chosen in preference to an alternative and is additionally measured by relative preference (*e.g.* fat vs. sweet). Laboratory choice paradigms can also be employed to measure the relative-reinforcing value (RRV) of various foods by discriminating the amount of work done to obtain food

when offered a choice between other food or non-food items (Lappalainen & Epstein, 1990). Thus under experimental conditions which potentially alter the motivation to eat it can be determined if a food stimulus becomes more or less reinforcing—the more work done to acquire the item, the more reinforcing it is said to be (Epstein, et al., 2006). Taken together, examining the wanting and liking and the reinforcing efficacy of foods—collectively operationalized henceforth as food reward—may provide a framework to understand factors influencing food choice, appetite, and to provide more personalized advice on body weight management.

A recent study employing the LFPQ task examined the impact of energy deprivation by means of exercise on food reward and *ad libitum* energy intake with two sessions, one at 50 minutes of high intensity exercise and then other without exercise (Finlayson, et al., 2009). Although there was no significant difference in the amount of food eaten by test day, it was found that after the exercise session there was a subgroup of "compensators" who ate more after exercise and also displayed a significant increase in "wanting"; specifically, after exercise there was an unconscious *implicit* desire for the "compensators" to eat high-fat sweet foods (Finlayson, et al., 2009). The main findings from a separate study noted that in a hungry state (3-4 hours acute energy deprivation) subjects "wanted" fat (particularly high fat) and savoury food more so than fat and sweet food, but this trend was reversed and subjects "wanted" low fat sweet after the completion of an *ad libitum* pizza lunch (Finlayson, et al., 2007b). Although to our knowledge there are no studies looking at RRV and exercise, results from an acute energy deprivation ranging in the time of ~13-20 hours in lean individuals indicated that in this relatively short period of deprivation the RRV of a palatable snack food (versus a sedentary activity) significantly increased from the fed state (Raynor, et al., 2003), and a positive relationship between the reinforcing value of food and energy intake has also been established (Epstein, Temple, et al., 2007; Epstein, Wright, Paluch, Leddy, Hawk, Jaroni, Saad, Crystal-Mansour, & Lerman, 2004).

Of late there has been much focus on genotypes thought to be linked with impaired dopamine signalling. The surge in studies of dopamine and the role of its transport and receptor genes in feeding and other reward-driven behaviours such as

ethanol consumption, gambling, drug-taking, and obesity strongly points to evidence of reward-related phenotypes (Noble, St Jeor, et al., 1994b). Support for the role of dopamine in human feeding behaviour is evidenced in part by the anorexigenic action of dopamine agonists (Goldfield, et al., 2007; Leddy, et al., 2004; Schertz, et al., 1996) and by the orexigenic action of dopamine antagonists (Roerig, et al., 2005; Ruetsch, et al., 2005). Dopamine availability is dependent on its release, transport (reuptake), metabolism, and receptor binding. Consequently, by looking at the genes involved at any one of these functional stages there is an opportunity to indirectly investigate brain dopamine levels—in effect looking at markers of neurotransmitter activity (Epstein, Temple, et al., 2007)—and how behaviour may be resultantly impacted.

One polymorphism believed to result in decreased dopamine signalling occurs due to an alteration in the gene for a postsynaptic dopamine receptor, DRD2. Specifically, there are three *Taq1* A variants (A1/A1, A1/A2, and A2/A2) and compared to carriers of the *Taq1* A2 allele, *in vivo* imaging had shown that people with the *Taq1* A1 allele have reduced brain dopamine signalling (Pohjalainen, et al., 1998). With further *in* vivo (PET imaging) evidence, the mechanism of action is thought to be mediated primarily with the association of the *Taq1* A1 allele with decreased DRD2 receptor density (Noble, et al., 1991). What this suggests is that carriers of the *Taq1* A1 allele experience reduced dopamine signalling in the brain; indeed, it has recently been demonstrated that decreased density of DRD2 is strongly associated with human obesity, in inverse proportion to BMI (Wang, et al., 2001). Linking physiology with behavior, current research has revealed that the reinforcing value of food –analogous to the “wanting” component of food reward—can not only influence energy intake, but the presence of the *Taq1* A1 allele of the dopamine receptor can interact with obesity to influence food reinforcement (Epstein, Temple, et al., 2007; Epstein, Wright, Paluch, Leddy, Hawk, Jaroni, Saad, Crystal-Mansour, Shields, et al., 2004). Subjects identified as high in food reinforcement who were carriers of the A1 allele consumed more food than participants high in food reinforcement without the A1 allele and participants low in food reinforcement with or without the A1 allele (Epstein, Temple, et al., 2007). Although these data are preliminary, what this suggests is that using a behavioral

genetic approach to understand the interaction between genotype, obesity, and food reinforcement can be viewed as a viable research venture.

Thus, individuals with the *Taq1* A1 allele are hypothesized to be less sensitive to stimulation of dopamine-regulated reward circuits—analogous to a reward-deficiency syndrome (Noble, Noble, et al., 1994)—and by enduring a hypodopaminergic state are more likely to seek reinforcers, whether it be food or drug (Blum, et al., 1996). In fact, this represents one view of dopamine's involvement in food reward and it fits well with much of the literature presented thus far. Although it is a very hedonistic view of feeding behavior, it is a tenable hypothesis that overeating has emerged as a compensatory mechanism to ameliorate a deficiency in the reward circuitry (Wang, et al., 2002). Others contend a somewhat opposing view that focuses on an individual's sensitivity to reward, as measured by a psychobiological questionnaire. It is argued that individuals with high levels (hyper-dopaminergia) of synaptic dopamine are more sensitive and have a greater capacity for reward, thereby making them more likely to engage in pleasurable behaviors (Davis & Fox, 2008). In the end, it is not clear whether the dopamine hypothesis of feeding is best explained by hypo-dopaminergic states as described by a reward deficiency syndrome, or by hyper-dopaminergic states as described by heightened reward sensitivity, or by both.

As a final introductory note, it must be accepted that contrary to the view that obesity is only about behavioural control (or lack thereof), that feeding behaviour is not necessarily a conscious process. The *Milieu Intérieur* is constantly responding to the ebb and flow of blood-borne (chemical) and mechanical (distension) signals and their sensory afferents; incentive stimuli in the external environment along with learned associations both external and internal add salience to edible objects; genotypic variations amongst several hundred genes predispose behavioural outcomes in particular environments; and the whole of the hierarchy from blood to brain necessitates access to the basal motor system to coordinate the action of hand-to-mouth. In the end this thesis is guided by the unresolved questions regarding how each level of the aforementioned hierarchy impacts appetite, feeding-related peptides, olfaction, food reward, and body weight regulation. This thesis will also serve as an analysis of the

degree to which “wanting” and “liking” may reflect underlying nutritional needs (*e.g.* chronic energy deprivation) or to what extent these components of food reward can be reflected independently of nutritional need state (*e.g.* weight-stable and energy replete). In combination with an expansive list of variables the goal is to track and define the prospective behavioural, genetic, and metabolic factors that may promote overconsumption and compromise appetite control under acute or chronic bouts of energy deprivation.

Significance of the Study

Perhaps a better title for this section would be *Significance of the Studies*. Altough there exists a constant and pervasive theme of various forms of energy deprivation and the resultant impact on food reward, this thesis is defined by three separate original studies: Study I looks at a 6 month energy deprivation of approximately 700 kcal/day by caloric restriction alone or by caloric restriction plus resistance training; Study II contrasts a 24 hour complete fast with the fed state; and Study III looks at an acute 3 day energy deprivation of approximately 700 kcal/day by caloric restriction alone or by aerobic exercise alone.

Early identification of traits (e.g. dietary restraint, disinhibition) that predispose some individuals to overconsume under similar food environments and describing speficic behaviours that are affected by acute and chornic energy deprivation will help to tailor individual interventions. If it can be shown that food restriction (as typically seen in dieting or with exercise) is positively related to food reward and *ad libitum* EI—thereby indentifying a tie between reward and overconsumption—then the questions raised in this thesis could afford evidence for the need to focus on motivational factors that influence eating. When the goal is to reduce body weight and combat problems of eating pathology, potential findings may demonstrate a need to focus on alternatives to feeding, thereby combating antecedent precursors to a lapse in control of appetite and energy intake.

Dissociating a psychological concept such as a reward into separable components of hedonic evaluation and motivation to obtain and consume does have ecological validity. In particular, the tools to be later described that will be employed to

measure food reward can offer valuable information regarding the determinants of food choice. There are a lot of data showing that if access to a preferred food (such as cheesecake) is reduced, then people will choose a less preferred—although still enjoyable—alternative (such as yogurt). Applied in light of behavioral economics theory, if the price of a good that is typically purchased is increased, then as cost increases there will be a certain point where the consumer will *substitute* with something similar, though less expensive. A knee-jerk solution would be to tax foods with "empty calories" such as high fructose corn syrup, which in fact is occurring in some U.S. States. A better solution would be to promote the development of ready-to-eat healthy snacks such as the conveniently peeled and washed baby carrots, and to implement such foods across school cafeterias nationwide. At the very least, a goal should be to decrease the disparity in prices between fresh produce and the lower cost highly processed foods.

When considering the millions of people that start dieting each year, the fact that food reinforcement increases with energy deprivation has obvious concerns. The very thing that is supposed to help with weight loss (*e.g.* decreased energy intake) can act to increase the motivation to eat energy dense food. Although there are no definitive data to show that increased meal frequency (independent of energy intake) promotes weight loss, it may still be a good approach to prescribe such a diet in an attempt to control for overeating due to increased saliency of palatable food. To be sure, irregular meal patterns such as skipping meals can reinstate abhorrent binge-eating behavior in individuals recovering from bulimia nervosa. Nonetheless, a reduced-calorie diet imposed with a greater frequency of meals may also leave individuals to choose a non-food activity that is reinforcing (*e.g.* hiking) in the place of impulsively eating due to a perceived deprivation in energy and increased food reinforcement. The increased food reinforcement—and its predicting power of *ad libitum* feeding—experienced by the obese may also highlight the importance of limiting access to food in a strategic manner.

Although there are no data about food reinforcement and success with weight loss, a valid hypothesis would be that for individuals high in food reinforcement (vs. low) a high meal frequency (*e.g.* 6 meals) diet of pre-packaged meals would result in improved adherence to the diet and better appetite control vs. low meal frequency (*e.g.*

3 meals). Regarding the forced-choice computer task that was developed in an attempt to measure *implicit* "wanting" and *explicit* "wanting" and "liking", there exists the potential to test the impact of various forms of energy deprivation on these reward-related variables. Useful information will no doubt emerge on how relative (macronutrient) preference or reaction time—two quantitative measures of implicit "wanting"—can be impacted by not only reduced energy intake but also by increased energy expenditure. A recent study examined the impact of energy deprivation by means of exercise on food reward and *ad libitum* energy intake with two counterbalanced sessions, one at 50 minutes of high intensity exercise and then other without exercise (Finlayson, et al., 2009). Although there was no significant difference in the amount of food eaten by test day for this group of lean women, it was found that after the exercise session there was a subgroup of "compensators" who ate more after exercise and also displayed a significant increase in *implicit* "wanting". After exercise there was an unconscious *implicit* desire for the "compensators" to eat high-fat sweet foods. What is interesting is that a separate study with similar exercise intensity (but longer duration) also demonstrated that lean female subjects overcompensated for the exercise session (vs. an equicaloric lower intensity exercise) when given access to an *ad libitum* for the remainder of the day (Pomerleau, et al., 2004). In both studies there was evidence that performing intense aerobic exercise results in increased energy intake, thereby acutely promoting *positive* energy balance. It may be that some individuals are more sensitive to substrate flux and the mobilization and utilization of glucose, making them more likely to select energy dense foods following intense aerobic work. Elucidating who would potentially be "compensators" from the onset of an exercise prescription, as measured by increased implicit "wanting", could not only help with recidivism but also with weight loss success. Extending these findings to obese individuals could potentially identify those who would benefit from performing a lower intensity exercise to attain/maintain weight loss.

For most of the developed countries meals are entrained to a schedule thereby eliminating what might be real signals of energy deprivation—this fact has raised to attention that such feeding may anticipate and prevent the development of significant metabolic changes (Woods, 1991). It is for this reason that an extreme (complete)

caloric deprivation was chosen for one of the studies in this thesis. This extreme caloric deprivation is part of what makes this thesis unique. There is simply no data on how such a drastic deprivation can impact olfaction, food reward, and food reinforcement. The study of feeding behaviour, and in particular the study of subjective hedonic experience, is central to understanding how appetite regulation can be compromised in certain individuals. Furthermore, with an integrated picture of physiologic and behavioural changes that can occur as a result of acute caloric deprivation—particularly with regard to food palatability and reward—what will emerge will be a better understanding of how palatable food can disrupt attempts at body weight regulation during moderate and extreme forms of dieting.

Definitions

1-Alliesthesia: From the words esthesia (meaning sensation) and allios (meaning changed). It is the modification of conscious sensations by changes in internal signals, reflected by afferent actions of peripheral receptors; plainly, a pleasant or unpleasant sensation depending on the subject's internal state.

2-Appetite: An internal state or a specific disposition to act. Appetite as it pertains to feeding can be conceptualized as the hunger-stimulated response to a particular food and implies knowledge of the item(s) to which the actions should be directed

3-Incentive Salience: A motivational process that does not involve a pleasure/hedonic component, but is limited to a drive process that defines the 'wanting' component of a reward. It is primarily believed to be influenced by dopaminergic neurotransmission.

4-Reinforcer: In a Skinnerian sense reinforcement can be viewed as behavioral measures of learning: it is the strengthening of an observed behavioral response (stimulus-response associations) or the strengthening of a learned behavioral response (stimulus-stimulus associations). A reinforcer can be a goal or a commodity.

5-Reward: A psychological process that is contingent on a reinforcing stimulus. Reward can be dissociated into two qualities of a food stimulus: 1) a hedonic component of "liking"/palatability, and 2) a reinforcing component that is sometimes described as "wanting"/incentive motivational component.

i)-“Liking”: Divided into *implicit liking,* which is measured objectively by affective facial reactions to various food stimuli, and *explicit liking,* which is measured by the subjective hedonic rating of orosensory pleasure (*e.g.* visual analogue scales).

ii)-“Wanting”: Divided into *intrinsic wanting*, which can be measured by choice tests, reaction time, or reinforcement, and *extrinsic wanting,* which can be defined by the intent or desire to consume a specific food and measured subjectively on a visual analogue scale.

ii) A) Relative-Reinforcing Value of Food: Describes how hard an individual is willing to work for food by measuring responses at a predetermined reinforcement schedule. It is *relative-reinforcing* due to the fact that there are alternatives, albeit in a forced choice methodology.

6-Satiation/Satiety: Satiation refers to the processes that lead to the termination of feeding, *e.g.*, a within-meal interval, whereas, satiety refers to the state of inhibition of feeding, *e.g.*, a between meal interval.

7-Sensory Specific Satiety: With continued exposure to the same food item there is a discernable and rather abrupt change in the overall hedonic rating, *i.e.*, the perceived sensory qualities of the item are not absolute though the change in liking is specific to the unchanging sensory characteristics of the item itself.

8-Work: Work will be defined as the willingness to perform simple button-presses on a computer. It is to be understood that the work performed during the RRV computer task is internal work, thereby indicating insignificant changes in energy expenditure as a result of performing this task.

CHAPTER II—REVIEW OF LITERATURE

This review of the literature pertinent to the submitted thesis is published as a book chapter titled **Reinforcement and Food Hedonics: A look at How Energy Deprivation Impacts Food Reward** published in The Handbook of Behavior, Food and Nutrition (Cameron, J., & Doucet, E. (2011). Reinforcement & Food Hedonics: A Look at How Energy Deprivation Impacts Food Reward. In C. R. Martin, V. R. Preedy & R. R. Watson (Eds.), *Handbook of Behavior, Food and Nutrition* (Vol. 1, pp. 3667). New York: Springer-Verlag New York Inc.). It has been included here modified from its final form as accepted by the editorial board because it remains, in the thesis author's opinion, a most appropriate review of literature for the submitted thesis. J.C. wrote the book chapter and created all of the original tables and figures and E.D. edited the work.

Reinforcement & Food Hedonics: A Look at How Energy Deprivation Impacts Food Reward

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Abstract

In a detailed analysis of the psychobiology of feeding behavior with particular attention to food reward, this chapter describes theories and data on reinforcement and taste perception. This chapter will also provide evidence for an explanation of what may account for the large variation in the individual response to a similar food stimulus, highlighting the role of peripheral feeding signals (*e.g.* the “hunger hormone” ghrelin or the adiposity-marker hormone leptin) and the role of the neurotransmitter dopamine. What is interesting is that when energy deprivation is prolonged not only does palatable food become more salient but items that would normally not be selected can also become attractive. The fact that this increased hedonic valence is similar for rodents and primates (including humans) is indicative of common (neuro)biological underpinnings. Although it will be argued that nutritional state—whether defined by acute or chronic energy deprivation—can impact the “liking” and “wanting” of a food stimulus, it will also be argued that humans also eat in the absence of energy needs due to simple Pavlovian learning. New evidence from studies on taste and olfaction are presented and it appears that, continuous with leptin’s role as a marker of energy reserves, when leptin levels are high (signalling adequate reserves) there is a corresponding decrease in sensitivity to taste and to olfactory stimuli. Merging together literature on the dopamine hypothesis of feeding and the *incentive salience hypothesis*, this chapter will also describe the current views of how the neurotransmitter dopamine impacts the motivational component of food reward. The pleasure/palatability component of food reward will also be described in a comprehensive format at the behavioral and neurophysiological level. A question that remains to be answered is the extent to which homeostatic components (nutritional need-states) can impact the quality of oro-sensory reward, thereby enhancing food hedonics and ultimately compromising appetite control.

Introduction

Why is it that amidst the plethora of seemingly conscious choices we make throughout our days that we often find it irresistible to reach for that next chip? After all, aren't *we* in control here? When asked such seemingly simple questions it appears as though problems such as helpless overeating—or just as germane, the command involved in dietary restriction—seems to be plainly a matter of psychological weakness and purposeful cognitive control. But, as will be discussed throughout this chapter, there is much more to feeding behavior and stable body energy reserves than self-control. In order to comprehend the physiological and psychological components involved in the uncomplicated act of eating (or not eating!) a bag of potato chips it is necessary to focus on the myriad of mechanisms that are in motion when anticipating, approaching, ingesting, and reflecting about food.

Upon closer investigation it will be argued in the following pages that a significant amount of the variation in the individual response to a similar food environment can begin to be explained not only at the gene level, but also by examining psychobiological differences in the evaluation of food *reward*. Specifically, by highlighting the potential differences in responding between lean and obese animals—spanning studies from rodents to primates (including humans)—both to the hedonic evaluation of food and to the reinforcing value of food, this chapter will attempt to describe some of the intricacies behind one the most integrated of all behaviors, feeding. Furthermore, another underlying theme will be the discussion of how energy deprivation, defined as either acute or chronic, can impact the two abovementioned components of food reward.

Beginning with the advent of agricultural practices and modernized with industrial production of food, modern-day humans are now unique to the animal kingdom in that feeding, for much of the affluent world, occurs for reasons other than sustained periods of energy deprivation. The pursuit of pleasure—or the hedonics (from the Greek word *delight*) of food—is what often guides feeding behavior. But what belies the physiology and psychobiology of what amounts to abhorrent feeding patterns still remains elusive. What is clear, however, is that when energy deprivation is prolonged there is a degree of

disinhibition with respect to appetite: not only does palatable food become more salient but items that would normally not be selected can also become attractive. A plethora of research has since emerged on how homeostatic-like elements—states of nutritional need—can alter the pleasure of a sensation of food. This concept was coined as *alliesthesia* and has since received much attention.

Often a combination of sight, smell, touch, and previous exposure to the food stimulus, the rewarding quality of food is therefore represented by an active process of the brain that is defined by a composite reaction to the food, and as a result is not simply a physical property of the taste stimulus itself (Berridge, 1996). Furthermore, the actual reward corresponds to, and is divided by, what are believed to be three separate psychological concepts ("*wanting*", "*liking*", and learning) which are underpinned by distinct neurobiological mechanisms. Each of these dissociable components describes independent qualities that define a rewarding stimulus, and each will be elaborated on in the following sections.

As a final introductory note, it must be clarified that contrary to the view that obesity is only about behavioral control (or lack thereof), that feeding behavior is not entirely a conscious process. The *Milieu Intérieur* is constantly responding to the ebb and flow of blood-borne (chemical) and mechanical (distension) signals and their sensory afferents; incentive stimuli in the external environment along with learned associations both external and internal add salience to edible objects; genotypic variations amongst several hundred genes predispose behavioral outcomes in particular environments; and the whole of the hierarchy from blood to brain necessitates access to the basal motor system to coordinate the action of hand-to-mouth. In the end this chapter is guided by the unresolved questions regarding how each level of the aforementioned hierarchy impacts the rewarding characteristics of feeding. This chapter will also serve as an analysis of the degree to which reinforcement/"wanting" and palatability/"liking" may reflect underlying nutritional needs (*e.g.* chronic energy deprivation) or to what extent these components of food reward can be reflected independently of need state (*e.g.* weight-stable and energy replete).

Part I: Reinforcement

1. Episodic Nature of Feeding: Beyond Homeostasis

Prior to beginning a discussion about the intricacies of defining and describing the rewarding attributes of a food stimulus there is a need to introduce the physiology of feeding in relatively general terms. At the behavioral level food intake is episodic (i.e. not continuous). This indicates that there must be physiologically distinct messengers that bring an animal to begin and to end a meal. In fact, nearly half a century of research has shown that there are hormonal signals released by the gastrointestinal tract—peripheral short-term feeding signals—resulting from, or prior to, a single bout of eating. These short-term signals are divided into orexigenic signals (e.g. the peptide hormone ghrelin) that convey the overall message to eat, and this in contrast with anorexigenic signals (e.g. the peptide hormones cholecystokinin and peptide YY) that convey the message of fullness (see **Table 1**). These peripheral feeding signals released from the physical act of consuming foodstuffs are first processed within the nuclei of the hindbrain (primarily, the nucleus of the solitary track, NTS). In a reciprocal manner, these stimuli are transmitted and converge to the real-time processing stations of the hypothalamus and associated cortico-limbic structures eventually leading to goal-directed motor programs that either facilitate or impede the further ingestion of food.

Table 1. A brief list of the peripheral feeding signals implicated in the short- and long-term modulation of energy intake. Of all the feeding signals, ghrelin is the only peptide hormone that is an orexigenic feeding signal.

Feeding Signal	Primary Site of Secretion	Effect on Food Intake
Long Term		
Insulin	Pancreatic β cells	Decreased Energy Intake
Leptin	Adipocytes	Decreased Energy Intake
Short Term		
CCK	Endocrine I cells of the proximal Small Intestine	Decreased Energy Intake
PYY_{3-36}	Enteroendocrine L cells of the ileum & colon	Decreased Energy Intake
GLP-1	Enteroendocrine L cells of the	Decreased Energy Intake

	proximal Small Intestine	
Ghrelin	Oxyntic X/A cells of the Stomach	Increased Energy Intake

Note: CCK is abbreviated for cholecystokinin; PYY_{3-36} is abbreviated for peptide YY; GLP-1 is abbreviated for glucagon-like peptide 1. Table was modified from (Cameron, et al., 2007) with permission.

Furthermore, there is extensive evidence that these peripheral meal-to-meal signals act directly on the arcuate nucleus of the hypothalamus by crossing the highly selective blood brain barrier, or indirectly at the arcuate through second messenger signalling. It must also be noted that there are metabolic signals such as excursions in blood glucose that can act to promote feeding. Classic work on glycaemia first demonstrated in rats was extended to humans and showed that arteriovenous differences (rate of utilization) of glucose correlated with hunger and energy intake (Van Itallie, et al., 1953). Although there is continued controversy on the action and mechanism of glucose's role in feeding, it appears that this metabolic signal also has downstream effects at the hypothalamus. The hypothalamus acts as a primary relay station that influences three major systems: the autonomic nervous system, the endocrine system, and the nested brain areas involved in motivational systems. As previously noted, when considering feeding behavior—on a very primitive level—this response can be deconstructed and thought of as motivational states (or drives) that are based upon bodily needs. More than this, whether it is to quench one's thirst or to eat in response to severe hunger pangs, these drives often force the body into action.

Overall, there are two main points to consider regarding the short-term regulation of energy intake: meal size and frequency. What is noticed in free-feeding laboratory conditions is that meal size predicts the interval until the following eating episode. This so-called "postprandial relationship" suggests that meal size is determined via adjustments to the interval to the next meal—not dependent on mere convenience or learned time cues. Conversely, and in most cases in the Western world, meals are scheduled at specific times of the day, resulting in no significant relationship between meal size and inter-meal interval (de Castro, 2000). In this so-called "preprandial

relationship" there is however a relationship between the inter-meal interval and meal size; what can be extrapolated is that under daily circumstances, the episodic quality of feeding is lead by associative learning. However, as the period of deprivation (inter-meal) increases there may be a shift to respond in a drive-induced manner. What this indicates is that there need not be a deprivation or homeostatic signal in order to initiate a drive state or to continue consummatory behavior; once an animal learns simple stimulus-response relationships, stimuli that were once meaningless become powerful cues with the potential to initiate goal-directed motor programs. Simply put, humans learn how and when to initiate feeding: we discover very early that the general feeling of malaise created by the rumbling of a hunger pang or light-headedness of hypoglycaemia are often associated with a lack of food. And humans, like snakes and snails, learn by reinforcement.

2. Reinforcement: Psychological Theory

Although there is no single definition of reinforcement, the concept at its most basic level—in a purely behaviorist sense—can be defined by an environment-behavior relation resulting in the strengthening of an association. As an example, a foraging animal may be experiencing some form of vitamin deficiency and randomly come across a novel food containing the deprived vitamin. Prior to having experienced the positive post-ingestive consequences of that food stimulus—without the paired experience of assuaging the metabolic requirement for the vitamin —there existed no incentive value to the object. Without previous exposure there is no goal, or direction to behavior. This definition becomes inadequate (or incomplete), however, given the evidence that the taste of sweet or salty food can be innately rewarding (Berridge, 1996), and therefore considered a "primary reward/primary reinforcer". As an example, newborn babies (and chimps and rats) demonstrate stereotypical responses of "liking" of sweet foods. In the above examples, then, sweet/salty foods are innate incentive stimuli, which are analogous to unconditioned stimuli, and the initially neutral food containing the vitamin (the conditioned stimuli) becomes a predictor of reward (Berridge, et al., 1998).

In the field of psychology some of the most used descriptors for a reinforcer label it as a goal and an incentive, or a stimulus that is approached or attained (Salamone, et

al., 2002). Furthermore, implicit in any definition of a reinforcer is the ability for a stimulus to motivate behavior once the stimulus reward association is imprinted. The motivation to obtain the goal object (e.g. food) is not merely an immeasurable psychological concept but can be categorized (e.g. anticipatory, appetitive, etc.) and the neurotransmitters mapped. In fact, the role of mid-brain dopamine projections will be argued as being one avenue for explaining the neurophychology of motivation—and specifically the incentive contribution to food reward—in the aetiology of what today me be considered maladaptive behavior.

3. Food as a Reward

The concept that food can serve as a natural *reward* is not hard to grasp in the subjective sense that many foods have the quality of inducing a sense of gratified pleasure. This is most easily measured in humans because an experimenter can simply ask a subject to rate this pleasure/palatability on an analogue scale, for example. But the first neurophysiological evidence for the role of food as a *reward* emerged from brain stimulation studies during the late 1960s and throughout the 1970s. These studies demonstrated that humans would work to obtain electrical stimulation of some sites of the brain (including the lateral hypothalamus (Olds, 1977; Rolls, 1975), which was by definition rewarding. What is more, the rewarding quality of the brain stimulation appeared to mimic the rewarding quality of food; interestingly, it was found that animals would work harder to obtain brain stimulation when hungry (Hoebel, 1969), but when an animal was fed to satiety, it was later found that the group of lateral hypothalamic neurons under observation ceased to respond to food (Rolls, et al., 1986). It must be noted that evidence offered with human brain *reward* stimulation suggested that while the experience was certainly rewarding—patients could be found compulsively self-stimulating over thousands of repeated presses—there was no evidence of self-described pleasure in either case (Heath, 1972; Portenoy, et al., 1986). Observations such as these helped to lay the framework that disentangled the concept that rewards must be pleasurable; it is part of the *incentive salience* hypothesis (described below) that attempts to verify that under various circumstances (*e.g.* addiction) a *reward* need not be both pleasurable and desired at the same time.

The work on brain stimulation was extended to psychomotor stimulants and eventually it was discovered that the rewarding effects of both of these sources of unnatural reward could be blocked by dopamine antagonists. Eventually it was later confirmed that food *reward*—a natural *reward*—could be similarly attenuated (Wise, et al., 1978), and thus began the explosion of studies examining how dopamine modulates feeding and food reward. The following subsections will describe some of the theoretical views of dopamine's role in feeding and reinforcement. The focus will then shift to an examination of the potential role that reinforcement (and dopamine) plays in obesity and how peripheral signals of energy deprivation (*e.g.* the "hunger hormone" ghrelin and the adiposity-marker hormone leptin) may modulate reinforcement through altered dopamine function.

3.1. The Dopamine Hypothesis of Feeding: From Anhedonia to Incentive Salience

The accepted hypothesis that is now entrenched in fields spanning from neuroscience to clinical psychology is what has been termed the dopamine hypothesis, or applied to food intake, the dopamine hypothesis of feeding. In brief, via dopamine signaling arising from the ventral tegmental area of the midbrain there is downstream communication with limbic and prefrontal cortex brain areas that act to focus attention on salient environmental stimuli and to promote learning associations, thereby facilitating specific behavioral output such as feeding. The thesis is that dopamine neurons form the backbone of the network of the brain's natural reinforcement system. Indeed, food consumption, in likeness with drug consumption, increases brain dopamine levels in not only in animals, but also in humans (Wang, et al., 2002). A more detailed account of the effects of altered levels of dopamine in the brain is presented in **Figure 1**. The *exact* role that dopamine plays in reward is, however, still open to debate.

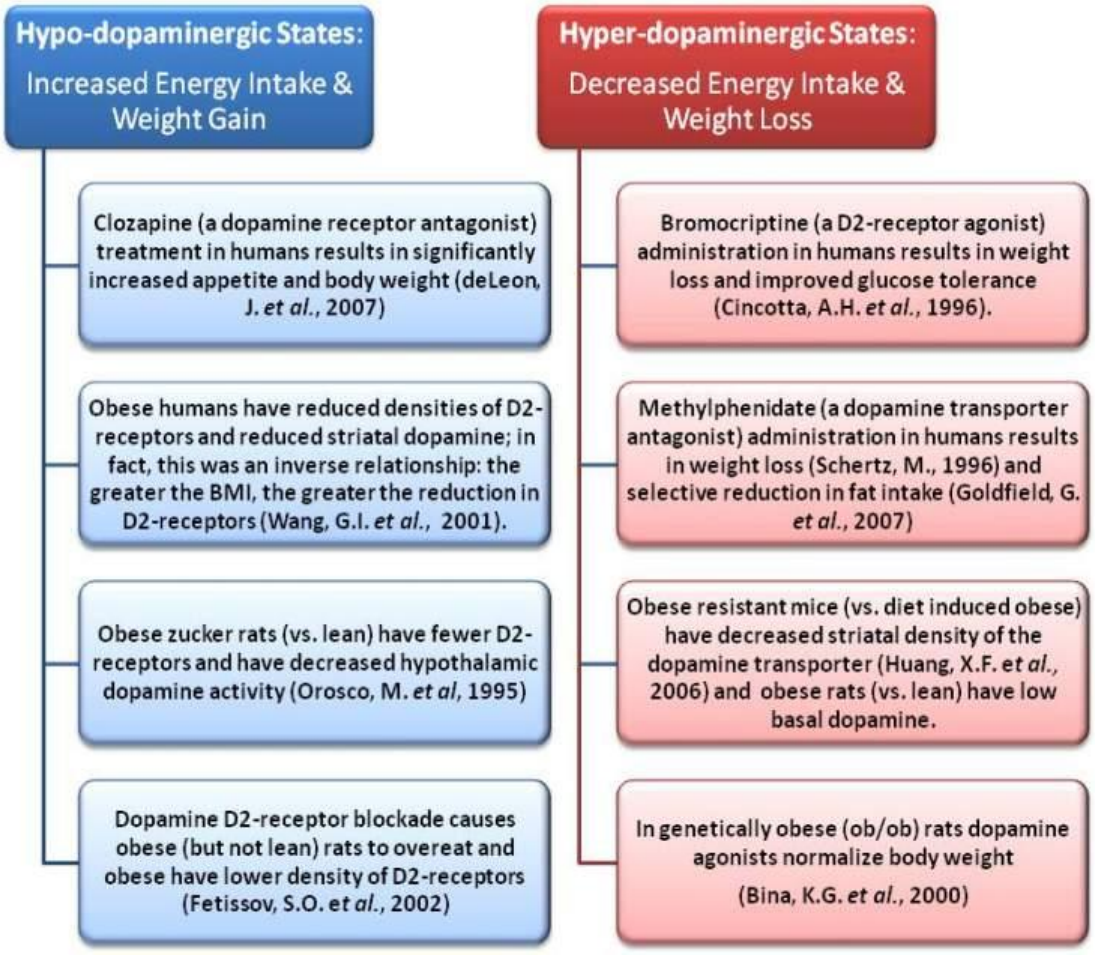

Figure 1: Key points describing dopamine's role in feeding behavior. A look at how altered levels of the neurotransmitter dopamine, by pharmacological or genetic manipulation, can impact normal feeding behavior and body weight regulation in humans and in rodents. Note that a dopamine receptor antagonist *blocks* dopamine signalling by post-synaptic inhibition; oppositely, a dopamine transporter antagonist *promotes* signalling by flooding the synapse with dopamine. References are, from left side (de Leon, et al., 2007; Fetissov, et al., 2002; Orosco, et al., 1995; Wang, et al., 2001) and from the right side (Bina, et al., 2000; Cincotta, et al., 1996; Goldfield, et al., 2007; Hauge, et al., 1991).

Dopamine is both a hormone and neurotransmitter that occurs in a wide variety of animals, including humans. It is one of the primary neurotransmitters in the mammalian brain, where it controls cognition, emotion, locomotor activity, food intake and endocrine

regulation (Missale, et al., 1998). In the brain there are three major dopaminergic pathways but the most relevant to this discussion is the reward related circuit—the mesolimbic pathway—originating in the mid-brain ventral tegmentum and extending to several limbic structures, including the nucleus accumbens, the amygdala, and the hippocampus (Berthoud, 2007). Part of the dopamine hypothesis of food intake is based on the initial work performed on animals subjected to the dopamine antagonist pimozide, which eventually led to the "Anhedonia (Greek for *without pleasure*) Hypothesis". This hypothesis stated, amongst other findings, that neuroleptics (specifically dopamine D2-receptor blockers) appeared to selectively blunt the rewarding impact of food stimuli by decreasing the pleasure of the reinforcer (Wise, 1982). While other groups have arrived at many of the same conclusions—in that dopamine is required for *normal* motivation and reward—there appears to be some disagreement regarding the precise role of dopamine in reinforcement or reward. All of the intricacies of the various theories cannot be covered here; however, the view that appears most persuasive has been painstakingly developed (for review see (Berridge & Robinson, 1998)) and asserts that dopamine is *not* necessary for 1) hedonic activation (*i.e.* normal affective reactions described as subjective/objective "liking") and, 2) for reward learning (*i.e.* relation between the conditioned stimulus and the unconditioned stimulus), but it *is* important for the attribution of the incentive salience of rewards. In fact, these three psychological processes are the foundation of the "incentive salience hypothesis", which offers a further description suggesting that these three processes are all dissociable qualities of reward that can be separated into components of "wanting" and "liking" (see **Figure 2**).

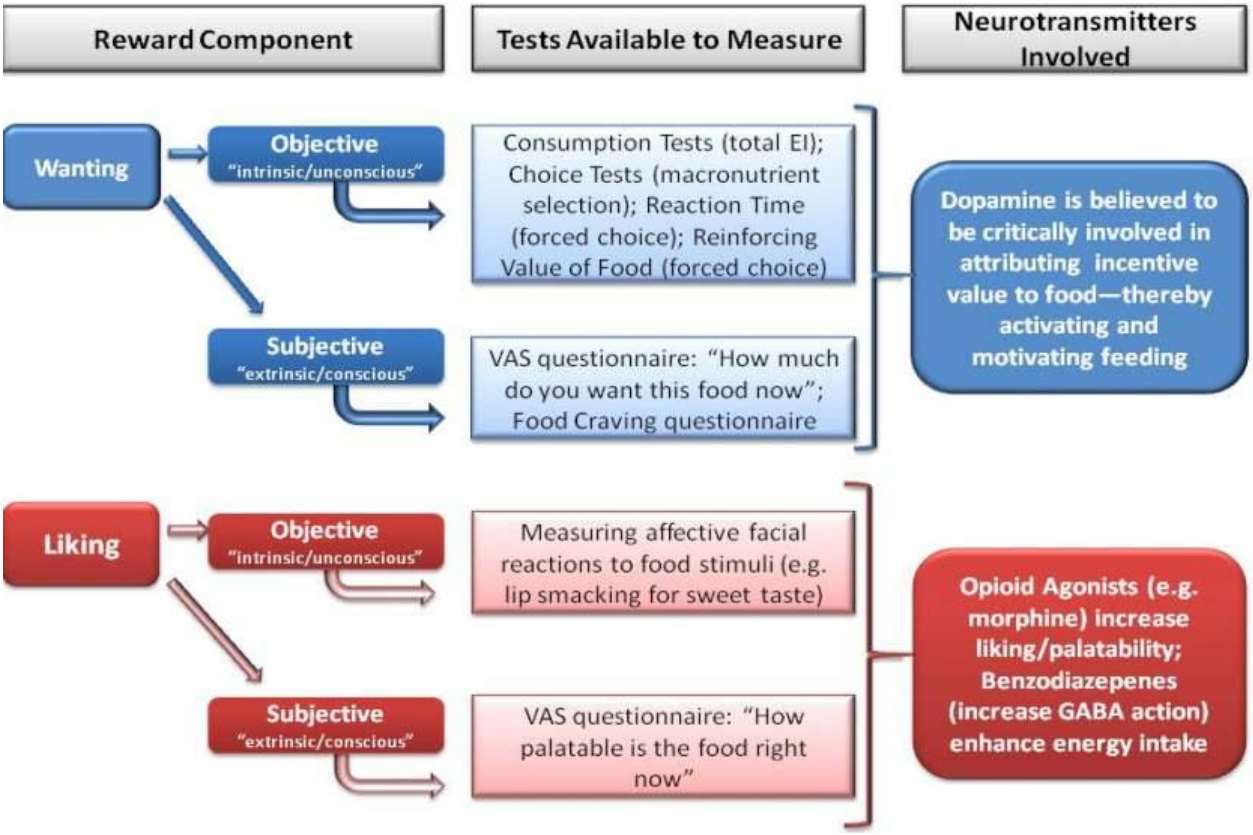

Figure 2. A representation of the dissociable qualities of food reward according to the "incentive salience hypothesis" and the means by which each process can be quantitatively assessed in the laboratory.

3.2. "Wanting" and "Liking": Measuring Perceptual and Motivational Qualities of Food Reward

According to the "incentive salience hypothesis", there is a change at the neurological level that describes *perceptual* (*e.g.* cognitive) and motivational (*e.g.* unconscious) components that accompany the shift from a stimulus being neutral to something that is attractive and can energize and motivate behavior (Berridge, et al., 2009). The relevance for the study of feeding is that tools can be devised that measure the level of attraction to a stimulus/reinforcer and how much behavior it will support. One method that has been designed to examine *explicit* "liking"/ "wanting" and *implicit* "wanting" of food is the recent development of a forced choice computer-based procedure (Finlayson, et al., 2007b). This task presents photographic food stimuli of twenty different items varying along two dimensions—fat (high or low) and taste

(savoury or sweet). From this computer task implicit “wanting” is measured by the speed with which one stimulus is chosen in preference to an alternative and is additionally measured by relative preference (*e.g.* fat vs. sweet).

In an attempt to determine whether acute energy deprivation influences food reward this forced choice paradigm was utilized at times pre- and post a standard lunch meal. The objective was to look for a state (hungry vs. satiated) dependent dissociation between “wanting” and “liking”. The main findings noted that in a hungry state (3-4 hours acute energy deprivation) subjects “wanted” fat (particularly high fat) and savoury food more so than fat and sweet food, but this trend was reversed and subjects “wanted” low fat sweet after the completion of an *ad libitum* pizza lunch. A separate study from the same group looked at the impact of meal-induced satiation on the dissociable qualities of food reward and discovered that once again implicit “wanting” for low fat sweet foods increased following a lunch meal and that “liking” for sweet foods did not decrease as much as that for fat foods (Finlayson, et al., 2008b). The general trend of decreased ‘liking’ as one progresses from a hungry to satiated state is consistent with the notion of alliesthesia, a concept that will be discussed in greater detail in ***Part II*** of this chapter.

Under study designs similar to those just discussed (state-dependent), recent results from neuroimaging studies offer promising descriptions of what might be occurring in areas of the brain implicated in reward. With fMRI analysis, it was discovered that satiated normal weight women show a stronger BOLD response in the striatum and OFC—reward centers that can be viewed in **Figures 4** and **5** in ***Part II*** of this chapter—when presented with images of low calorie foods. When hungry, however, these brain areas showed a stronger bold response to high calorie foods (Siep, et al., 2009). In a separate fMRI study subjects were tested 3 hours after the ingestion of a standardized meal by either infusing the “hunger hormone” ghrelin or a saline control prior to looking at food pictures (Malik, et al., 2008). Ghrelin (and not saline) increased the response to food cues in the amygdala, the OFC, and the striatum. Consequently, as demonstrated by these fMRI data, the state-dependent results reported with the forced choice paradigm reported in the previous paragraph may be in part explained by a homeostatic-like influence on reward processing. To be sure, a study combining the

"wanting/liking" computer task with fMRI neuroimaging and peripheral injections of ghrelin/leptin or neuropharmacological manipulation of dopamine would be of the highest impact. Unfortunately no such work has yet been performed.

3.3. The Relative-Reinforcing Value of Food & Energy Deprivation: Do Dopamine-Related Polymorphisms Impact Feeding?

In yet another definition of a reinforcing stimulus, one can describe a reinforcer as a stimulus that increases the rate of a behavior that it follows. The reinforcing value of a stimulus refers to how much behavior the stimulus will support (Epstein, Leddy, et al., 2007). This can be objectively observed as the increased willingness—a quantitative measure—to work at a progressive ratio computer task to obtain a desirable food stimulus (vs. some alternative). As an example, a computer can be set up with a screen that alternates between 2 different choices (typically a healthy food and palatable snack food) that can be navigated with a mouse pad. It is called the relative-reinforcing value of food because the probability of earning food points varies across schedules and is contingent on performing simple button presses on a computer joystick. The reinforcement schedule typically remains at a variable ratio (VR2) for all 5 trials for the healthy food, but increases progressively for the palatable snack food at VR2, VR4, VR8, VR16, and VR32 across the five trials. Thus, on average the reinforcement schedule for the healthy food is set to reinforce every second button push (VR2), and this remains the same across all trials; the reinforcement schedule for the snack food doubles across each trial, such that in the final trial (VR32) snacks were reinforced on every 32nd button press. Essentially, if subject 1 stops responding for the snack food at 16 button presses per point and subject 2 continues to respond for snack points at 32 button pushes per point, then the snack food is said to be twice as reinforcing for subject 2, as they must work twice as hard to obtain the reinforcer.

What is interesting is that comparing obese and lean individuals with this relative-reinforcing value of food paradigm, it was noted that not only did obese subjects work harder for palatable food items (vs. sedentary activities or healthy foods), but they also demonstrated an increased willingness to work for the reinforcer. In fact, this increase in the reinforcing value of food predicted *ad libitum* intake in the obese independently of

rated pleasantness (Saelens, et al., 1996; Temple, et al., 2008). What this suggests is that the measure of motivation to work to obtain, *i.e.* implicit "wanting" as measured by button-presses, can in some instances be a better predictor of energy intake than explicit subjective accounts of "liking". It appears as though at the behavioral level the combination of consommatory and appetitive motivation can trump orosensory reward. Indeed a troubling finding is the observance that not only do the obese find food more reinforcing (they work more for food than for sedentary activity compared to lean) (Saelens & Epstein, 1996), but obese persons also find high-fat foods more reinforcing than low-fat foods when compared to normal weight controls (Epstein, et al., 1991). The reinforcing value of food, in turn, can influence how much food is eaten at an *ad libitum* buffet. What is more, individuals who are categorized as high in food reinforcement (*i.e.* spend more time at a variable ratio task working for palatable food vs. bland or non-food items) eat more in an all-you-want-to-eat environment compared to individuals low in food reinforcement (Epstein, Wright, Paluch, Leddy, Hawk, Jaroni, Saad, Crystal-Mansour, & Lerman, 2004). It is unclear, however, whether these findings represent a cause or consequence of excess energy reserves.

One almost inescapable consequence of obesity is sustained periods of energy depletion or dieting. It is well known from psychological studies of reinforcement that food deprivation (and even drug deprivation) increases the reinforcing value of food. Obese and lean individuals alike pass through perturbations in body weight as a result of the prolonged interplay between energy intake, energy expenditure, and the overall involvement of gene and environment interactions. Indeed, results from an energy deprivation in lean individuals ranging in the time of ~13-20 hours indicated that in this relatively short period of deprivation the reinforcing value of a palatable snack food significantly increased from the baseline measure in the fed state (Raynor & Epstein, 2003). As an ecologically relevant example, if a lean person begins to regularly skip meals, then food is likely to become more reinforcing when it is finally approached. It is unknown if this phenomenon would persist with chronic periods of energy deprivation, but one could postulate that such behavior would lead to body weight gain according to the connection between the reinforcing value of food and energy intake (Raynor &

Epstein, 2003). Obese individuals experiencing the same feeding patterns theoretically would be even more vulnerable to weight gain, as food is already more reinforcing to begin with. What is fascinating is evidence suggests that polymorphisms of genes involved in the normal regulation of the neurotransmitter dopamine may be involved in a "high-food reinforcement" phenotype, or even related to excess energy reserves.

As a member of the catecholamine family, dopamine is synthesized from the amino acid tyrosine (produced in the liver from phenylalanine), mainly by nervous tissue and the medulla of the adrenal glands. Following the synthesis of dopamine (see **Figure 3**) there is vesicle packaging in the nerve terminal that prepares this monoamine neurotransmitter for synaptic release. When a dopaminergic neuron is sufficiently excited, dopamine is released into the synaptic cleft where it interacts with the postsynaptic receptors causing the depolarization of the postsynaptic cell and initiating a new action potential. Dopamine availability is dependent on its metabolism, release, transport, and receptor binding. Consequently, by looking at the genes involved at any one of these stages there is an opportunity to indirectly investigate brain dopamine levels—in effect looking at markers of neurotransmitter activity (Epstein, Temple, et al., 2007)—and how behavior may be resultantly impacted.

The dopamine transporter gene (SLC6A3) codes for a membrane spanning dopamine transporter protein (DAT) that mediates reuptake of dopamine from the synapse into surrounding neurons. There are multiple alleles for this DAT protein and it appears that the 10-repeat homozygous polymorphism is associated with increased dopamine transporter density and transport (Fuke, et al., 2001) when compared with the 9-repeat/10- repeat allele. The hypothesis posits that due to simple mendellian genetics, people who received the same 10-repeat allele (*i.e.* 10/10 genotype) from both parents have lower levels of postsynaptic dopamine. This is further evidenced from *in vivo* PET studies in humans showing that individuals with the 9-repeat/10-repeat genotype displayed a mean 22% reduction of DAT protein availability compared with 10-repeat homozygous individuals (Heinz, et al., 2000). Individuals with the 10/10 genotype can also be at increased risk to obese. It was found that African Americans with the 10/10

genotype had an odds of having BMI values ≥ 30 kg/m^2 that were 5.2 times greater than African Americans with the 9/9 or 9/10 genotype (Epstein, et al., 2002).

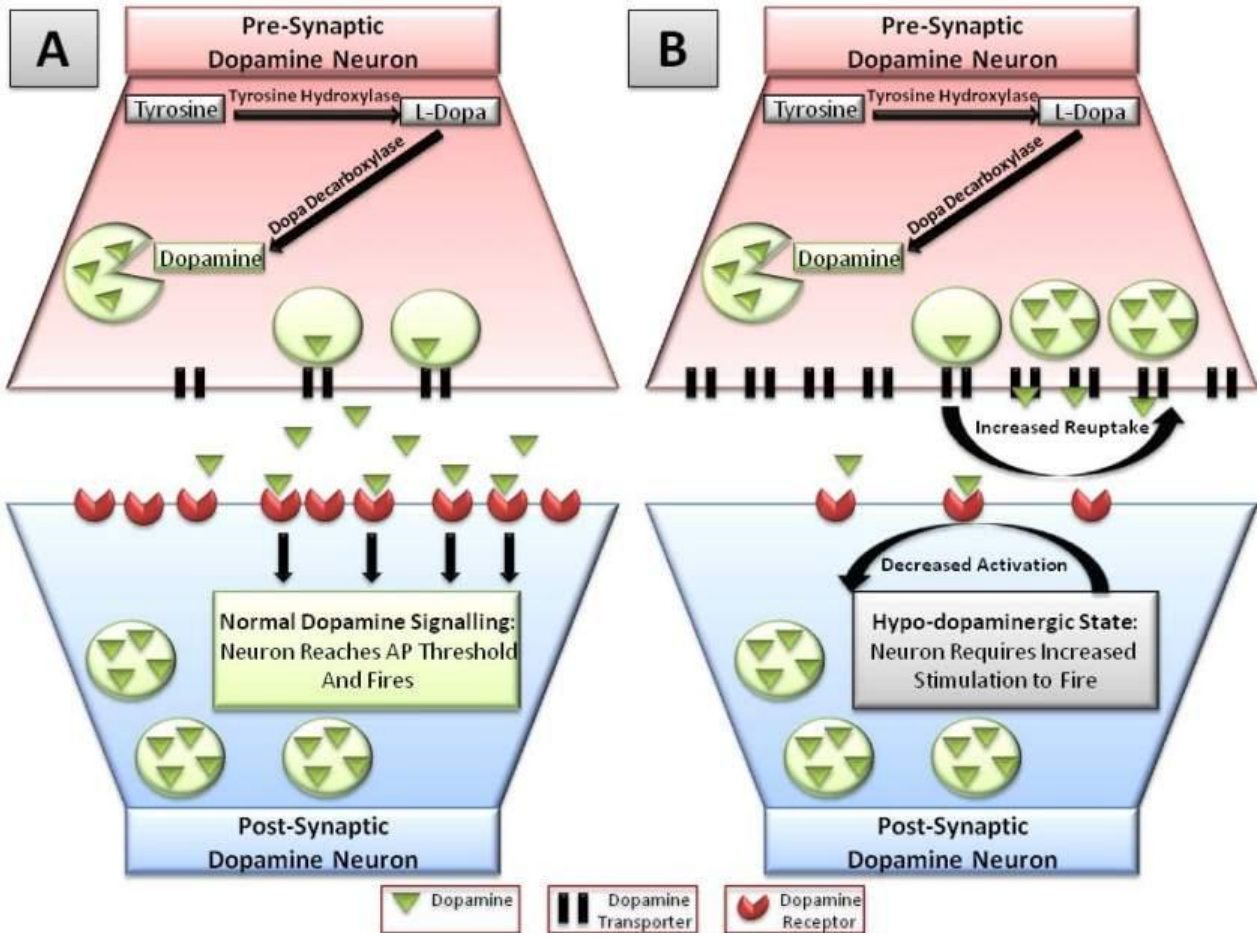

Figure 3. A stylized representation of midbrain dopamine neurons representing (**A)** a typical neuron with a normal propagation of action potential (AP), and (**B**) a neuron with impeded post-synaptic signalling (a "hypo-dopaminergic state") due to polymorphisms of the dopamine receptor and transporter. The dopamine transporter is responsible for the reuptake of dopamine and the 10/10 allele is hypothesized to result in decreased synaptic dopamine due to *increased* transporter density (*i.e.* increased reuptake). Also believed to be involved in impaired dopamine signalling, the Taq1A allele of DRD2 results in *decreased* density of dopamine D2-receptors (see **B**).

Another polymorphism believed to result in decreased dopamine signalling occurs due to an alteration in the gene for a postsynaptic dopamine receptor, DRD2. Specifically, there are three *Taq1* A variants (A1/A1, A1/A2, and A2/A2) and compared to carriers of the *Taq1* A2 allele, *in vivo* imaging had shown that people with the *Taq1* A1 allele have reduced brain dopamine signalling (Pohjalainen, et al., 1998). With

further *in* vivo (PET imaging) evidence, the mechanism of action is thought to be mediated primarily with the association of the *Taq1* A1 allele with decreased DRD2 receptor density (Noble, et al., 1991). What this suggests is that carriers of the *Taq1* A1 allele experience reduced dopamine signalling in the brain; indeed, it has recently been demonstrated that decreased density of DRD2 is strongly associated with human obesity, in inverse proportion to BMI (Wang, et al., 2001). Linking physiology with behavior, current research has revealed that the reinforcing value of food –analogous to the "wanting" component of food reward—can not only influence energy intake, but the presence of the *Taq1* A1 allele of the dopamine receptor can interact with obesity to influence food reinforcement (Epstein, Temple, et al., 2007; Epstein, Wright, Paluch, Leddy, Hawk, Jaroni, Saad, Crystal-Mansour, Shields, et al., 2004). Subjects identified as high in food reinforcement who were carriers of the A1 allele consumed more food than participants high in food reinforcement without the A1 allele and participants low in food reinforcement with or without the A1 allele (Epstein, Temple, et al., 2007). Although these data are preliminary, what this suggests is that using a behavioral genetic approach to understand the interaction between genotype, obesity, and food reinforcement can be viewed as a viable research venture.

Taken together, individuals with the *Taq1* A1 or the 10/10-repeat alleles are hypothesized to be less sensitive to stimulation of dopamine-regulated reward circuits—analogous to a reward-deficiency syndrome (Noble, Noble, et al., 1994)—and by enduring a hypodopaminergic state are more likely to seek reinforcers, whether it be food or drug (Blum, et al., 1996). In fact, this represents one view of dopamine's involvement in food reward and it fits well with much of the literature presented thus far. Although it is a very hedonistic view of feeding behavior, it is a tenable hypothesis that overeating has emerged as a compensatory mechanism to ameliorate a deficiency in the reward circuitry (Wang, et al., 2002). Others contend a somewhat opposing view that focuses on an individual's sensitivity to reward, as measured by a psychobiological questionnaire. It is argued that individuals with high levels (hyper-dopaminergia) of synaptic dopamine are more sensitive and have a greater capacity for reward, thereby making them more likely to engage in pleasurable behaviors (Davis & Fox, 2008). In the

end, it is not clear whether the dopamine hypothesis of feeding is best explained by hypo-dopaminergic states as described by a reward deficiency syndrome, or by hyper-dopaminergic states as described by heightened reward sensitivity, or by both.

4. Peripheral Feeding Signals Impact Brain Dopamine: Evidence that Energy Deprivation can Impact the Motivational Component of Food Reward

A promising path has been paved with respect to adiposity signals and their possible role in mediating food reward. When leptin is administered intraventricularly in rodents, it attenuates the rewarding impact of food-restriction-sensitive stimulation (Fulton, et al., 2000), where animals significantly decrease rates of brain stimulation reward. What is interesting is that this rewarding effect is potentiated by chronic food deprivation and that the ability of this deprivation to enhance brain stimulation reward is proportional to the degree of weight loss (Carr, et al., 1993). In effect, with greater weight loss animals will continue pressing the lever for ever smaller amounts of stimulation, which is translated into a leftward shift in the rate-frequency curve. Further, intracerebroventricular leptin administration causes a rightward shift in this curve, restoring the reward value to pre-deprivation levels (Fulton, et al., 2000), but to initially respond to the deprivation, an absolute weight loss of approximately 10% was required. It may be that long-term adiposity signals like leptin actively signal reward pathways of current body reserves, thereby intrinsically making food more attractive—more rewarding—when a significant loss of body energy reserves or a similar signal (decreased leptin) is detected.

New developments into the study of leptin and ghrelin—signals of energy surfeit and deficit, respectively—have indicated that these feeding signals may play complimentary roles in the dopamine hypothesis of feeding. Mesolimbic brain circuits have recently been shown to express the long form of the leptin receptor (the main leptin receptor, OB-Rb); more than this, it has been demonstrated that OB-Rb mediated signaling modified dopamine signaling and food intake. When leptin was administered directly to the ventral tegmental area (VTA)—the central hub of dopaminergic neurons—rats decreased food intake; oppositely, when the OB-Rb was knocked-out in this area, feeding was increased, especially of highly palatable chow (Hommel, et al., 2006). In

contrast, but consistent with its role in feeding, when ghrelin is injected into the VTA rats show a robust dose dependent feeding response (Naleid, et al., 2005). Taken together, what emerges is the neurological foundation that connects dopaminergic pathways of reward with short- and long terms feeding signals. Leptin inhibits the firing of VTA dopamine neurons and ghrelin triggers tonic dopamine release, resulting in decreased and increased feeding, respectively. Food reward appears to be impacted by homeostasis and dopamine plays an integral role in the appetitive motivation to feed. Another possible role for dopamine that cannot be discounted is that it has indirect downstream action on another neurotransmitter. Indeed, there is evidence that ghrelin can impact the opioid system in the rat (Sibilia, et al., 2006). The point here is that dopamine is only one piece of the grand puzzle that describes the intricate network underlying food reward.

Part II: Food Hedonics

1. Palatability: The Multimodal Representation of Taste

Taste perception is transmitted by cranial nerves, which propagate information about the touch, temperature and pain sensation on the tongue (Kringelbach, 2004). The afferent taste signal progresses from cranial nerves to the NTS, continuing to the thalamus and then to the primary taste cortex; higher order taste assimilation is believed to be accomplished by the connections of the primary taste cortex with the secondary taste cortex (*i.e.* the orbitofrontal cortex) (Baylis, et al., 1995). With respect to the pleasantness of the taste of foods, the responsiveness of the taste neurons in the NTS and in the primary taste cortex (see **Figure 4**) do not seem to be affected by states of deprivation and repletion (Rolls, et al., 1988; Yaxley, et al., 1988). What this implies is that these areas do not reflect the hedonics of feeding, but instead represent sensory qualities of food independent of motivational state. Therefore the identity and intensity of food taste is made explicit in the primary taste cortex, but it is with the rich interconnectivities of the orbitofrontal cortex (OFC) that the hedonic component of the rewarding value of food finally is coded. In short, evidence from primates indicates that the identity of a taste and its intensity are represented separately from its pleasantness (Rolls, 2007).

The OFC can be subdivided into two regions: the posterior region, restricted to the limbic functions and considered part of the limbic system, and an anterior region, restricted to inhibitory control over the amygdala (Borod, 2000). In order for peripheral feeding related signals to influence food pleasantness—and by convention, food reward—this must occur from the processing of the stimuli at the level at or beyond the secondary taste cortex (Rolls, et al., 1990). Efferent connections from the OFC include the amygdala, the hippocampus, the lateral hypothalamus, the striatum, and the ventral tegmental area (Kandel, et al., 2000). What is interesting is that each of these brain areas is influenced by ghrelin, leptin and dopamine. Interconnected within this network of neuromodulators and feeding-related signals lies what is believed to be a path towards describing reward signaling, or in the context of energy deprivation, *alliesthesia* (discussed below).

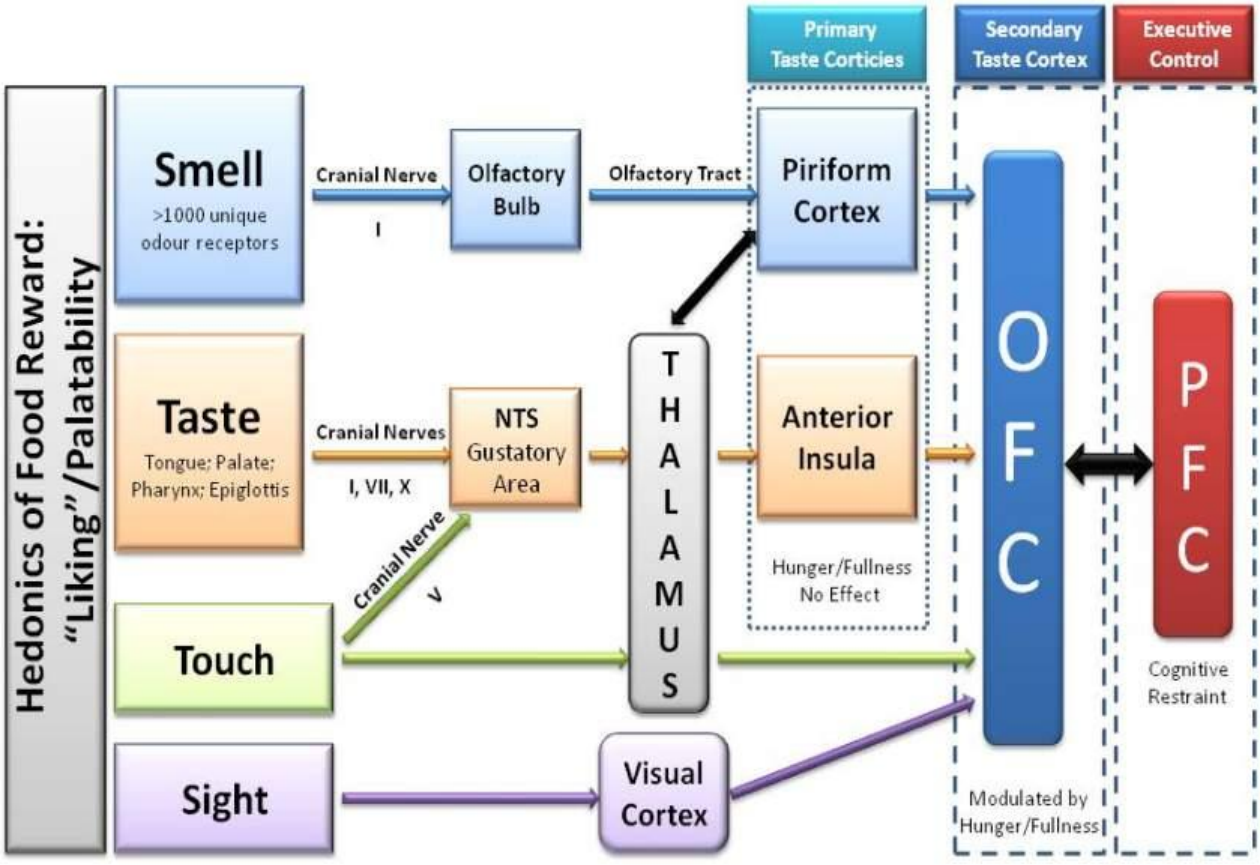

Figure 4. A schematic diagram of pathways demonstrating the multimodal representation of taste and olfaction in the brain. Note that cranial nerve I is the olfactory nerve; cranial nerve VII

is the facial nerve; cranial nerve X is the vagus nerve; and cranial nerve V is the trigeminal nerve (which transmits the mouthfeel of fats and oils).

Pioneering work performed on primates indicated that responses from a group of OFC neurons to glucose taste decreased to zero when the monkey ate glucose to satiety; concurrently, over the course of administration of glucose the animal's behavior changed from positive to negative affective reactions (Rolls, et al., 1989). Furthermore, it was demonstrated that this decrease in neural activity was specific to the ingesta, *i.e.* the authors discovered a piece of the neural foundation of *sensory specific satiety* (Rolls, et al., 1989). What remains unclear is the role that information from the primary taste cortex and from adjacent limbic areas impacts the change in pleasantness of a food stimulus that occurs with continuous exposure over a meal or from re-feeding after chronic energy deprivation.

Another area that requires clarification is to what degree the decisions regarding when, what, and with whom to eat are conscious. Indeed, much of the expression of appetite and eating is not explicit, and therefore outside of introspection. Studies from rats, to chimps, to human infants have demonstrated that 'liking' of sweet foods can be implicit—and this was accomplished by measuring stereotypical facial reactions to the sweet stimuli (Steiner, et al., 2001). Sweet tastes elicit a positive hedonic pattern of evaluation primarily described by tongue protrusions (licking/smacking of lips) and paw licking (Grill, et al., 1985). Since 'liking' is considered a basic evaluative reaction of the brain with objective behavioral indicators it can be implicitly measured by affective measures to the food *reward*; alternatively, the hedonic impact of 'liking' can be explicitly measured in humans by describing their reaction to the food stimulus (*e.g.* analogue scales). A pleasant stimulus is a rewarding stimulus and for the most part feeding is a rewarding action. The "liking" component of food reward has its biological underpinnings in at least two major neurotransmitter families defined by opioid, and endocannabinoid systems. The best studied in humans is the opioid modulation of palatability. Specifically, opioid receptor antagonists (*e.g.* naltrexone and naloxone) reduce the pleasantness of foods (Bertino, et al., 1991; Drewnowski, et al., 1992) and agonists (*e.g.* DAMGO and morphine) increase the pleasantness (Atkinson, 1987; Levine, et al.,

1987). This hedonic pleasure/palatability component of reward is represented in the brain mainly by pallidal circuits (Yeomans, et al., 2002) and by circuits within the shell of the nucleus accumbens (Pecina, et al., 2005) (see *striatum* in **Figure 5**).

2. Peripheral Feeding Signals and Taste Processing

There are limited data regarding the potential role of gut peptides (peripheral feeding signals) in taste processing. Studies on rats have indicated that PYY_{3-36}, a hormone released by L-cells of the distal colon, produced a dose-dependent conditioned taste aversion to a sweet solution (Chelikani, et al., 2006; Halatchev, et al., 2005). Similarly, another peptide released by intestinal L-cells, GLP-1, also has the potential to produce a robust conditioned taste aversion to saccharine (Thiele, et al., 1997). What is intriguing is that CCK, a 'satiety hormone' like PYY and GLP-1, can actually produce a conditioned flavour *preference* at low doses in rats (Perez, et al., 1991). The authors interpreted this preference to the positive post-ingestive consequences of satiety. It is possible that the taste aversion with PYY and GLP-1 could be an artifact of nausea that has been reported in human subjects; nonetheless, there is evidence that these gut-peptide messengers are involved in taste-processing. The hormone produced by the OB gene, leptin, has also been demonstrated to have a role in the hedonic evaluation of food. Specifically, leptin receptors were identified in taste cells and exogenous administration of leptin inhibited sweet taste responses in lean mice but not in db/db mice (lacking a functional leptin receptor) (Ninomiya, et al., 2002). Evidence of a separate role for leptin in *olfactory processing* has also emerged from data on rats showing how nutritional status impacts olfactory perception. Specifically, intracerebroventricular leptin administration (mimicking satiety) dose-dependently increases consumption of an aversive odorized drink, suggesting that leptin decreases odor sensitivity (Julliard, et al., 2007). Taken together, leptin appears to play a role in *taste* and *olfactory* processing that is dependent on nutritional status. What this suggests is that when rodents are energy-replete and leptin levels are high there is a corresponding decrease in sensitivity to taste and to olfactory stimuli. Although there are limited data, human studies have also indicated that leptin is expressed and may play a functional role in the salivary glands and the oral cavity; the expression of the long form

of the leptin receptor has also been discovered in the membranes of glandular cells and in the salivary ducts (Bohlender, et al., 2003).

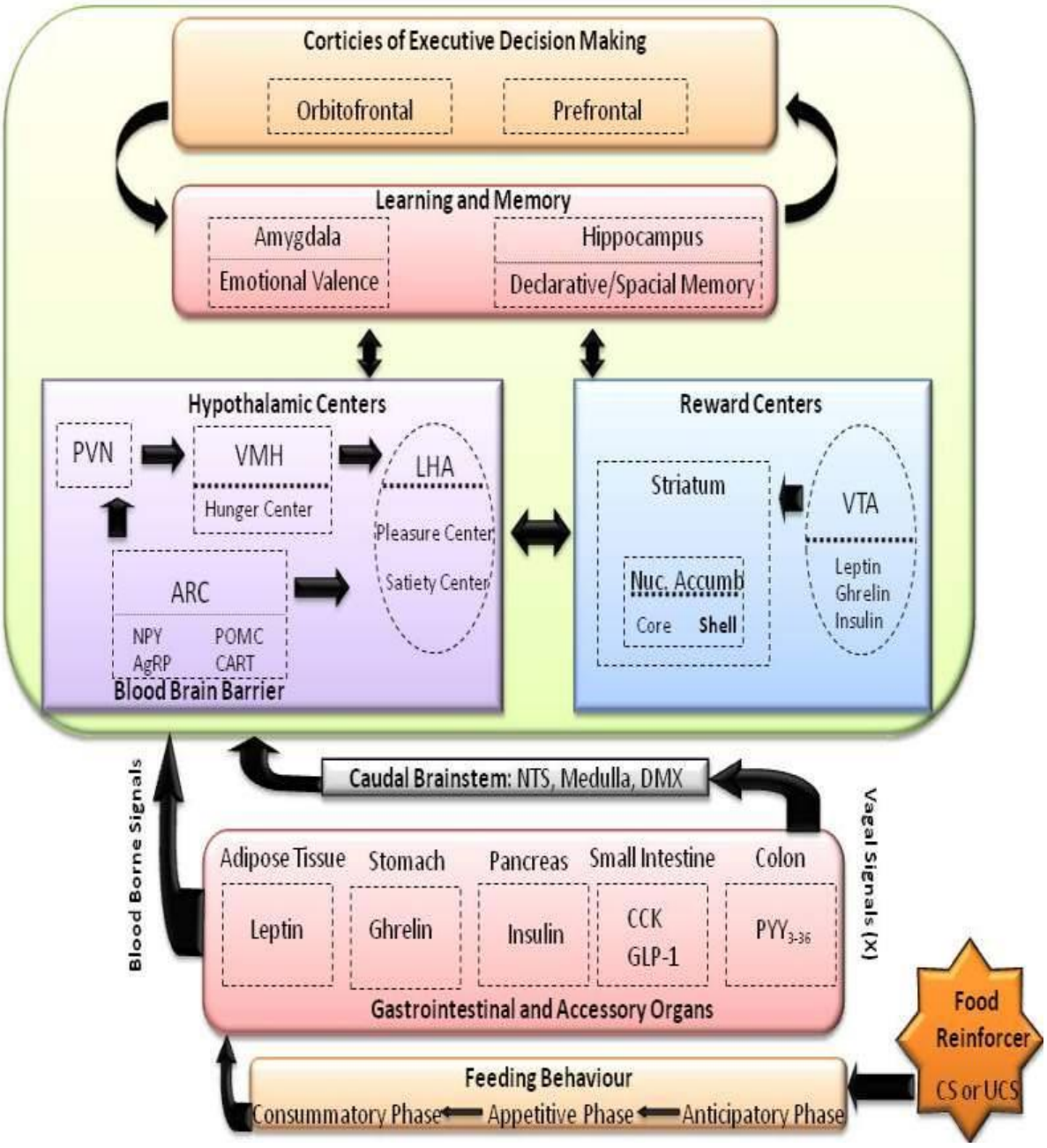

Figure 5. An integrated summary of all the concepts discussed throughout this chapter: with the introduction of a food reinforcer begins the appetitive motivation to seek and finally to consume the food stimulus. When fasted for an extended period of time peripheral feeding-related signals from gastrointestinal and accessory organs are proposed to drive feeding behavior in a powerful manner via the ARC and the activation of neuropeptides that trigger hunger signals (*e.g.* NPY

and AgRP). Conversely, when in the sated state, the nucleus accumbens and VTA may continue to promote consummatory behavior in the absence of hunger, *i.e.*, as conditioned associations and learning interact with the reward pathways to increase implicit wanting. Equally as plausible, the palatability of food signalled by the Insular and Orbitofrontal corticies could also trigger eating in the absence of hunger. Abbreviations: PVN=paraventricular nucleus; LHA=lateral hypothalamic area; ARC=arcuate nucleus; NPY=neuropeptide Y; AgRP=agouti-related peptide; POMC=pro-opiomelanocortin; CART=cocaine and amphetamine-related transcript; Nuc. Accumb=Nucleus accumbens. Modified from (Cameron & Doucet, 2007) with permission.

The role that *serum* leptin may play in food hedonics in humans has only been superficially investigated. In a group of six men and twenty women offered a standardized high carbohydrate breakfast, palatability was positively correlated to fasting serum leptin independently of BMI and body fat mass (Raynaud, et al., 1999). One interpretation of these results is that palatability would be independent of need state, as those who had the highest leptin levels (indicating caloric surfeit) rated food as tasting most pleasant. Contrarily, these results could be demonstrative of the large variation in body composition in this study. With nearly half of the subjects being obese there could be a resistance to leptin that could not be easily detected.

In a study examining the impact of fasting leptin concentration on energy intake and macronutrient preference it was found that high fasting serum leptin was associated with lower preference for chocolate as well as lower energy intakes and specifically fat intake (Karhunen, et al., 1998). These findings remained significant after adjusting leptin concentrations for body fat mass and dietary underreporting. In a more recent study consisting of a chronic energy deprivation (8 week weight loss trial) and a repeated measure looking at food hedonics pre- and post weight loss, there was no significant relationship between serum leptin and rated pleasantness (Cameron, et al., 2008). What this study did demonstrate, however, was that after the 8 weeks of caloric deprivation (-700kcal/day) subjects rated the same foods as more pleasant to taste. Of note, both types of food presented to the subjects—vegetables and fruits vs. desserts—increased in hedonic valance. After weight loss both healthy food and “junk” food tasted better.

3. Energy Deprivation and Palatability: Evidence from Alliesthesia

A question that remains to be answered is the extent to which homeostatic components (a need-state) can impact the quality of oro-sensory reward, thereby enhancing food hedonics. Much of the research on this topic uses a pre-load paradigm that assesses the impact of a pre-test snack on the subsequent *ad libitum* energy intake and rated palatability. The rationale behind this test supposes that if palatability is dependent on a need-state, then a pre-load of high energy density (vs. low) would have a greater impact on palatability of the *ad libitum* meal (*i.e.* meal becomes less pleasant following energy dense preload). There are conflicting data, but it appears that the short term manipulation of satiety does not reliably impact palatability. For example, subjects not only rated food as being less pleasant following a high energy preload (vs. low), but they consumed less total weight- and calories in *ad libitum* feeding following the preload (Johnson, et al., 1993) and similar findings were noted in a separate study (Booth, et al., 1982).

On the other hand, several studies employing similar preload paradigms have demonstrated a lack of change in palatability with need state (Birch, et al., 1986; Yeomans, et al., 1998). Specifically, when a soup preload was covertly manipulated with maltodextrin and administered 30 minutes prior to *ad libitum* feeding, subjects did not rate the palatability lower than the trial where plain soup was consumed (Yeomans, et al., 1998). However, with the added maltodextrin it was noted that subjects had lower hunger and higher fullness ratings prior to beginning *ad libitum* feeding. Similarly, a study of children aged 3-5 and adults 25-35 years old showed no change in pleasantness with respect to preload energy density, but both groups displayed sensory specific satiety during the lunch feeding (Birch & Deysher, 1986). While the preload paradigm may be examining possible short-term signaling of need (free)-state, a far better manipulation that is unfortunately studied even less is increasing the deprivation state (*e.g.* chronic energy deprivation). As alluded to in much of this chapter, feeding repeatedly takes place without any measurable changes in body energy reserves, and most often according to learned cues (see **Figure 5**). In order to truly examine how the '*internal milieu*' impacts food reward there most likely needs to be a prolonged perturbation in energy balance over days and weeks.

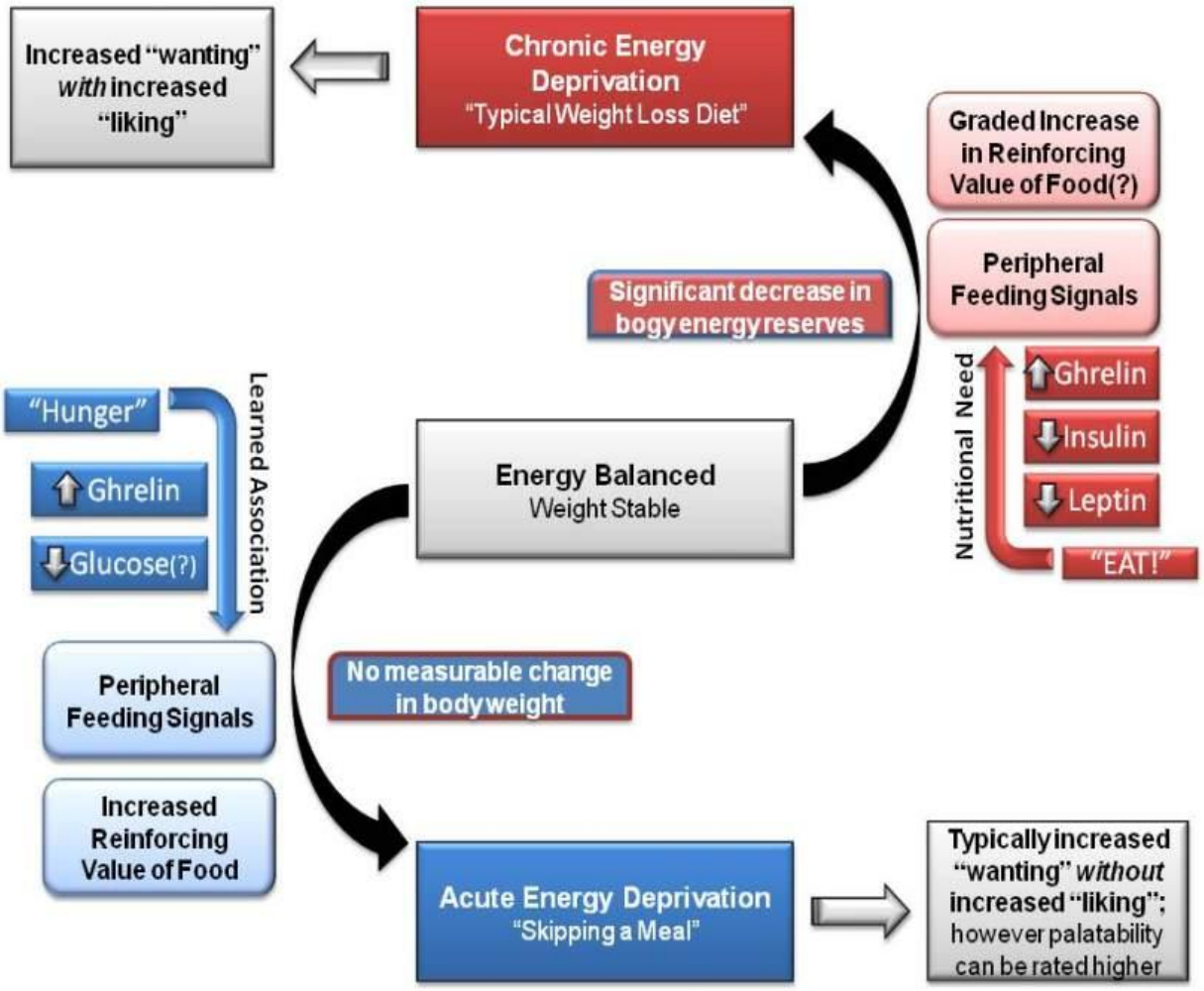

Figure 6. *Key Features* representing the Ying and Yang of food reward as a function of energy deprivation. When there is no measurable change in body weight, it is believed that peripheral signals act as *cues* to feed, but chronic energy deprivation results in exaggerated responses from peripheral signals. It may be that significant changes in body energy reserves changes mere cues into powerful signals that motivate appetitive and consommatory feeding behavior.

Some of the pioneering efforts examining energy deprivation and palatability were conducted by Michel Cabanac, the coiner of the term alliesthesia. In his seminal paper on hedonics (1971) he tested changes in gustative and olfactive perception at the subjects' normal weight, at 10% below this weight, and then again after the subject returned to normal weight. To reduce the body weight by 10% the energy intake was limited to 500-800 kcal per day until the weight was achieved, and this reduced weight was maintained for several weeks prior to the test day. In short, alliesthesia was

measured by the change in subjective pleasantness ratings for multiple ingestions of sweet tasting sucrose solutions. When subjects were at their normal weight and after ingesting 200 milliliters of a 25% aqueous solution of glucose, these stimuli became unpleasant (negative alliesthesia). However, when subjects lost 10% of their body weight alliesthesia could no longer be demonstrated, and 50 grams of glucose was insufficient to cause the negative affective ratings. Finally, the return to normal body weight restored the change in sensation from pleasant to unpleasant.

Corroborating these results are findings from deprivation periods of much shorter duration. By manipulating the period of energy deprivation with two separate test days, one day with a 3.5 hour period of deprivation and another with an overnight fast of approximately 12-15 hours, Speigel et al. (1989) found that the deprivation period impacted palatability, consistent with alliesthesia. These results were consistent with obese and lean subjects. Germaine to this chapter are two major findings from this study: 1) the longer deprivation period resulted in increased palatability ratings and increased rate- and quantity of food eaten *ad libitum*, and 2) obese persons experienced a greater increase in palatability than lean persons, but lean individuals increased *ad libitum* feeding more than obese individuals.

Summary Points

- Variation in the individual response to a similar food environment can begin to be explained not only at the gene level, but also by examining psychobiological differences in the evaluation of *food reward*.
- When energy deprivation is prolonged there is a degree of disinhibition with respect to appetite: not only does palatable food become more salient but items that would normally not be selected can also become attractive.
- Short-term feeding-related signals are divided into orexigenic signals (*e.g.* the “hunger hormone” ghrelin) that convey the overall message to eat, and this in contrast with anorexigenic signals (*e.g.* CCK and $PYY_{3\text{-}36}$) that convey the message of fullness/satiety.

- Leptin appears to play a role in taste and olfactory processing that is *dep*endent on nutritional status: when energy-replete and leptin levels are high there is a corresponding decrease in sensi*tivi*ty to taste and to olfactory stimuli.
- There need not be a depri**v**ation or homeostatic signal in order to initiate a drive state or to continue consummatory behavior; humans *learn* how and when to initiate feeding.
- The dopamine hypothesis states that dopamine neurons form the backbone of the network of the brain's natural reinforcement system acting to focus attention on salient environmental stimuli and to promote learning associations. **L**eptin and ghrelin may play complimentary roles in the dopamine hypothesis of feeding.
- *Incentive salience theory* suggests that dopamine is *not* necessary for subjective/objective "liking") *nor* for reward learning, but it *is* important for the attribution of the incentive salience of rewards.
- The measure of motivation to work to obtain, *i.e.* implicit "wanting", can in some instances be a better predictor of energy intake than explicit subjective accounts of "liking".
- *In vivo* imaging suggests that individuals with the *Taq1* A1 (dopamine transporter) or the 10/10-repeat (dopamine receptor) alleles can be less sensitive to stimulation of dopamine-regulated reward circuits—analogous to a reward-deficiency syndrome—by imposing a hypodopaminergic state.
- The "liking" component of food reward has its biological underpinnings in at least two major neurotransmitter families defined by opioid and endocannabinoid systems.
- A question that remains to be answered is the extent to which homeostatic components (nutritional need-states) can impact the quality of oro-sensory reward, thereby enhancing food hedonics.

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PART ONE CONTINUED: OBJECTIVES AND HYPOTHESES

Objectives and Hypotheses

In four separate original research reports, acute deprivation periods of 1 day (24 hour fast) or 3 days (25% energy deprivation), and a chronic deprivation period of 6 months will be analyzed in order to elucidate whether or not there are measurable differences in metabolic and behavioural responses to negative energy balance. The objectives were to quantitatively assess, using a psychological systems approach, how appetite, plasma peptides, olfaction, food reward, and *ad libitum* feeding respond to varying degrees of energy deprivation. Due to the fact that this manuscript is composed of three separate studies (and four original research papers) the objectives and hypotheses will be presented accordingly.

Article I— The TaqIA RFLP located downstream of the dopamine D_2 receptor gene (DRD2) is associated with attenuated intervention-induced fat mass loss and increased carbohydrate intake in post-menopausal obese women

While obesity in general, and weight loss in particular, are no doubt influenced by polygenic factors, there has not been an effort to examine whether polymorphisms of TaqIA or DAT1 may impact the inter-individual variation in weight loss by two separate modalities. Accordingly, the primary objective was to investigate the potential association of the TaqIA RFLP allele or the DAT1 VNTR to weight loss in overweight/obese post-menopausal women who completed a 6-month weight loss intervention using caloric restriction with or without the addition of a resistance training program. The secondary objective was to elucidate if there existed genotype associations with energy intake or energy expenditure variables. It was hypothesized that individuals carrying the A1 allele or the 10/10 DAT1 allele would respond with reduced body weight loss to the caloric restriction intervention and that individuals carrying the A1 allele would display an enhanced carbohydrate preference and attenuated physical activity energy expenditure.

Article II— Fasting for 24 hours improves nasal chemosensory performance and food palatability in a related manner

In a within-subjects randomized study the primary objective was to examine the modulation of nasal chemosensory performance, using a validated smell test, and to

examine if changes in smell have a measurable impact on food palatability. It was hypothesized that compared to their fed state, fasted individuals would demonstrate improvements in smell performance (odor threshold, discrimination, and identification) and exhibit heightened hedonics consistent with the concept of alliesthesia. It was also hypothesized that the improved smell performance would coincide with higher palatability ratings of a fixed-energy meal, i.e., it was anticipated that the deltas for olfaction and palatability will be positively related.

Article III— The Impact of a 24 hour fast on food reward and intake during *ad libitum* feeding,

The main objective of this randomized cross-over repeated measures study was to examine the impact of a 24 hour complete fast (vs. fed state) on two dimensions of food reward—specifically, on the 'wanting' (both implicit and explicit and the relative-reinforcing value of food)—and on the 'liking' (explicit) for an array of preferred foods in order to elucidate hedonic influences on *ad libitum* feeding, food preference, and the motivation to eat. It was hypothesized that preferred snack food would increase in relative-reinforcing value (versus preferred fruit) and this increase would correlate with decreased (faster) reaction time to choose foods with increased energy density in the implicit wanting task from a separate computer program. It was further hypothesized that under the fasting condition there would be a lack of evidence of negative alliesthesia, along with significantly higher appetite and *ad libitum* energy intake.

Article IV— Deprivation by diet alone or by aerobic exercise alone: How modality of an acute intervention can differently impact appetite horomones, olfaction, food reward, and ad libitum feeding

This study wass a randomized, counter-balanced crossover study consisting of 3 arms: first there was a control arm (CON) after which subjects were randomly assigned with counterbalancing to one of the following: deprivation by diet alone (DER) arm, or the deprivation by aerobic exercise alone (DEX) arm. The objectives were to examine, using a psychobiological systems approach, how modality of an acute 3 day energy deprivation differently impacts, olfaction, food reward, *ad libitum* feeding, and appetite hormones. It was hypothesized that independent of modality of deprivation that relative

to the control there would be, improvements in smell performance, increased food reward and *ad libitum* feeding, and a decline in leptin and increase in ghrelin. It was also hypothesized that the increased food reward would prove to be a predictor of *ad libitum* EI and that relative to the deprivation by aerobic exercise alone, the deprivation by diet alone would produce the largest changes in appetite and *ad libitum* EI.

Assumptions, Limitations and Delimitations

Presumably, all participants will answer honestly to all pre-screening questions, follow the requested protocol, and answer truthfully to all questionnaires that are presented to them. In order to guard against any responding bias it was made clear to participants that anonymity and confidentiality were some of the highest priorities of our research.

Assumptions exist with the reliability of the various food manufacturers' nutritional information that is posted for the consumer. Also, it is assumed that the 6 month shelf-life indicated by the manufacturer of the smell test is accurate, and the tool maintains its demonstated reliability as such.

Due to the fact that much of the dietary reporting is accrued via personal testimonial documented by the participants, there exists the persistent subjective self-reporting limitation observed in most studies involving feeding behaviour (i.e. significant amounts of data are collected beyond the confinement of the laboratory). In an effort to better manage the self-reporting limitation a newly developed food menu in the study looking at energy deprivation by diet alone or exercise alone was employed. By supplying all of the food for the study the responder bias should be less of a limiting factor.

As might be expected, utilizing a convenience sample as opposed to a random sample of participants significantly diminishes the population validity; hence, the generalizability of the results of each of the four samples will be applicable to a smaller range of individuals, as described in the discussion in each individual study.

A delimiting factor was the single time measure for blood sampling of the peptires leptin and ghrelin. As with most research there were constraints with research funds and due to the relative overlap of the diurnal rhythyms of leptin and ghrelin a single fasted sample was deemed an acceptable proxy of both hormones.

CHAPTER III—METHODOLOGY

Methods used for the data collection process for each of the studies conducted are herein presented in article format in four separate original research articles. To also include them here in a separate section would be redundant for the reader, and it was therefore deemed appropriate to describe the methodology within each corresponding article.

PART TWO: RESULTS OF THE STUDY AND DISCUSSION

Part TWO—RESULTS AND DISCUSSION

The following chapters present the results and discussions of each of the original studies in article-style format. Overall there are 3 separate studies that compose the original work of my thesis. For Article I the data was previously collected in a collaborative study by the MONET group looking at, amongst other variables, heart health in post-menopausal women (study I). From this cohort, I analyzed the blood samples and performed the genotyping which corresponded to the work performed in Article I. This was the only study (and only article) that I did not design and perform the data collection. Articles II and III were both from the same data set (*i.e.* study II), and Article IV was from a separate data set altogether (*i.e.* study III).

Article I, titled The TaqIA RFLP is associated with attenuated intervention-induced body weight loss and increased carbohydrate intake in post-menopausal obese women is published in the journal *Appetite* (Jameason D. Cameron, Marie-Ève Riou, Frédérique Tesson, Gary S. Goldfield, Rémi Rabasa-Lhoret, Martin Brochu and Éric Doucet. (2012). The TaqIA RFLP is associated with attenuated intervention-induced body weight loss and increased carbohydrate intake in post-menopausal obese women. *Appetite, Epub ahead of print*).

Articles II and III are two papers that were produced from the same study. Article II, titled Fasting for 24 hours improves nasal chemosensory performance and food palatability in a related manner is published in *Appetite* (Cameron, J.D., Goldfield, G.S., & Doucet, E. (2012). Fasting for 24h improves nasal chemosensory performance and food palatability in a related manner. *Appetite, 58 (3), 978-981*). Article III, titled The Impact of a 24 hour fast on food reward and intake during *ad libitum* feeding: evidence of increased reward from food and food-related cues is currently submitted to the journal *Applied Physiology, Nutrition, and Metabolism*.

Article IV, titled Deprivation by diet alone or by aerobic exercise alone: How modality of an acute intervention can differently impact olfaction, food reward, *ad libitum* feeding, and appetite horomones, is currently being prepared for submission for the *American Journal of Clinical Nutrition.*

Chapter IV: Article I

Running Head: The TaqIA RFLP is associated with attenuated intervention-induced body weight loss and increased carbohydrate intake in post-menopausal obese women

This article is published in the September 2012 issue of the journal *Appetite.*

Cameron, J. D., Riou, M. E., Tesson, F., Goldfield, G. S., Rabasa-Lhoret, R., Brochu, M., & Doucet, E. (2012). The TaqIA RFLP is associated with attenuated intervention-induced body weight loss and increased carbohydrate intake in post-menopausal obese women. Appetite.

J.C. performed the DNA extraction and PCR analysis from blood previously collected. J.C wrote the manuscript, and M-E.R., F.T., G.G, R.R-L., M.B., and E.D. all contributed in the editing of the manuscript.

The TaqIA RFLP is associated with attenuated intervention-induced body weight loss and increased carbohydrate intake in post-menopausal obese women.

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Keywords: DRD2 / ANKK1; TaqIA RFLP; Obesity; Weight-loss; Diet; Exercise; Post-Menopausal Women

Abstract

Introduction: Polymorphisms of the dopamine receptor D2 (DRD2) gene have been associated with obesity phenotypes. Our aim was to examine if the genotype of TaqIA Restriction Fragment Length Polymorphism (RFPL) was related to an attenuated weight loss response or to changes in energy expenditure (EE) and food preference before and after weight loss.

Methods: Obese post-menopausal women (age=57.1±4.6 yr, weight=85.4±15.4 kg and BMI=32.8±4.5 kg/m²) were genotyped for TaqIA (n=127) by using PCR-RFLP analysis and categorized as possessing at least one copy of the A1 allele ($A1^+$) or no copy ($A1^-$). Women were randomized into 2 groups, caloric restriction (CR) and caloric restriction + resistance training (CRRT) and in this study were further classified as follows: $A1^+$CR, $A1^+$CRRT, A1-$^-$CR and $^-$A1$^-$CRRT. Body composition, total daily EE, physical activity EE, Resting EE (REE), and energy intake were obtained at baseline and post intervention using DXA, doubly-labelled water, indirect calorimetry, and 3-day dietary records, respectively.

Results: Overall, all of the anthropometric variables and REE significantly decreased post-intervention ($p<0.001$). Women in the CRRT group lost significantly more fat mass (FM) than the CR women ($p<0.05$). There were significant time by group by allele interactions for attenuated body weight (BW), BMI, and FM loss for $A1^+$ (*vs.* $A1^-$) in CRRT ($p<0.05$) and for increased % carbohydrate intake ($p<0.01$).

Conclusion: TaqIA genotype was associated with body weight loss post-intervention; more specifically, carriers of the A1 allele lost significantly less BW and FM than the $A1^-$ and had increased carbohydrate intake in the CRRT group.

1. Conflict of Interest: None Disclosed

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Introduction

Of late there has been much focus on genotypes thought to be linked with impaired dopamine signalling. The surge in studies of dopamine and the role of its transport and receptor genes in feeding and other reward-driven behaviours such as ethanol consumption, gambling, drug-taking, and obesity strongly points to evidence of reward-related phenotypes (Noble, St Jeor, et al., 1994a). Support for the role of dopamine in human feeding behaviour is evidenced in part by the anorexigenic action of dopamine agonists (Goldfield, et al., 2007; Leddy, et al., 2004; Schertz, et al., 1996) and by the orexigenic action of dopamine antagonists (Roerig, et al., 2005; Ruetsch, et al., 2005). Dopamine is also involved in motor control and motivation (Salamone, et al., 2005), and there is a body of evidence to suggest that dopaminergic activity in the brain is related to voluntary physical activity (for review see (Knab, et al., 2010)). Dopamine availability is dependent on its release, transport (reuptake), metabolism, and receptor binding. Consequently, by looking at the genes involved at any one of these functional stages there is an opportunity to indirectly investigate brain dopamine levels—in effect looking at markers of neurotransmitter activity (Epstein, Temple, et al., 2007)—and how behaviour may be resultantly impacted.

The TaqIA Restriction Fragment Length Polymorphism (RFPL) (rs1800497) is found within the ankyrin repeat and kinase domain containing-1 gene (ANKK1) (Neville, et al., 2004), located 10.5 kb downstream of the DRD2 gene in chromosome band 11q23.1 (Manning, et al., 2002). This polymorphism is a single nucleotide C/T change; the T allele is referred to as A1, the C allele as A2, individuals hetero- or homozygous for the T allele are referred to as $A1^{+}$, and individuals homozygous for the C allele are referred to as $A1^{-}$. This polymorphism occurs in ANKK1 exon 8, and results in a glu713-to-lys (E713K) non-conservative substitution; to date, this substitution has not been associated with a change in ANKK1 structural integrity, substrate-binding specificity, or function, however, dopamine-related endophenotypes have been associated with the TaqI RFLP (Rodriguez-Jimenez, et al., 2006). Moreover, ANKK1 and DRD2 genes have been shown to overlap, sharing halotypic blocks, and furthermore it was shown that ANKK1 expression was significantly upregulated by the powerful dopamine receptor

agonist apomorphine (Hoenicka, et al., 2010). Although the precise relationship between TaqIA RFLP and DRD2 remains uncertain, there is convincing evidence that $A1^+$ individuals have a 30-40% reduction in D2 receptor density and availability *in vivo* (Jonsson, et al., 1999; Noble, et al., 1991; Pohjalainen, et al., 1998; Thompson, et al., 1997); it must be noted, however, that there are data showing no significant differences in dopamine binding potential between $A1^+$ carriers and $A1^-$ (Laruelle, et al., 1998). Decreased striatal density of dopamine receptors has been shown via PET studies to be related to obesity (Volkow, et al., 2008), even proportional to body mass (Wang, et al., 2001). In line with the above noted hypo-responsiveness of dopamine transmission, evidence also suggests that the $A1^+$ allele is related to body mass (Noble, Noble, et al., 1994; Spitz, et al., 2000; Thomas, et al., 2001) but other groups have failed to find such a relationship (Davis, Levitan, et al., 2008; Jenkinson, et al., 2000; Southon, et al., 2003).

The TaqIA polymorphism has also been associated with a number of impulsive/addictive behaviours such as alcoholism (Munafo, et al., 2007), smoking (Noble, 1998), and overeating leading to obesity (Noble, Noble, et al., 1994; Spitz, et al., 2000). Another phenotype associated with the TaqIA polymorphism is food preference. In a sample of obese men and women it was shown that 64.3% of those who preferred carbohydrates (as opposed to foods high in fat or protein) were carriers of the A1 allele, compared to the 21.1% of carriers who preferred either high fat or protein foods (Noble, Noble, et al., 1994). What is interesting are the recent findings, using fMRI to measure responsivity of brain reward circuitry to palatable food cues, suggesting that the $A1^+$ allele is involved in consummatory and anticipatory feeding behavior. Specifically, individuals identified as having weaker striatal activation post ingestion of a palatable food (Stice, et al., 2008) or simply after imagining a palatable food (Stice, et al., 2010) had a greater risk of weight gain at 1 year, but only if they were an $A1^+$ carrier (vs. $A1^-$).

While obesity in general, and weight loss in particular, are no doubt influenced by polygenic factors, to our knowledge there has not been an effort to examine whether polymorphisms of TaqIA may impact the inter-individual variation in weight loss by two separate modalities. Accordingly, our primary objective was to investigate the potential

association of the TaqIA RFLP allele with body weight loss in overweight/obese post-menopausal women who completed a 6-month weight loss intervention using caloric restriction (CR) with or without the addition of a resistance training (RT) program. Our secondary objective was to elucidate if there existed genotype associations with energy intake (EI) or energy expenditure variables (EE). It was hypothesized that individuals carrying the A1 allele would respond with reduced body weight loss to the caloric restriction intervention and that individuals carrying the A1 allele would display an enhanced carbohydrate (CHO) preference and attenuated physical activity energy expenditure (PAEE).

2. Methods

This is a secondary analysis of the Montreal Ottawa New Emerging Team weight loss intervention which was designed to reduce body weight (BW) by 10 % and consisted of a 6-month intervention randomising participants to CR with or without resistance training (RT) (Brochu, et al., 2009). The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Université de Montréal, Comité d'éthique de la Faculté de Médecine. Written informed consent was obtained from all subjects/patients. By design, there were twice as many women randomised in the CR group compared to the CR+RT group, as women who completed the 6-month CR weight loss intervention were asked to participate in a 12-month follow-up with or without RT.

2.1 Subjects

A total of 147 Caucasian women aged 46 to 70 years were included in this study, of which data from 127 were included herein due to missing anthropometric data. Women were eligible to participate if they met the following criteria: *1)* body mass index (BMI) ≥27 kg/m^2, *2)* cessation of menstruation for more than 1 year and a follicle-stimulating hormone level ≥30 U/l, *3)* sedentary (<2 hours per week of structured exercise), *4)* non-smokers, *5)* low to moderate alcohol consumption (<2 drinks/day), *6)* free of known inflammatory disease and *7)* no use of hormone replacement therapy. On physical examination or biological testing, all participants had no history or evidence of: *1)* cardiovascular disease, peripheral vascular disease or stroke, *2)* diabetes (75g oral

glucose tolerance test), *3)* known renal and liver disease, *4)* asthma requiring therapy, plasma cholesterol > 8 mmol/L, *5)* systolic blood pressure > 160 mmHg or diastolic blood pressure > 100 mmHg, *6)* history of alcohol or drug abuse, *7)* previous history of inflammation disease or cancer, *8)* orthopedic limitations, *9)* body weight fluctuation of ± 2 kg in the last six months, *10)* untreated thyroid or pituitary disease and *11)* medications that could affect cardiovascular function and/or metabolism. After reading and signing the consent form, each participant was submitted to a series of tests.

2.2. Intervention Design and Procedure

2.2.1. Caloric restriction intervention (CR)

The women entered a medically supervised weight loss program for 6 months aimed at reducing BW by 10 %, as previously described (Brochu, et al., 2009). To determine the level of CR, 500 to 800 kcal were subtracted from baseline resting energy expenditure (REE; determined by indirect calorimetry) multiplied by a physical activity factor of 1.4, corresponding to a sedentary state (Tremblay, et al., 2004). Macronutrient diet composition was prescribed as follows: 55 %, 30 % and 15 % of EI, respectively from carbohydrates, fat and protein. Each participant met with the study dietitian to receive the diet prescription. Also, participants in both study groups were invited to meet bi-monthly with the dietitian for nutrition classes, 1-1.5 hours in duration.

2.2.2. Resistance training intervention (RT)

As previously described (Brochu, et al., 2009) each training session included a warm-up of low intensity walking on a treadmill for 10 min. The 6-month RT program consisted of four progressive phases and was performed weekly on 3 non-consecutive days [phase 1: introduction to training (3 weeks, 15 repetitions, 2 to 3 sets per exercise, 90-120 seconds between sets); phase 2 (5 weeks, 12 repetitions, 2 to 3 sets per exercise, 90 seconds between sets); phase 3 (9 weeks, 8 to 10 repetitions, 2 to 4 sets per exercise, 120-180 seconds between sets) and phase 4 (8 weeks, 10 to 12 repetitions, 3 to 4 sets per exercise, 60-90 seconds between sets)]. Each exercise session was individually monitored by qualified trainers.

2.3 Measurements

2.3.1. Anthropometric measurements

Body composition (fat mass (FM) and fat-free mass (FFM)) and BW were assessed by dual energy X-ray absorptiometry (DXA) using a daily calibrated GE-LUNAR Prodigy module (GE Medical Systems, Madison, WI). Briefly, subjects laid on an examination table, wearing standard issue hospital gowns, while a low-intensity X-ray scanned their entire body; height (Perspective Enterprises, Michigan, USA) and BW (BWB-800AS Digital Scale; Tanita Corporation of America Inc) was also measured while wearing standard issue hospital gowns. BMI [body weight (kg)/Height (m^2)] was also calculated.

2.3.2. Energy Intake

To measure free-feeding EI 3-day self-reports (see (Strychar, et al., 2009)) were employed at baseline and then 1 month post intervention (after completing 1 month of weight stability). Briefly, participants were given detailed instruction on how to report all foods and beverages consumed over a one week period represented by 2 weekdays and 1 weekend day. Upon handing in the completed self-reported food records, a registered dietitian reviewed the records with the women and reconciled any ambiguities in reporting or obtained more detailed information of EI.

2.3.3. Energy Expenditure

Daily energy expenditure was determined from doubly labeled water (DLW) over a 10-d period (Schoeller, et al., 1982). The DLW ($^2H_2{}^{18}O$) experiments generated 5 urine samples per subject: a pre-dose sample was collected before administration, two samples (16 to 24 hours later) were obtained after the $^2H_2{}^{18}O$ dose had initially equilibrated in the body, and two more samples were collected 10 days later, as previously described (Karelis, et al., 2010). All samples were measured in triplicate for ^{18}O-water and ^2H-water. An Isoprime Stable Isotope Ratio Mass Spectrometer connected to a Multiflow-Bio module for Isoprime and a Gilson 222XL Autosampler (GV Instruments, Manchester, UK) were used for daily energy expenditure measurements. Data processing was performed with MassLynx 3.6 software. Stability tests were performed each day before testing giving a standard deviation of 0.026 % for deuterium and 0.004 % for ^{18}O (Lavoie, et al., 2010; St-Onge, et al., 2007). In addition, REE and

PAEE were measured by indirect calorimetry and total daily energy expenditure (TDEE) was then calculated as previously described (Conus, et al., 2004).

2.3.4. TaqIA genotyping

The genotyping of TaqIA was done by polymerase chain reaction (PCR) using with forward primer: 5′-GAC GGC TGG CCA AGT TGT CTA-3′ and reverse primer: 5′-GTC GAC CCT TCC TGA GTG TC-3′ to amplify a 304 bp fragment spanning the polymorphic TaqIA site. Analysis of the TaqIA polymorphisms was done as previously described (Spitz, et al., 1998). The amplified products were incubated with 1 µl of the Taq1 (*Thermus aquticus* YT-1, *invitrogen*) restriction enzyme for 22 hours at 65ºC. The restriction products were visualized on a 2% agarose gel (run for 60min at 100V) stained with ethidium bromide. The A1 allele corresponds to the uncut amplicon of 304 bp, the A2 allele is characterized by two fragments of 177 and 127 bp. Of the 127 samples, 119 were analyzed in duplicate, while 8 samples were only analyzed once due to insufficient volume of plasma; all samples run in duplicate verified no difference in allele type (i.e. 100% precision), so it was deemed acceptable to include the 11 non-duplicated samples in the overall analysis.

2.3.5 .Analytic Plan

A between subjects two-way analysis of variance (ANOVA) was conducted to determine if there were any differences between exercise groups (CR *vs.* CRRT) or genotype ($A1^+$ *vs.* $A1^-$). Effects of the TaqIA polymorphism on anthropometric (i.e. BW, BMI, FM, and FFM), EI (i.e. mean EI, %CHO, %fat, %protein, and %alcohol), energy expenditure (i.e. TDEE, REE, and PAEE), and variables were analyzed using two ($A1^+$ *vs.* $A1^-$) X 2 (caloric restriction *vs.* caloric restriction and resistance training) x 2 (baseline *vs.* post-intervention) repeated measured ANOVAs. This analysis also allows the determination of how genotype influences changes in EI over time ($A1^+$ *vs.* $A1^-$) x (baseline *vs.* post-intervention), as well as which exercise modality was more effective in producing changes in variables of interest [(CR *vs.* CRRT) x (baseline *vs.* post intervention)]. Due to the limited data on rs1800497 genotype differences and possible differences in BW or CHO preference, for example, we chose not to control for multiple statistical

comparisons so as to not overlook findings that could otherwise help to bring light to better describing this polymorphism. Differences with *p*-values ≤ 0.05 were considered statistically significant and values are presented as means ± standard deviation unless indicated otherwise. Statistical analyses were performed using SPSS version 17 (Chicago, SPSS Inc.).

3. Results

At baseline, the average woman was 57.1±4.6 years of age, BW of 86.5±14.5 kg, BMI of 33.1±4.4kg/m^2, FFM of 46.2±6.8kg, FM of 40.4±9.3kg, and %FM of 46.4±4.5. There were no significant differences in initial BW or body composition at baseline neither by group nor by genotype.

TaqIA was in Hardy-Weinberg equilibrium and the frequencies of the TaqIA alleles were distributed as follows: six subjects (4.7%) were homozygous for the A1 allele, sixty subjects (47.2%) were heterozygous, and sixty-one (48.0%) were homozygous for the A2 allele. Due to the low frequency of A1 homozygous subjects, and in order to maintain consistency with previous research (Blum, et al., 1996; Epstein, Temple, et al., 2007), the TaqIA genotypes were dichotomized to $A1^+$ and $A1^-$.

As seen in **Table 1** a significant 3-way interaction of time by group by allele, F (1,123)=4.2, p=0.04, was found for attenuated BW loss whereby $A1^+$ carriers lost less BW than non A1 carriers in the CRRT group only ($A1^+$CRRT: 81.1±12.0kg to 76.7±13.4kg; $A1^-$CRRT: 86.1±16.6kg to 79.1±18.4kg, p<0.05). Similarly, significant 3-way interactions of time by group by allele were discovered only for the CRRT group for attenuated FM loss, F (1,123)=6.0, p=0.01, whereby $A1^+$ carriers lost less FM than non A1 carriers ($A1^+$CRRT: 37.2±8.5kg to 32.8±9.3kg; $A1^-$CRRT: 41.1±10.7kg to 34.9±13.5kg, p<0.05), and lastly for an attenuated decrease in BMI, F (1,123)=4.7, p=0.03, whereby $A1^+$ carriers lost less BMI than non A1 carriers ($A1^+$CRRT: 31.7±3.6kg/m^2 to 29.4±4.2kg/m^2; $A1^-$CRRT: 32.4±5.2kg/m^2 to 31.0±6.0kg/m^2, p<0.05) (see **Table 1**).

Similarly, the 3-way interaction on CHO intake was significant (p=0.019) whereby $A1^+$ carriers exhibited an increase in %CHO intake over time ($A1^+$CRRT: pre-intervention 46.2±5.1% to post-intervention 52.5±8.5%, p <.05) in the CRRT group, and the $A1^-$ exhibited a significant decrease in %CHO intake over time in the CRRT group ($A1^-$ CRRT: pre-intervention 51.4±6.7% to post-intervention 47.6±9.9%, p<0.01), F (1, 33)=7.93, p=0.008 (see **Table 1**). No significant differences in %CHO were found over time between carriers of the $A1^+$ and $A1^-$ genotypes.

In the entire sample, all of the anthropometric variables and REE significantly decreased post-intervention (p<0.001). No significant effects of allele were noted for REE, PAEE, or TDEE (see **Table 1**).

4. Discussion

This study utilized a theoretically driven approach to elucidate possible relationships between the rs1800497 genotype believed to alter dopaminergic activity and the predisposition to respond differently to standardized weight loss modalities. Overall there was a significant association between the TaqIA genotype and BW loss, FM loss, and decrease in BMI post-intervention, but only for the women in the CRRT group. Regarding the secondary hypotheses, there was an association between the TaqIA genotype and macronutrient intake, whereby $A1^+$ individuals in the CRRT group demonstrated an increase in CHO intake whereas $A1^-$ carriers showed a decrease in CHO intake post weight loss. There was no difference by genotype pre- and post-intervention in CHO intake in the CR group. $A1^+$ individuals exhibited smaller reductions in BW, FM, and BMI pre- and post-intervention in the CRRT group compared to the $A1^-$ participants, whereas no interactive effects were found in the CR group.

Ongoing research on the genetics of obesity has found more than 430 genes, markers, and chromosomal regions discovered to be related to obesity phenotypes (Snyder, et al., 2004) and estimates of the heritability of BMI are between 40-70% (Barsh, et al., 2000). Strong evidence also suggests that changes in FM and fat distribution, especially during negative energy balance (*vs.* overfeeding) (Bouchard, et

al., 1997), are largely determined by genetic factors. In a study examining the potential role of TaqIA polymorphism in “neurobesigenics”, a clinical subtype of Reward Deficiency Syndrome, 122 non-Hispanic Caucasian obese subjects (17 males and 105 female, mean age 42.3 yrs) with age matched non-obese controls were genotyped for TaqIA polymorphism (Chen, et al., 2012). It was found that the $A1^+$ genotype was present in 67% of the obese and only in 33% of the controls and that there was a significant positive association with percent body fat. Furthermore, a recent study demonstrated that parent-child concordance of the TaqIA genotype predicts similar parent-child weight loss response to a diet/exercise intervention (Epstein, et al., 2010). Specifically, if parent and child were concordant for the A1 allele, the child showed double the zBMI change at 6 months into the intervention and over four times the change at 12 months in comparison to a child who was not concordant for the A1 allele. In our sample of obese/overweight post-menopausal women we report similar findings whereby individuals with the $A1^+$ genotype had a lesser change in BMI (*vs.* $A1^-$) in the CRRT group (see **Table 1**). A similar significant finding was also noted in our group for concordance of the $A1^+$ allele and overall weight loss, but again only in the CRRT group.

In a recent study examining the efficacy of a vegan diet intervention and the potential impact of the TaqIA polymorphism on outcome variables it was found that relative to individuals with the $A1^-$ genotype, Caucasian $A1^+$ carriers demonstrated significantly more CHO intake (Barnard, et al., 2009). Though there was no genotype interaction with weight loss in the overall sample, when race was considered, however, African American A1 carriers did experience significantly less weight loss than the $A1^-$ individuals TaqIA genotype (Barnard, et al., 2009). Although the vegan diet intervention was by caloric restriction alone the negative energy balance (500-1000kcal/day) was very close to that prescribed in our intervention. Accordingly, these results dovetail nicely with our findings and those of previous groups (Noble, 2000; Noble, Noble, et al., 1994). Another very likely candidate phenotype that could have operated concurrently with CHO preference is a food reinforcement phenotype. Literature shows that acute (2-4 hrs.) and chronic (2 months) energy deprivation can increase the reinforcing value of palatable foods (Cameron, et al., 2008; Saelens & Epstein, 1996); what is more, studies

have revealed that food reinforcement can not only be a good predictor of *ad libitum* EI, but the presence of the A1 allele can interact with obesity to influence food reinforcement (Epstein, Temple, et al., 2007; Epstein, Wright, Paluch, Leddy, Hawk, Jaroni, Saad, Crystal-Mansour, Shields, et al., 2004). Subjects identified as high in food reinforcement who were carriers of the A1 allele consumed more food than participants high in food reinforcement without the A1 allele and participants low in food reinforcement with or without the A1 allele (Epstein, Temple, et al., 2007). In our sample of women we did not, however, have access to any measure of food reinforcement or reward, but given no study has examined how the chronic effects of energy deprivation involved in an intervention impacts food reinforcement in those with the A1 allele, this remains a fruitful area of inquiry as it may provide a mechanism for the current finding of increased CHO intake in these polygenetic individuals.

Another of our secondary hypotheses was that the TaqIA polymorphism would be related to lower levels of EE. Existing literature clearly demonstrates that dopamine deficient mice are hypoactive and dopamine receptor knockout mice demonstrate significantly less spontaneous movement (Baik, et al., 1995). Further, there is considerable evidence to show that genetic variation contributes to the interindividual variation in responsiveness to exercise training and that genotype-by physical activity interactions may play a measurable role in following health-related outcomes (Rankinen, et al., 2012). In this light and in concert with findings that lower levels of Nonexercise Activity Thermogenesis (NEAT) can contribute to BW gain (Levine, et al., 2005), it was hypothesized that lower EE in $A1^+$ individuals would account for part of the attenuated weight loss. Although there was no association between genotype and EE variables (see **Table 1**), this finding is to our knowledge novel and is the first to examine the potential for a energy expenditure-related phenotype using well validated measures of EE (i.e. doubly-labelled water).

The current study is among the first study to examine how variants of the Taq1A allele influence changes in body composition and EI in a 6-month weight loss trial in obese adults. Also, we are the first to show that the TaqIA genotype was associated with attenuated BW and FM losses in a group receiving diet and exercise interventions.

Consistent with our hypotheses and with previous research, we also found that being a carrier of the A1 allele was associated with an increase in CHO intake (only in the CRRT group) compared to non A1 carriers. Limitations of the study include the fact that although overweight or obese, the studied population was a homogeneous population of "healthy" non-diabetic Caucasian women, thus we are uncertain if our results generalize to all obese women in the targeted age range. Also, due in part to the significant differences in allele frequency and genotype prevalence that generally occur due to racial/ethnic variation (Chang, et al., 2009), there is also a need to be mindful of ethnic variation (or lack thereof) when interpreting the Taq1 A genetic data. Regarding the EI variables, there exists the limitation of self-reporting and the well-known under-reporting phenomenon (Karelis, et al., 2010). Finally, our observed associations between rs1800497 and FM and CHO intake must be interpreted recalling the less-stringent analysis of multiple comparisons.

In summary, in support of the main hypothesis there was an association between TaqIA polymorphism and the amount of BW loss; more specifically, there were group by genotype interactions where carriers of the A1 allele (*vs.* $A1^-$) lost significantly less BW, FM and BMI in the CRRT group. The secondary hypothesis was partially confirmed, whereby a post-weight loss increase in CHO intake emerged for $A1^+$ carriers in the CRRT group; however there were no significant effects on any measure of EE. Future research should be directed at longitudinally examining the interplay of dopamine-related polymorphisms, nutritional status, and food reward in order to have a better understanding of potential gene-environment interactions and how each of these variables may differentially affect feeding behaviour and obesity at large.

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Table 1. Subjects' characteristics by weight loss modality and TaqIA genotype.

Characteristics	Pre				Post				Time	Group	Allele	Time* Group	Time* Allele	Time* Group* Allele
	$A1^+$		$A1^-$		$A1^+$		$A1^-$							
	CR	CRRT	CR	CRRT	CR	CRRT	CR	CRRT						
Anthropometric														
n	**49**	**17**	**44**	**17**	**49**	**17**	**44**	**17**						
Body Weight (kg)	86.1±15.6	81.1±12.0	86.8±15.3	86.1±16.6	80.5±15.0	76.7±13.4	82.3±14.3	79.1±18.4	.001	.28	.41	.47	.44	.04
BMI (kg/m^2)	32.9±4.7	31.1±3.6	33.1±4.3	32.4±5.2	30.8±4.5	29.4±4.2	31.4±4.2	31.0±6.0	.001	.41	.17	.38	.42	.03
Fat Mass (kg)*	39.4±9.8	37.2±8.5	39.9±9.2	41.1±10.7	34.9.±9.9	32.8±9.3	37.1±9.7	34.9±13.5	.001	.51	.27	.03	.97	.01
Fat Free Mass (kg)*	46.6±7.7	43.9±4.9	46.6±7.3	44.9±7.2	45.6±6.6	43.9±5.1	45.1±6.4	44.1±6.0	.001	.19	.88	.06	.20	.74
Energy Expenditure (kcal/day)														
n	**35**	**15**	**30**	**17**	**35**	**15**	**30**	**17**						
TDEE	2458±395	2561±296	2493±392	2551±476	2380±360	2435±410	2446±365	2484±345	.06	.38	.63	.69	.58	.87
REE*	1352±216	1332±157	1367±232	1287±157	1280±200	1262±169	1309±230	1245±183	.001	.26	.91	.60	.28	.72
PAEE	905±295	953±225	905±275	1009±299	898±274	915±263	916±295	991±279	.73	.21	.44	.69	.80	.99
Energy Intake														
N	**15**	**8**	**5**	**9**	**15**	**8**	**5**	**9**						
Mean 3-day(kcal/day)	1822±398	1671±421	2198±494.7	23010±772	1670±421	1777±292	1780±367	1748±448	.002	.349	.256	.415	.297	.901
%CHO	49.6±4.9	46.2±5.1	43.8±6.4	51.4±6.6	49.9±6.7	52.5±8.5	50.5±6.1	47.6±9.9	.115	.264	.420	.448	.407	.019
%Fat	30.8±3.7	33.7±4.4	34.9±5.4	30.9±6.2	29.8±6.3	27.3±4.6	29.5±5.5	28.9±3.5	.005	.448	.622	.704	.991	.082
%Protein	17.9±3.8	15.8±1.9	17.7±2.5	15.1±1.7	18.1±3.1	17.1±2.2	18.4±2.5	19.8±1.5	.005	.192	.539	.034	.233	.374
%Alcohol	1.6±2.4	4.2±5.1	3.5±6.7	2.5±2.9	2.1±3.9	2.9±5.7	1.6±3.3	3.7±6.5	.549	.409	.951	.595	.973	.045

TDEE is total daily energy expenditure; REE is resting energy expenditure; and PAEE is physical activity energy expenditure; Values are Mean ± SD.n=48 for A1+ in CR for FM, FFM and REE; n=43 for A1- in CR for FM, FFM & for REE in this group n=44n=17 for A1+ and A1- in CRRT for REE

CHAPTER V: Article II

Running Head: Fasting for 24 hours improves nasal chemosensory performance and food palatability in a related manner

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J.C. performed all data collection and J.C., G.G., and E.D. were involved in the elaboration of the experimental design and collaborated to write the manuscript.

Fasting for 24 hours improves nasal chemosensory performance and food palatability in a related manner

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Keywords: Olfaction Sniffin' Sticks Fasting Energy Deprivation Food Hedonics

Abstract

Changes in smell function can modify feeding behaviour but there is little evidence of how acute negative energy balance may impact olfaction and palatability. In a within-subjects repeated measures design, 15 subjects (9 male;6 female) aged 28.6±4.5 yrs. with initial body weight (BW) 74.7±4.9 kg and body mass index (BMI) 25.3±1.4 kg/m^2 were randomized and tested at Baseline (FED) and Post Deprivation (FASTED) for nasal chemosensory performance (*Sniffin' Sticks*) and food palatability (Visual Analogue Scale). Significant main effects for time indicated improvements in the FASTED session for odor threshold, odor discrimination, and total odor scores (TDI), and for increased palatability. There were significant positive correlations between initial BW and the change in odor threshold (r=.52) and TDI scores (r=.53). Positive correlations were also noted between delta identification score and delta palatability (r=.68). When the sample was split by sex, only for females were there significant correlations between delta palatability and: delta BW (r=.88); delta odor identification (r=.94); and delta TDI score (r=.85). Fasting for 24 hours improved smell function and this was related to increased palatability ratings and initial BW. Further studies should confirm the role of BW and sex in the context of olfaction, energy deprivation and palatability.

1. Conflict of Interest: None Disclosed

Introduction

Energy deprivation impacts not only food intake and hedonics but also olfaction. Alliesthesia aptly describes how the subjective evaluation of an unchanging food stimulus can be modified by hunger state (Cabanac, 1971) and more recent evidence suggests that the effects of hunger state can similarly impact the pleasantness of food odors (Plailly, et al.). Although we do not yet understand the mechanisms by which alliesthesia changes for food and odor stimuli (e.g. from positive to negative when sated), energy deprivation is indeed linked to changes in olfactory bulb activity (Apelbaum, et al., 2005) and to changes in olfactory sensitivity in the rat (Aime, et al., 2007).

Previous research in human subjects has shown that freely selected meals were preceded by increased, and followed by decreased, olfactory sensitivity and that skipping the meal altogether further increased sensitivity (Goetzl & Stone, 1947). Hunger state and olfactory sensitivity has recently been revisited in human subjects and results showed that acuity to a neutral odor was greater in a high (compared to low) hunger state (Stafford & Welbeck, 2011) and that individuals with a higher rather than a lower BMI had poorer sensitivity to a neutral odor (Richardson, et al., 2004; Stafford & Welbeck, 2011). Indeed, the relationship between body weight status and nasal chemosensory performance remains to be better understood and furthermore only a small number of studies have focused on more standardized paradigms to examine the integral role of olfaction with regards to feeding behaviour in the context of energy deprivation and re-feeding.

To examine the modulation of olfactory sensitivity and its potential impact on food palatability we employed the well validated “Sniffin’ Sticks” (Hummel, et al., 2007) tests and imposed an acute 24 hour complete fast on healthy subjects. We hypothesized that compared to their fed state, fasted individuals would demonstrate improvements in smell performance (odor threshold, discrimination, and identification) and exhibit heightened hedonics consistent with the concept of alliesthesia. It was also hypothesized that the improved smell performance would coincide with higher

palatability ratings of a fixed-energy meal, i.e., we anticipated that the deltas for olfaction and palatability would be positively related.

Methods

Participants

Fifteen subjects (9 men and 6 women) aged 28.5±4.5 yrs. with an initial body weight of 74.7±4.9 kg and BMI 25.3±1.4 kg/m^2 participated in this study. Subjects were free from any illnesses and medication that could have influenced the outcome of the experiment and met the following inclusion criteria: non-diabetic, non-smokers, not pregnant, weight stable for ≥6 months (±2 kg) and aged between 18 and 40 years. Only pre-menopausal women with a regular menstrual cycle (28-35 days) were recruited including those using oral contraceptives. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Ottawa Research Ethics Committee. Written informed consent was obtained from all subjects.

Design and Procedure

During the initial screening visit to the laboratory, participant's height and body weight (BW) were measured in the pre-prandial state and informed consent was obtained. In order to minimize the hormonal effects on main outcomes, measurements for women were scheduled between days 1-5 of the menstrual cycle, where ovarian hormones are at their lowest levels. As such, women had at least a 1 month period, and men at least a 2 week wash-out period between the FED and FASTED conditions. The order of testing was then randomized such that half of the subjects first underwent the FED session and half first underwent the FASTED session.

Subjects arrived at 0730 fasted from 1900 the prior evening for the FED session. For the FASTED session, subjects arrived at 0730 and were fasted from 1200 the previous day; subjects were only allowed water during the 24 hours that defined the fasting interval (1200-1200). Upon arrival, BW was recorded and a calorically-clamped breakfast was administered (only for the FED session). In both the FED and FASTED sessions nasal chemosensory performance was measured at 1030, followed by a calorically-clamped lunch at 1200 and a visual analogue scale (VAS) rating of palatability.

Measurements

Height (HR-100 Height Rod; Tanita Corporation of America Inc. Arlington Heights, IL) and BW (HR-100; BWB-800AS, Tanita Corporation, Arlington Heights, IL., USA) were measured after voiding and while wearing a standard hospital gown.

Nasal chemosensory performance was measured with Sniffin' Sticks (Burghart Instruments, Wedel, Germany), a 3-test battery of odorized pens that measure odor detection threshold, odor discrimination, and odor identification, each on 16 point scales and added together to form an aggregate total odor score (threshold, discrimination, identification: TDI), as previously described (Hummel, Erras, et al., 1997). Briefly, the pens for the threshold test contained 16 concentrations (1-16, strongest to weakest) of n-butanol and subjects were presented with a triplet of pens, the other two being blanks (aqua-conservans). Subjects were required to identify the pen with n-butanol and the threshold score was determined by a single up-down staircase method, as previously described (Hummel, Erras, et al., 1997). In the discrimination task, subjects were again asked to identify the pen that smells different when presented with 16 triplets, the difference from threshold being that 2 pens have the same odor and one has a unique odor. The identification test consists of a forced-choice booklet of 16 pages, each with a choice of 4 odors. The subject is presented with 16 different pens (odors) and is required to circle the odor in the booklet that they believe identifies the pen.

For the FED session subjects were presented with a calorically-clamped breakfast which had to be consumed in 15 min. The meal consisted of 2 pieces of whole wheat toast (*D'Italiano*®, 147 kcal), 17 grams of peanut butter (*Kraft* Smooth Peanut Butter®, 101 kcal), 15 grams of raspberry jam (*Smuckers*®, 50 kcal), and 250 ml of water for a total of 298 kcal. At 1200 in the FED and FASTED sessions, subjects were presented with a lunch, which had to be consumed in 30 min. The meal consisted of 2 slices of cheese pizza (*Michelina's* Zap 'Ems Gourmet®, 781 kcal).

Palatability, or the hedonic value of food, was rated on a 150 mm VAS that was adapted from Hill and Blundell (Hill, et al., 1984). Immediately following the consumption of the pizza slices (~1215) subjects were presented with the following question: "How palatable was the meal?" (Not at all-Extremely).

Statistical Analysis

To test for differences in anthropometric variables, olfaction and food palatability across FED and FASTED sessions, one way repeated measures of Analysis of Variance (ANOVA) where time (FED vs. FASTED) represented the within-subjects (repeated measures) and sex as a between subject factor. Bivariate correlations were used to determine the strength of the relationship between the changes in anthropemetric variables and the change in olfaction and in food palatability; partial correlations controlling for age and sex were then used to follow up significant relationships. Statistical analyses were performed using SPSS version 17 (Chicago, SPSS Inc.). Statistical significance was denoted as $p<.05$ for all tests.

Results

There were no significant changes in any anthropometric variables. There were significant main effects for time (FED vs. FASTED) for three of the four measures of nasal chemosensory performance: odor threshold score improved, $F(1,13)=11.5$, $p=0.005$; odor discrimination improved, $F(1,13)=35.1$, $p=0.001$; and TDI score improved, $F(1,13)=33.3$, $p=0.001$. A significant 2-way interaction of time (FED vs. FASTED) by sex, $F(1,13)=6.9$, $p=0.02$, was found for the TDI score such that women demonstrated a greater mean increase in score than men (see **Figure 1**, panel **D**). There was also a significant main effect for palatability. The mean increase in palatability rating for the standardized lunch meal was 22.5mm, $F(1,13)=13.0$, $p=0.003$; no effect of sex was noted., h

Bivariate correlations revealed significant positive correlations between initial BW and delta odor threshold score ($r=.52$, $p<0.05$) and initial BW and delta TDI score ($r=.53$, $p<0.05$); positive correlations were also noted between delta identification score and delta palatability ($r=.68$, $p<0.05$). All above relationships remained significant when partial correlations were performed controlling for age and sex.

Discussion

In this study we show that nasal chemosensory performance and food palatability improve after 24 hours of energy deprivation, and perhaps most novel, that changes in olfaction were positively related with changes in palatability and with initial BW. The role that energy deprivation plays in food hedonics has been well documented and evidence suggests that short term (Yeomans, 1996) and longer term (Cameron, et al., 2008)

energy deficits increase the palatability of similar test foods. On the other hand, although there is a growing interest in studying the role of olfaction in energy deprivation paradigms and appetite research, there is only preliminary data with more standardized measures of chemosensory performance. In line with our reported main effect of time (FED vs. FASTED) for change in odor scores, a study looking at high (12 hour fast) vs. low (fed) hunger state and olfaction found that individuals had significantly improved *Sniffin' Sticks* scores in the high hunger state (Stafford & Welbeck, 2011). The current study was to our knowledge the first to show a link, using *Sniffin' Sticks*, between changes in olfaction and changes in palatability, and the positive relationship between BW and changes in olfactory identification scores.

Along with a progressive deterioration in smell sensitivity with age, the sex differences in smell capability are well documented (Hummel, et al., 2007), where females generally demonstrate superior smell sensitivity (Kobal, et al., 1996). In our sample, females displayed superior improvements in TDI scores in response to 24 hours of complete food deprivation (**Figure 1**). Because we controlled for menstrual cycle phase, it is possible that there are factors other than sex hormones that may explain such differences.

An interesting finding reported here is the fact that independent of sex the initial BW was associated with the overall change in threshold and TDI scores; thus there seems to be more room for smell improvements over the course of an acute energy deprivation for larger individuals. This is not necessarily in contradiction to previous findings indicating that severity of obesity was progressively related to olfactory dysfunction (Richardson, et al., 2004) or that obese subjects have been found to have impaired smell function—obese children aged 10-16 demonstrated significantly lowered thresholds for odor detection and identification compared to non-obese controls (Obrebowski, et al., 2000). Indeed, consistent with our results is the recent finding that elevated insulin that mimics a feeding episode diminishes smell function (Ketterer, et al.). Overall this suggests that the severe but acute metabolic challenge of a 24 hour complete fast may also modulate olfaction, and due to contradictory results in the existing literature—some have shown superior odor identification for higher versus lower BMI (Simchen, et al., 2006) or for lower versus higher hunger state (Albrecht, et

al., 2009)—that controlling for the test odorant itself is equally as important as body weight status or level of energy deprivation when examining alliesthesia to food and odor cues.

We showed that nasal chemosensory performance and palatability ratings improved as a result of a 24 hour fast and females demonstrated larger improvements in overall olfactory performance (TDI scores) under such conditions. Initial BW was positively related to improved odor detection threshold and TDI scores. Given the limitation of a small sample size, objective measures of olfaction must be further studied in light of these findings and especially due to the potential dysregulation of appetite due to enhanced saliency of food in times of energy deprivation.

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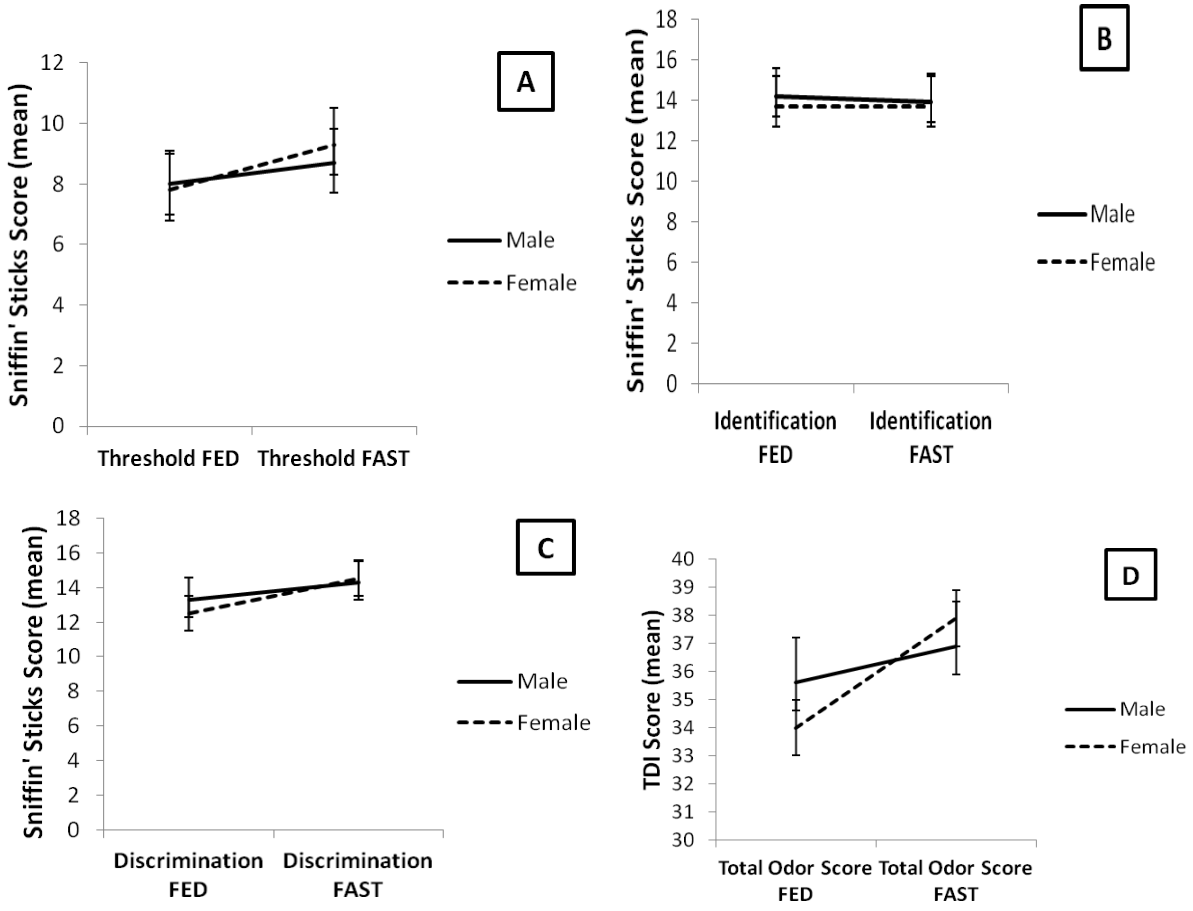
Figure Legend

Figure 1. Demonstrating the changes in nasal chemosensory performance from the baseline (FED) to the experimental (24 hr. FASTED) session for men and for women. The odor detection threshold significantly improved with fasting, indicating subjects were able to detect the non-food test odorant (n-butanol) at smaller concentrations; odor discrimination and TDI (threshold, discrimination and identification scores combined) also significantly improved under the fasting condition in both men and women. A group by sex interaction is noted in panel **D**, whereby women demonstrated larger increases in TDI from FED to FASTED ($p<0.05$).

Figure 1.


A
Sniffin' Sticks Score (mean)
Threshold FED
Threshold FAST
Male
Female
B
Sniffin' Sticks Score (mean)
Identification FED
Identification FAST
Male
Female
C
Sniffin' Sticks Score (mean)
Discrimination FED
Discrimination FAST
Male
Female
D
TDI Score (mean)
Total Odor Score FED
Total Odor Score FAST
Male
Female

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Chapter VI: Article III

Running Head:

The Impact of a 24 hour fast on food reward and intake during *ad libitum* feeding: evidence of increased reward from food and food-related cues

This paper is currently submitted to the journal *Applied Physiology, Nutrition, and Metabolism*

J.C. performed all the data collection and J.C., G.G, G.F., J.B., and E.D. were involved in the elaboration of the experimental design and collaborated to write the manuscript.

The Impact of a 24 hour fast on food reward and intake during *ad libitum* feeding: evidence of increased reward from food and food-related cues

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Keywords: **Fasting Food Preference Food Reward Hedonics**

Abstract

Introduction: We examined the impact of a 24 hour complete fast (vs. fed state) on two measures of food reward—specifically, on 1) 'wanting' as measured by response to food images & the relative-reinforcing value of food (RRV), and 2) 'liking' as measured by response to food images & the hedonic evaluation foods consumed.

Methods: In a within-subjects repeated measures design, 15 subjects (9 male; 6 female) aged 28.6±4.5 yrs with initial body weight (BW) 74.7±4.9 kg and body mass index (BMI) 25.3±1.4 kg/m^2 were randomized and counterbalanced to normal feeding (FED) and 24-hour fast (FASTED) conditions. Trait characteristics were measured with the Three-Factor Eating Questionnaire and Sensitivity to Reward (SR) questionnaires. Two computer tasks measured food reward: 1) RRV progressive ratio task, 2) explicit liking and wanting (LFPQ); also measured were *ad libitum* energy intake (EI; buffet) and food palatability (visual analogue scale).

Results: There were no significant anthropometric changes between conditions. Appetite scores, hedonic ratings of palatability, and *ad libitum* EI all significantly increased under the FASTED condition ($p<0.05$). Under the FASTED condition there were significant increases in the RRV of snack foods; similarly, explicit wanting and liking significantly increased for all food categories. Across-meal negative alliesthesia was noted only for savory foods under FASTED.

Conclusion: We observed increased food reward measured by RRV and LFLQ during *ad libitum* feeding after a 24-hour fast. Although there was a 30% increase in EI under the FASTED condition, positive alliesthesia was demonstrated by heightened hedonic ratings under the FASTED condition.

1. Conflict of Interest: None Disclosed

Introduction

In one of the best known and most intensive controlled studies of human feeding ever conducted it was shown that after 24 weeks of energy restriction (approximately 1500 kcal/day) that subjects demonstrated uncontrollable energy intake (EI) and preoccupations with sweet foods (Franklin, et al., 1948). Similar findings have been reported with acute periods (1 day) of energy deprivation with clinical populations (Hetherington, et al., 2000; Telch, et al., 1996). However it is still unclear what factors underpin compensatory eating in the early stages of fasting. There are conflicting data, but it appears that the short-term manipulation of satiety, most often achieved with a pre-load paradigm, does not reliably impact palatability (Booth, et al., 1982; Johnson & Vickers, 1993; Yeomans, et al., 1998). While the preload paradigm may be sensitive to possible short-term signaling of need (free)-state, a better manipulation has been less well studied and involves increasing the deprivation state *through* sustained energy deprivation. In support of the external validity of a sustained energy deprivation, it can be pointed out that approximately 14% of Americans have reported using short-term fasting as a means of losing weight (French, et al., 1994); furthermore, the relevance of studying subjects under periods of deprivation longer than a few hours is reflected in the observation that day-to-day feeding repeatedly takes place without any measurable changes in body energy reserves. Such feeding may respond to learned cues (De Castro, 1996), and not necessarily to a need-state.

In his seminal paper on hedonics (1971), Michel Cabanac tested changes in gustative and olfactive perception at the subjects' normal weight, at 10% below this weight, and then again after subjects returned to normal weight. Alliesthesia was measured by the change in subjective pleasantness ratings for multiple ingestions of sweet tasting sucrose solutions under different states of hunger. When subjects were at their normal weight and after ingesting 200 milliliters of a 25% aqueous solution of glucose, these sweet stimuli changed from pleasant to less pleasant (negative alliesthesia). However, when subjects lost 10% of their body weight, negative alliesthesia could no longer be demonstrated. Finally, the return to normal body weight restored the change in sensation from pleasant to unpleasant.

Energy deprivation not only influences the palatability—or liking—of a particular food stimulus, but also the desire—or wanting—to engage a food stimulus. Early research in food reinforcement showed that wanting could change in a state-dependent (i.e. hungry vs. satiated) manner (Bulik, et al., 1994; Epstein, et al., 2003; Raynor & Epstein, 2003). Several groups have demonstrated that, consistent with alliesthesia, the hedonic liking evaluation of ingested foods can be influenced by hunger-state (Blundell, et al., 1988; Laeng, et al., 1993) or by a state of reduced body energy reserves (Cameron, et al., 2008; Frankham, et al., 2005). State-dependent evidence also exists for affective reactions to *food images*, which have been shown to be higher with heightened hunger in both men and women (Rolls, et al., 1983; Stoeckel, et al., 2007; Uher, et al., 2006). It is unclear, however, what impact a complete 24 hour fasting challenge would l have on the direction of wanting and liking or whether fasting causes changes in appetite, food reward and *ad libitum* EI.

The main objective of this randomized cross-over study was to examine the impact of a 24 hour complete fast (vs. fed state) on food reward—specifically, wanting and liking for an array of preferred foods in order to elucidate hedonic and nutrient-status (need-state) on subsequent *ad libitum* feeding. Three separate procedures were employed to measure food reward and provide evidence for alliesthesia: 1) Visual analogue scales (VAS) measuring subjective ratings of hunger, fullness and palatability immediately following the ingestion of foods, 2) A progressive ratio computer task measuring the relative-reinforcing value (RRV) of preferred foods with a concurrent schedule paradigm (see (Epstein & Leddy, 2006; Lappalainen & Epstein, 1990)), and 3) The Leeds Food Preference Questionnaire task (LFPQ) measuring separate and concurrent assessments of explicit liking and wanting for an array of food images (Finlayson, et al., 2007a). It is important to note that all three of these procedures are sensitive to changes in hunger and satiety (short term withholding of food). It was hypothesized that under the fasting condition there would be significantly higher appetite scores and *ad libitum* EI, and that correlations would exist between these changes. It was further hypothesized that relative to the fed state, snack food would increase in RRV (versus fruit); and that relative to the fed state there would be increased explicit wanting and liking. Finally, it was hypothesized that the meal-induced

attenuation of explicit liking for the fasted condition would be less pronounced. That is, negative alliesthesia would be weak or absent.

Methods

3.1 Subjects

Fifteen volunteers (9 male; 6 female) aged 28.6±4.5 yrs. with initial body weight (BW) 74.7±4.9 kg and body mass index (BMI) 25.3±1.4 kg/m^2 participated in this randomized cross-over study, once in the fed state (FED) and once after performing a 24-hour complete fast (FASTED). Subjects were free from any illnesses and medication that could have influenced the outcome of the experiment and met the following inclusion criteria: non-diabetic, non-smokers, not pregnant, weight stable for ≥6 months (±2 kg) and aged between 18 and 40 years. Only pre-menopausal women with a regular menstrual cycle (28-35 days) were recruited including those using oral contraceptives. Characteristics of subjects under control (fed) and experimental (fasted) conditions are presented in **Table 1**. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Ottawa Research Ethics Committee. Written informed consent was obtained from all subjects.

3.2. Design and Procedure

3.2.1 Screening visit

The study began during the initial (screening) visit to the laboratory, where informed consent was obtained and then height and BW were measured. Each subject was then asked to complete a questionnaire to indicate his or her favorite snack food and favorite fruit/or vegetable. For this decision, they were asked to circle five choices each from a comprehensive list of common products (chips, candies, cakes, and chocolate bars) and common fruits/vegetables, or if the choice was not on the list, they were asked to indicate what their favorite food items were. In this manner each subject would have his single favorite snack food and fruit/or vegetable as reinforcers for the RRV paradigm. Note that no subjects choose a vegetable as their preferred food item, so all non-snack foods were fruits. In order to ensure subjects liked the foods used in the LFPQ, each food item was presented with a likert rating scale from 1-5, 1 being “I do

not like at all" and 5 being "I like it a lot". Items rated at less than 3 were replaced with a food choice in the same category (e.g. high fat sweet replaced a high fat sweet food, etc.). Also, subjects were required to complete the Three Factor Eating Questionnaire (TFEQ) and the Sensitivity to Reward (SR) questionnaires. In order to minimize the hormonal effects on main outcomes, measurements for women were scheduled between days 1-5 of the menstrual cycle, where ovarian hormones are at their lowest levels (Davidsen, et al., 2007). As such, women had at least a 1 month period, and men at least a 2 week period between the FED and FASTED sessions.

3.2.2. Experimental manipulation of FED and FASTED states

Subjects arrived at 0730 fasted from 1900 the prior evening for the FED session. For the FASTED session, subjects arrived at 0730 and were fasted from 1200 the previous day; subjects were only allowed water during the 24 hours that defined the FASTED interval (1200-1200). Upon arrival BW was measured. For the FED session subjects were presented with a calorically-clamped breakfast at approximately 0900, which had to be consumed in 15 minutes. Appetite measures (VAS) were taken fasted, and then every hour after completing the breakfast meal. Similarly, food palatability was measured immediately after the standardized breakfast and lunch, and after the food reinforcers for the RRV computer task, and after completing the *ad libitum* dessert. This was followed by the computer task measuring RRV at 1100 (see **Figure 1**). The second computer task—the LFPQ—measured explicit liking and wanting for food and was employed once at ~1145, prior to eating lunch, and then once again immediately following lunch. A standardized (calorically-clamped) lunch was served at ~1200. At approximately 1230, after consuming lunch and after performing the post lunch LFPQ computer task, subjects were offered an *ad libitum* dessert buffet with a 30-minute time limit to eat as much or as little as desired. All sessions ended immediately after completing the buffet and the VAS.

3.3 Measurements

3.3.1. Anthropometric Measures

Height (HR-100 Height Rod; Tanita Corporation of America Inc. Arlington Heights, IL) and BW (HR-100; BWB-800AS, Tanita Corporation, Arlington Heights, IL.,

USA) were measured after a 12 hour overnight fast, after voiding, while wearing a standard hospital gown.

3.3.2. Questionnaires: TFEQ and SR

The TFEQ was administered during the initial screening process to determine the subject's individual attitude towards eating. This 51 item questionnaire is an instrument that assesses three important attitudes to food: chronic dietary restraint, disinhibition (the vulnerability to lose control and over-consume), and susceptibility to hunger (Stunkard, et al., 1985). The SR was assessed by one scale of the previously validated Sensitivity to Punishment and Sensitivity to Reward Questionnaire (Torrubia, et al., 2001). The SR scale comprises 24 forced-choice items reflecting the respondent's approach responses under various conditions of reward and is proposed to measure individual differences in reward sensitivity. The scale items reflect both the anticipation of reward (*e.g.* Does the good prospect of obtaining money motivate you strongly to do some things?) and pleasure experienced from rewarding activities (*e.g.* Does your attention easily stray from your work in the presence of an attractive stranger?).

3.3.3. Appetite and palatability: visual analogue scales (VAS)

Appetite ratings were measured using a pen and paper on a 150-mm visual analogue scale (VAS) adapted from Hill and Blundell (Hill, et al., 1984). Desire to eat, hunger, fullness and prospective food consumption (PFC) were rated using the following questions: 1) "How strong is your desire to eat?" (Very weak- Very strong); 2) "How hungry do you feel?" (Not hungry at all- As hungry as I have ever felt); 3) "How full do you feel?" (Not full at all- Very full), and 4) "How much food do you think you could eat?" (Nothing at all- A large amount). Hedonic measures of palatability were similarly measured immediately following the ingestion of the personalized snack and fruit reinforcers (~1045), and also immediately following each slice of pizza (~1200), and finally after the *ad libitum* dessert (~1300). The trapezoid method was employed to measure the area under the curve (AUC) for all appetite-related variables, as previously described (Drapeau, et al., 2007).

3.3.4. Standardized Breakfast and Lunch

During the FED condition subjects were presented with a standardized breakfast which had to be consumed in 15 minutes. The meal consisted of 2 pieces of whole

wheat toast (*D'Italiano*®, 147 kcal), 17 grams of peanut butter (*Kraft* Smooth Peanut Butter®, 101 kcal), 15 grams of raspberry jam (*Smuckers*®, 50 kcal), and 250 ml of water for a total of 298 kcal. At 1200 in the FED and FASTED sessions, subjects were presented with a standardized lunch, which had to be consumed in 30 minutes. The meal consisted of 2 slices of cheese pizza (*Michelina's* Zap 'Ems Gourmet®, 781 kcal). The *ad libitum* buffet consisted of 5 snack food items and 4 fruit/or vegetable items that were individualized—that is, the buffet items were previously indicated as being favourite food items on the food questionnaire from the RRV task. All items were presented on white foam plates and in standardized quantities (*e.g.* 70g potato chip serving, 100g apple serving, 100g candy bar serving, etc.). Subjects were allotted 30 minutes to eat as much or as little of the buffet as they wanted and were told they could request more servings of any item. All food items were weighed to the nearest 0.1 gram (Scout Pro SP2001; Ohaus Corporation) and the difference calculated and subsequently analyzed with Food Processor SQL software (version 9.6.2.; ESHA Research).

3.3.4. Food reward: RRV and LFPQ computer tasks

The reinforcing value of a stimulus can be objectively observed as the increased (or decreased) willingness—a quantitative measure—to work (button presses) at obtaining food points via a progressive ratio computer task to obtain a desirable food stimulus (vs. some alternative). Specifically, for this study, computer software was used to set up a split screen that alternated between two different choices (a healthy food and palatable snack food) and was navigated with a mouse/pad (see (Epstein, et al., 2012)). Food points were earned by selectively working for the food item of choice. The reinforcement schedule was set at variable ratio (VR2) for all 5 trials for the fruit (as follows: VR4, VR6, VR8, VR10, VR12); for the snack foods the schedule was also set as variable ratio, but the ratio for the response rate doubled each session (as follows: VR4, VR8, VR16, VR32, VR64). Subjects were told that it would be harder to obtain snack food points as trials progressed, but were not informed of the exact rate of change of the reinforcement schedule. Note that 1 point was equivalent to 1 gram of the snack or fruit. Prior to performing the RRV task subjects were required to ingest a "primer" of the foods they picked as their favourite—approximately a 50kcal portion of

the snack and fruit reinforcers. Each subject was then shown what each food portion would look like if they worked entirely for the snack food or entirely for the fruit; this was done to help control for reward expectation (Robbins, et al., 1996) and previous research has shown that a primer can increase the ability to show differences in food reinforcement (Reiss, et al., 1996).

The LFPQ is a computerised task designed to allow the separate and concurrent (paired) assessments of explicit liking and wanting and 'implicit wanting' for each stimulus from the same array of foods. This stimuli used in the task were photographic images of food varying in fat content (high or low) and taste (savoury or sweet). These dimensions can be separated into four categories: high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW), and low fat sweet (LFSW). Each of the four categories is represented by five food items for a total of twenty different food stimuli. For the explicit measures of liking and wanting, the 20 foods were rated according to 'how pleasant would you find the taste of this food right now?' and 'how much do want some of this food right now?'. This procedure has been extensively validated and can discriminate between appetitive states, individual phenotypes such as binge eaters (Finlayson, et al., 2011), and exposure to short term exercise (Finlayson, et al., 2009).

3.4 Statistical Analysis

To test for differences in anthropometric variables, appetite, *ad libitum* EI, palatability, RRV, and LFPQ across FED and FASTED sessions, repeated measures Analysis of Variance (ANOVAs) where time (FED vs FASTED) represented the within-subjects effects and sex represented the between subjects effects. Bivariate correlations were used to determine the strength of the relationship between the changes in food reward and the change in EI, and the relationship between TFEQ and SR trait variables and food reward and EI; partial correlations controlling for age and sex were then used to follow up significant relationships. Statistical analyses were performed using SPSS version 17 (Chicago, SPSS Inc.). Results are presented as means ± SD. All variables were normally distributed and effects were considered significant at $p<0.05$.

Results

As expected there were no significant changes in BW or BMI between FED and FASTED conditions (see **Table 1**). Mean scores (maximum and minimum in parentheses) for Three-Factor Eating Questionnaire (TFEQ) scores were 9 (±3.9) (4-17), 7.5 (±3.6) (2-13), and 7.6 (±3.0 (3-12), for dietary restraint, dietary disinhibition, and susceptibility to hunger, respectively; the mean Sensitivity to Reward (SR) score was 13.4 (± 3.8) (9-21).

There were significant differences (time effects) under the FASTED condition for all four measures of AUC (mm) for appetite (see **Table 1**). Relative to FED, *ad libitum* EI and all hedonic measures of palatability significantly increased under the FASTED condition. There were no significant sex or time x sex effects for variables of appetite or food hedonics (see **Table 1**).

As indicated by higher mean snack points ($p<0.001$) and higher mean snack responses ($p<0.05$) for the RRV task, significant time effects were noted for the RRV measure of food reward where preferred snack food was more reinforcing under the FASTED compared to the FED condition (see **Table 2**). There were no significant sex or time x sex effects for either RRV measure.

There were significant time effects as indicated by increases in the explicit liking scores from FED to FASTED in the pre-meal LFPQ scores for the following: HFSA ($p<0.05$) and LFSW ($p<0.05$). Similarly, there were significant time effects for post-meal scores as indicated by increases in the explicit liking scores from FED to FASTED for the following: HFSA ($p<0.05$), HFSW ($p<0.01$), and LFSW ($p<0.05$) (see **Table 2**). In the FED condition there were significant across-meal decreases in explicit liking for all four food categories: HFSA ($p<0.05$), LFSA ($p<0.001$), HFSW ($p<0.001$), and LFSW ($p<0.001$). In the FASTED condition there were only decreases in explicit liking for HFSA ($p<0.001$) and LFSA ($p<0.001$), while no significant decreases were noted for the two sweet categories (see **Figure 2**). There were significant time effects as indicated by increases in all the explicit wanting scores for each specific food category from FED to FASTED in the pre-meal LFPQ scores: HFSA ($p<0.05$), LFSA ($p<0.001$), HFSW ($p<0.005$), and LFSW ($p<0.05$). Similarly, significant time effects were noted for the post-meal explicit wanting rating from FED to FASTED, showing increases for each category: HFSA ($p<0.01$), LFSA ($p<0.05$), HFSW ($p<0.005$), and LFSW ($p<0.01$) (see

Table 2). The only noted sex effect for the LFPQ variables was for post-meal wanting of HFSA, where men wanted HFSA foods more than women post-meal; there were no time x sex interactions for any LFPQ variables.

Bivariate correlations revealed that the noted increase in *ad libitum* EI under the FASTED condition was positively correlated with the change in the AUC appetite score for desire to eat ($r=0.55$, $p=0.04$). Partial correlations controlling for sex revealed: that TFEQ disinhibition scores were positively correlated with snack points earned under the FED condition ($r=0.56$, $p<0.05$); and that under the FASTED condition, disinhibition was positively correlated with snack points ($r=0.55$, $p<0.05$) and snack responses ($r=0.52$, $p<0.05$).

Discussion

This study demonstrated evidence of strong acute changes in appetite motivation and in the rewarding qualities of preferred foods after a 24-hour fast. The increased appetite scores for AUC desire to eat were significantly related to increased FASTED EI. In addition, dietary disinhibition was positively related to snack points earned and to higher responses made for the energy dense snack items in the RRV task. Food was rated with higher post-ingestive subjective liking scores (VAS). In the FASTED condition food images were perceived both as more liked and wanted both pre and post lunch, indicating alliesthesia for orosensory and visual stimuli. Across-meal measures of the LFPQ for liking in the FASTED condition demonstrated that sweet foods maintained a strong hedonic saliency by failing to show the decrease in liking scores from pre- to post-meal noted in the FED condition (i.e. lack of negative alliesthesia). Also, the results from the LFPQ were quite consistent with the observed increase in the RRV of palatable energy dense foods. This indicates that the acute overcompensation from short term fasting may arise from a heightened reward saliency as well as from an increased hunger motivation. The LFPQ and the RRV indicate that fasting changes the direction of eating towards sweet and energy dense foods. Both of these would signal the presence of calories to compensate rapidly for the energy lost in fasting. Consequently, the changes observed would be biologically functional.

There are few studies that have looked at the acute impact of complete fasting on appetite and *ad libitum* EI. Intuitively, one would anticipate that energy deprivation

results in increased hunger ratings. But evidence suggests that as the fasting period is carried over from hours to days the appetite response can normalize to levels previously noted in the fed state after approximately 4-5 days of fasting (Lappalainen, Sjoden, et al., 1990). Similar seemingly discrepant results have been found with less dramatic methods of energy deprivation—namely very low calorie diets—where on average subjects reported decreased appetite during the intervention--described as “less food less hunger” (Wadden, et al., 1987). In a separate study with a fasting period (19 hours) comparable to our design, subjects without clinical pathology did not eat significantly more when allowed access to *ad libitum* feeding (Hetherington, et al., 2000). Compared to FED, the appetite response to the current 24-hour fast significantly influenced all four AUC appetite measures. Most affected was hunger, which increased by 69%, followed by 50% and 30% increases in PFC and desire to eat, respectively, and a 47% decrease in fullness. In other words, this level of fasting (not surprisingly but contrary to some observations) markedly increased the motivation to eat. After 24 hours of fasting we demonstrate a significant 30% increase in mean *ad libitum* EI (kcal) that was positively correlated with increased AUC desire to eat scores. A similar study found significantly increased appetite scores following a 36-hour fast but they noted that when *ad libitum* feeding was reinstated there was only a 20% increase in EI, and this was unrelated to the changes in appetite (Johnstone, et al., 2002).

An interesting feature is the evidence for positive alliesthesia for preferred food stimuli —subjects rated palatability of the dessert significantly higher under the FASTED condition even after eating 30% more energy (see **Table 1**). Corroborating these results are findings from deprivation periods of much shorter duration. By manipulating the period of energy deprivation with two separate test days, one day with a 3.5 hour period of deprivation and another with an overnight fast of approximately 12-15 hours, Speigel et al. (1989) found that the deprivation period influenced palatability, consistent with the concept of alliesthesia. A strong feature of the current findings lies in the observation that although neither the macronutrient nor the item content of the dessert buffet were controlled between subjects, there were no significant differences in percent fat or carbohydrate intake between the *ad libitum* EI in the FED and FASTED conditions (see **Table 1**). This outcome is comparable to controlling for macronutrient intake and

allows for a clearer understanding of overall changes in liking, either measured by VAS or by the LFPQ, (not contaminated by changes in food preferences) thereby permitting a closer correspondence with previous work on alliesthesia.

Another interesting feature is whether differences in underlying traits (*e.g.* TFEQ or SR) or differences in food reward can be attributed to the energy deprivation or to the noted increase in motivation and palatability. The RRV task employed in the current study demonstrated that relative to FED, there were FASTED increases in the reinforcing value of palatable food as evidenced by significantly higher points earned and responses made for the snack item versus the preferred fruit (see **Figure 2**). To our knowledge there is only one other study using the RRV task under comparable levels of energy deprivation; after ~13 hours of fasting food also became more reinforcing for normal weight unrestrained females (Raynor & Epstein, 2003). What is interesting is the recognition that this increase in the reinforcing value of food can predict *ad libitum* EI, independently of rated liking or hedonic ratings of foods (Epstein, et al., 2011; Epstein, Temple, et al., 2007). Furthermore, we have replicated recent findings that trait scores for TFEQ disinhibition are related to food reinforcement (Epstein, et al., 2012). Although snack foods were significantly more reinforcing under the FASTED condition, whereby subjects increased the number of button-pushes (responses) to obtain more of the palatable snack item, the current study found no indication that this increased desire or motivation to obtain more energy dense foods impacted the increased *ad libitum* EI also noted under the FASTED condition.

The LFPQ is different from the RRV task in that it allows the separate and concurrent assessments of explicit liking and wanting for the same target stimuli (Finlayson, et al., 2007a). In our sample, explicit wanting and liking of food images increased under the FASTED relative to FED condition. , Both pre-meal scores and post-meal scores significantly increased for food categories (the exception being LFSW). In previous work with the LFPQ measuring wanting and liking, once in a hungry or satiated state (Finlayson, et al., 2008a), it was shown that liking for sweet foods did not decrease as much as that for fat foods. A comparable effect was seen in this study in the FED condition. However under the FASTED condition only savory foods decreased in liking (negative alliesthesia), while sweet foods maintained significantly

heightened hedonic liking from pre- to post-lunch meal (see **Figure 2**). That is, the preference for sweet foods is conserved when satiety is imposed on a fasted state. Limited data on alliesthesia to food images exists; no effect of deprivation on ratings of liking has been reported (Drobes, et al., 2001), while others have reported higher liking ratings under hungry versus sated subjects (Jiang, et al., 2008; Rolls, et al., 1983; Stoeckel, et al., 2007; Uher, et al., 2006). More research is needed to clarify the role that sex has on these evaluations, and how the degree of deprivation may differently impact responses to food or food-related cues.

Limitations for the current study include the fact that the fasting period was out of the laboratory and therefore may have provided opportunities for lack of compliance for the fasting protocol. Also, there was a relatively small number of subjects and statistical power may have been a little low, especially for the gender comparisons. Strengths of the study are the within subjects randomized crossover design, the fact that women were tested in the same phase of the menstrual cycle (not easy to accomplish), and that the food items were personalized.

As a result of a 24-hour complete fast, preferred foods were both wanted and liked significantly more than in the fed state. Scores for appetite were dramatically affected, pushing up hunger, desire to eat and PFC, while simultaneously attenuating fullness; also, the change in AUC score for desire to eat was positively related to *ad libitum* EI under the FASTED condition. Higher sensitivity to reward and disinhibition scores correlated with responding for palatable snack food stimuli in the RRV task, further indicating that RRV has strong ties with impulsivity. Although there was a 30% increase in EI under the FASTED condition, positive alliesthesia was demonstrated by heightened hedonic ratings following this much larger eating episode compared to the FED condition. The results from the LFPQ were in agreement with the abovementioned finding, whereby after 24 hours of fasting, foods increased in explicit wanting and liking, and there was evidence of a lack of negative alliesthesia under the fasted condition specifically to the sweet category of taste. Under the conditions of this study, a 24 hour complete fast, following equivalent re-feeding, maintained an influence of the need state on homeostatic and hedonic aspects of appetite control. Such changes make self-control of eating difficult for dieters.

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Table and Figure Legends

Table 1. Subjects' characteristics (mean ± SD) under FED (control) and FASTED (experimental) conditions.

Table 2. Food reward as measured by the Relative-Reinforcing Value (RRV) of food paradigm (i.e. snack points and snack responses) and as measured by the Leeds Food Preference Questionnaire (LFPQ) paradigm (i.e. explicit liking and wanting of food images). Following the ingestion of a 50kcal primer of a preferred snack and preferred fruit, RRV was measured at one time point. Explicit liking and wanting were measured once prior to ingestion a standardized test meal and once again immediately following the test meal.

Figure 1. Study protocol. Note that in order to perform the 24 hour fast under the FASTED condition, subjects did not eat a standardized breakfast; the fasting period was slightly less than 24 hours due to the standardized consumption (100kcal: 50 kcal snack and 50 kcal fruit) of the primers used in the RRV task.

Figure 2. Results from the LFPQ computer task for explicit liking FASTED and FED conditions showing the change in across-meal mean liking score (± SD) for each of the four food categories: high fat savory (HFSA), low fat savory (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW). In the FED state (panel **A**) across-meal liking for all four categories deceased, indicating negative alliesthesia; under the FASTED condition (panel **B**) only for sweet foods was there a lack of negative alliesthesia.

Table 1.

Characteristics	FED	FASTED	Time	Sex	Time x Sex
			P value		
Anthropometric					
Body Weight (kg)	74.4±4.9	74.2±4.9	0.12	0.10	0.60
BMI (kg/m^2)	25.2±1.4	25.0±1.4	0.09	0.56	0.69
Appetite (AUC)					
Desire to Eat	555.1±316.9	719.2±123.3	0.05	0.32	0.79
Hunger	415.6±169.5	703.5±121.8	0.001	0.36	0.08
Fullness	560.5±44.4	295.6±100.1	0.001	0.32	0.12
PFC	492.4±43.5	741.6±131.5	0.001	0.14	0.75
Food Hedonics					
Snack	120.5±7.6	135.1±4.9	0.005	0.70	0.94
Fruit	112.3±7.1	130.9±5.0	0.02	0.25	0.44
Pizza 1	107.9±29.9	119.6±23.9	0.05	0.90	0.98
Pizza 2	95.6±37.2	118.6±28.9	0.005	0.90	0.20
Dessert	121.1±8.6	132.2±4.9	0.06	0.67	0.91
AUC	436.6±79.4	502.6±68.8	0.001	0.58	0.26
Ad Libitum EI					
Total EI (grams)	300.0±42.4	388.8±55.2	0.001	0.021	0.30
Total EI (kcal)	491.1±99.6	854.9±104.6	0.001	0.003	0.35
Energy Density	1.65±0.2	2.4±0.2	0.002	0.55	0.76
%Sugar	17.6±1.9	29.0±7.5	0.06	0.19	0.71
%Fat	6.5±1.1	12.2±3.7	0.21	0.42	0.35
%Protein	2.4±0.35	4.7±9.0	0.05	0.76	0.26

Area under the curve (AUC) was calculated with the trapezoid method including all variables under the Food Hedonics category. Note that PFC is prospective food consumption; EI is energy intake in kilocalories (kcal); and Energy Density is kcal/gram

Table 2.

Characteristics	FED	FASTED	Time	Sex	Time x Sex
			P value		
RRV					
Snack Points	16.9±9.3	25.6±12.4	0.008	0.90	0.26
Snack Responses	381.9±202.2	613.5±344.3	0.03	0.99	0.14
Pre-Meal Liking					
HFSA	54.4±18.6	67.5±16.4	0.03	0.18	0.68
LFSA	48.8±18.1	62.1±14.1	0.07	0.90	0.34
HFSW	61.8±23.6	70.6±19.1	0.16	0.43	0.50
LFSW	55.2±17.3	64.4±18.2	0.04	0.16	0.36
Post-Meal Liking					
HFSA	29.7±20.6	43.9±17.6	0.02	0.07	0.74
LFSA	24.8±15.8	34.1±18.2	0.08	0.14	0.73
HFSW	50.6±27.6	67.3±18.8	0.008	0.43	0.28
LFSW	44.3±21.4	56.3±17.9	0.01	0.96	0.46
Pre-Meal Wanting					
HFSA	49.8±18.8	68.9±18.2	0.015	0.44	0.72
LFSA	45.2±19.1	64.2±13.9	0.008	0.97	0.29
HFSW	50.3±22.2	69.1±20.4	0.004	0.29	0.25
LFSW	47.6±15.1	59.9±17.9	0.02	0.26	0.38
Post-Meal Wanting					
HFSA	21.8±20.5	40.2±22.1	0.006	0.012	0.72
LFSA	18.7±16.9	31.6±20.3	0.018	0.06	0.82
HFSW	38.3±23.4	62.8±22.0	0.002	0.14	0.95
LFSW	34.7±18.9	52.9±18.9	0.001	0.70	0.17

Note: HFSA=high fat savory; LFSA=low fat savory; HFSW=high fat sweet; and LFSW=low fat sweet.

Figure 1.

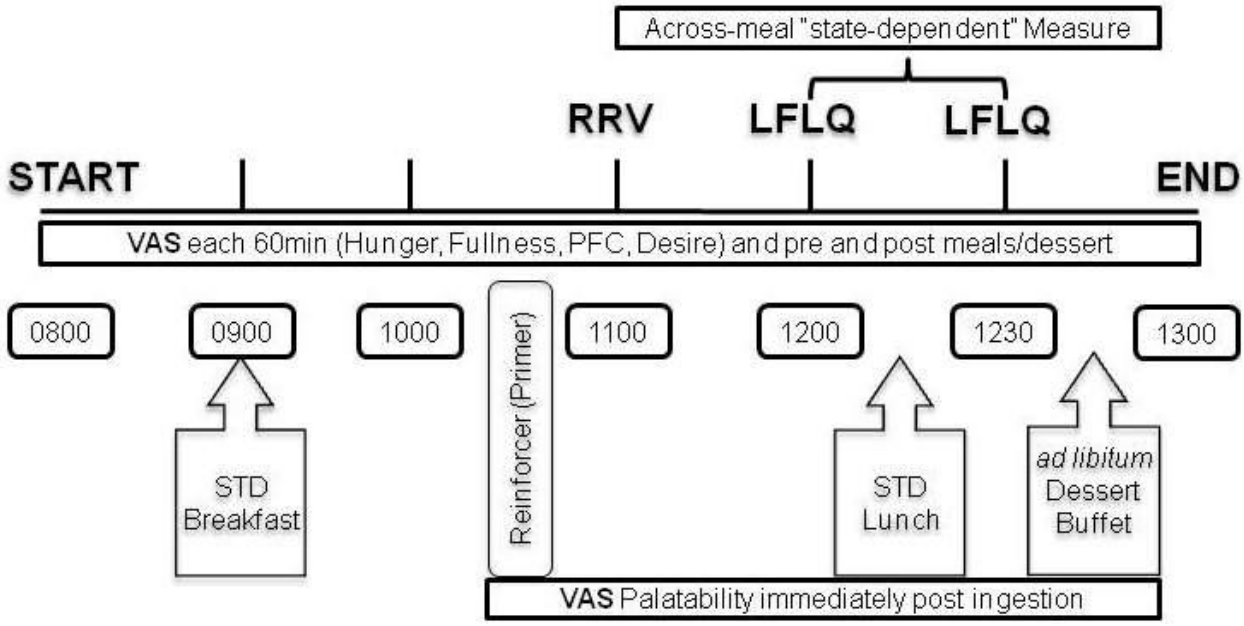
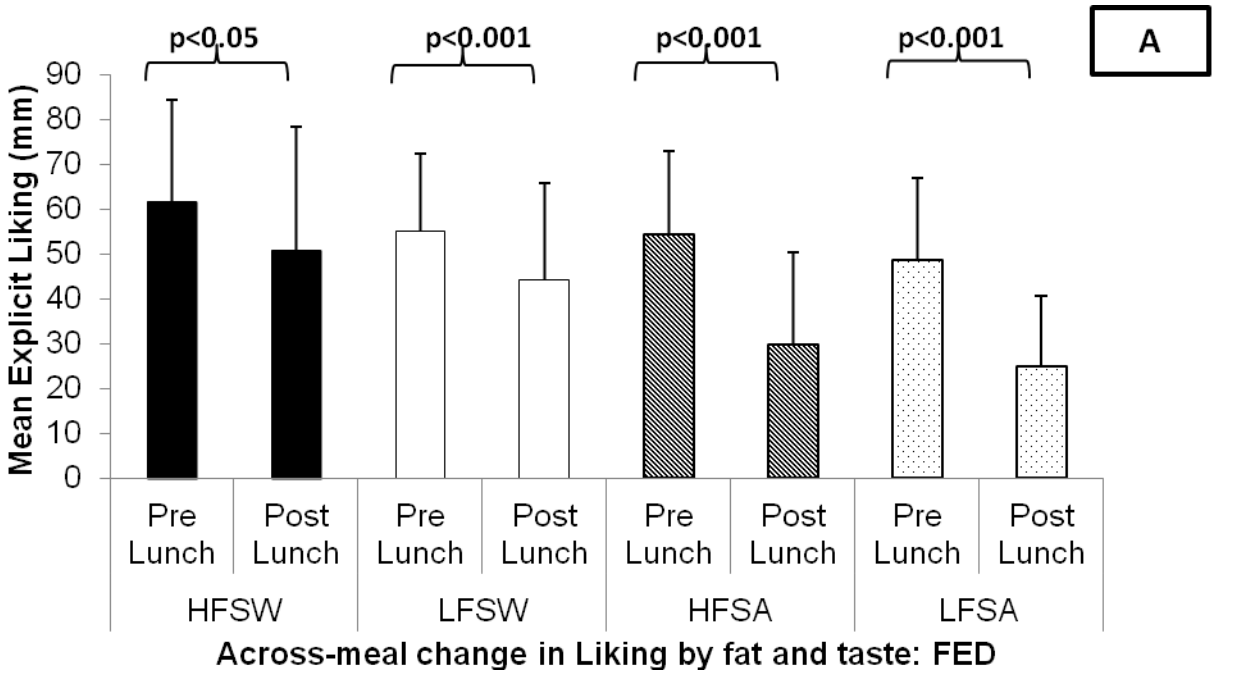
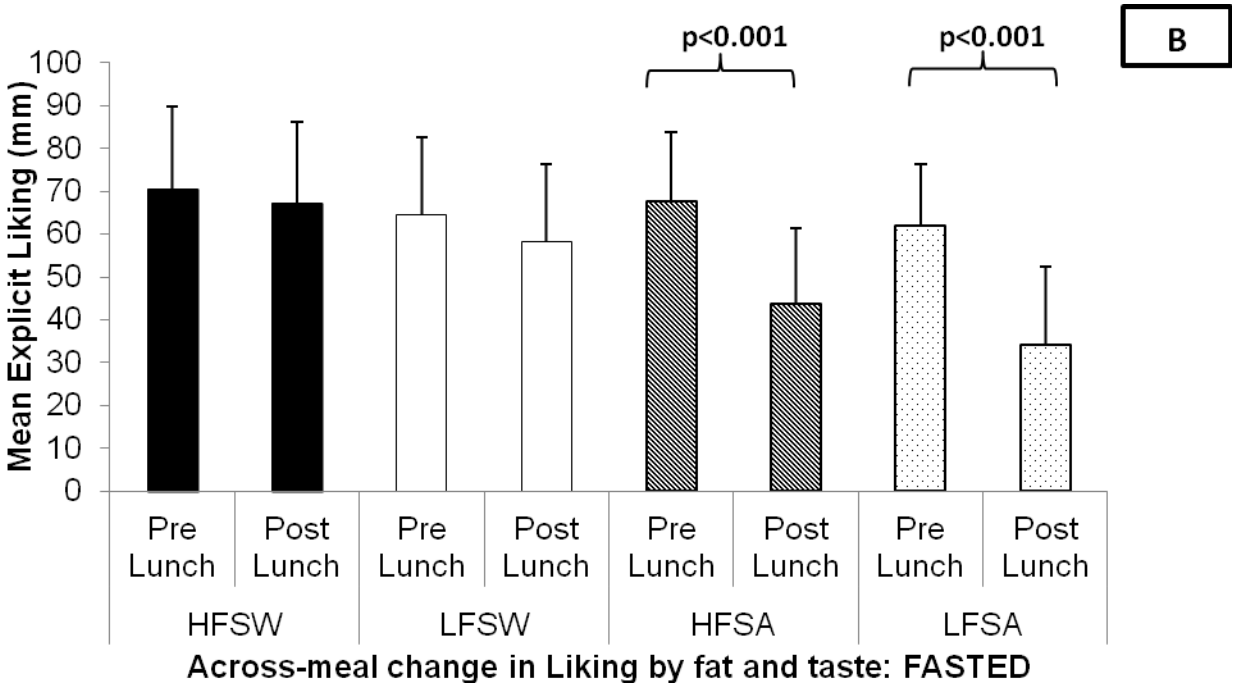

Across-meal "state-dependent" Measure
RRV
LFLQ
LFLQ
START
END
VAS each 60min (Hunger, Fullness, PFC, Desire) and pre and post meals/dessert
0800
0900
1000
1100
1200
1230
1300
STD Breakfast
Reinforcer (Primer)
STD Lunch
ad libitum Dessert Buffet
VAS Palatability immediately post ingestion

Figure 2.


A
p<0.05
p<0.001
p<0.001
p<0.001
Mean Explicit Liking (mm)
90
80
70
60
50
40
30
20
10
0
Pre Lunch
Post Lunch
Pre Lunch
Post Lunch
Pre Lunch
Post Lunch
Pre Lunch
Post Lunch
HFSW
LFSW
HFSA
LFSA
Across-meal change in Liking by fat and taste: FED


B
p<0.001
p<0.001
Mean Explicit Liking (mm)
100
90
80
70
60
50
40
30
20
10
0
Pre Lunch
Post Lunch
Pre Lunch
Post Lunch
Pre Lunch
Post Lunch
Pre Lunch
Post Lunch
HFSW
LFSW
HFSA
LFSA
Across-meal change in Liking by fat and taste: FASTED

Chapter VII: Article IV

Running Head: Deprivation by diet alone or by aerobic exercise alone: how modality of an acute intervention can differently impact olfaction, food reward, *ad libitum* feeding, and appetite hormones.

J.C. performed all the data collection and J.C., M-E.R., G.G., G.F, J.B. and E.D. were involved in the elaboration of the experimental design and collaborated to write the manuscript. Claudia Casimiro performed the assays for leptin and ghrelin.

DEPRIVATION BY DIET ALONE OR BY AEROBIC EXERCISE ALONE: HOW MODALITY OF AN ACUTE INTERVENTION CAN DIFFERENTLY IMPACT APPETITE HORMONES, OLFACTION, FOOD REWARD, AND *AD LIBITUM* FEEDING.

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Keywords: Olfaction Sniffin' Sticks Diet Exercise Energy Deprivation Food Hedonics Food Reward

Abstract

Introduction: There is a paucity of evidence regarding whether modality of acute energy deprivation can differently impact smell function, food reward, and *ad libitum* feeding. It is also unclear if modality of energy deprivation may differently impact changes in hormones involved in appetite.

Methods: Ten male subjects aged 23.7±5.1 yrs. with initial body weight 83.2 ± 11.5 kg were included in this repeated-measures cross-over design. They were tested before (CON1) and after 3 days (CON4) under normal feeding conditions; after randomization subjects performed 2 acute -25% daily needs energy deficits induced by diet only (DER) or by exercise only (DEX), and tested before (DER1 and DEX1) and after 3 days (DER4 and DEX4) of intervention. The repeated measures were CON4, DER4, and DEX4. Body weight, smell performance (*Sniffin Sticks*), relative-reinforcing value of food (RRV; computer task), and intrinsic/extrinsic wanting and liking (LFPQ; computer task), and plasma concentrations of leptin and ghrelin (ELISA) were measured on all test days. Body composition (DXA), *ad libitum* energy intake (EI) (buffet), and palatability (visual analogue scale) were measured only on CON4, DER4, and DEX4.

Results: Body weight and FFM significantly decreased from CON4 to DER4. For the repeated measures, relative to CON4 there were improvements in the DER4 session for: odour threshold (7.6±1.2 vs. 8.75±1.0) and total odour scores (TDI) (33.8±2.7 vs. 35.9±2.4); relative to CON4, the DEX4 session had improvements only in odour threshold score (7.6±1.2 vs. 8.3±1.6). Relative to CON4 there was a significant increase in snack points earned in both the DER4 (15.5±4.3 vs. 24.7±6.5) and DEX4 (15.5±4.3 vs. 35.6±4.8) sessions; subjects earned significantly more snack points in the DEX4 session relative to DER4. There were DER4 increases in EI (654±216 vs. 1186±313 kcal) and palatability (402.6±56.9 vs. 467.1±61.1mm: area under curve) when compared to CON4; relative to CON4, palatability increased under DEX4 (402.6±56.9 vs. 482.0±46.0mm). EI was higher for DER4 (1186±313 vs. 713±176 kcal) compared to DEX4. There were no significant effects for condition or time for plasma concentrations of leptin or total ghrelin. Correlations indicated that the improvements in TDI from CON4 to DER4 were related to increased EI (r=0.67). All results presented are significant at $p<0.05$.

Conclusion: These data show that independent of modality that acute deprivation can impact smell function.

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Conflict of Interest: None Disclosed.

Introduction

All else being equal, interventions either decreasing energy intake (EI) or increasing energy expenditure (EE) (or both) should produce a negative energy balance and result in weight loss. Commensurate with sustained energy deprivation—acute or chronic—there are varying, and often graded, responses that the human body experiences: changes in metabolic, autonomic, neuroendocrine, and behavioural factors, all of which define how each individual differently responds to a theoretically similar absolute level of energy deprivation. As an example, a recent carefully controlled 12 week weight loss program of five days per week working on a treadmill at 500kcal (2093kJ) per session found that individual variability in weight loss was between -14.7 to +2.7kg (Caudwell, et al., 2009). This finding highlights the fact that there are both physiological and psychological—or psychobiological (Blundell, 1992; Harshaw, 2008)—differences that may emerge when individuals, for example, are further examined for differences in appetite, feeding-related peptides, olfaction, and the rewarding value of food. Thus there needs to be a more integrated approach when attempting to discern the underlying changes that accompany sustained energy deprivation.

Olfaction plays an integral role in locating food sources, determining which food items are acceptable to consume, and largely contributes to the sense of taste. Although research has suggested a role for energy deprivation in the modulation of smell function (Albrecht, et al., 2009; Goetzl & Stone, 1947), it remains to be determined how this may impact feeding and overall body weight status. For example, using the well validated Sniffin' Sticks (Hummel, et al., 2007) to measure odour detection threshold, odour discrimination, and odour identification it was shown that individuals demonstrated higher (better) scores after 12 hours (Stafford & Welbeck, 2011) and 24 hours (Cameron, et al., 2012b) fasting versus in the fed state, but there was not a relationship with increased *ad libitum* energy intake (Cameron, et al., 2012b). It remains to be determined, however, with respect to olfaction, what role is played by the underlying metabolic changes that occur with energy deprivation, and whether

measured changes in smell function can impact feeding and appetite differently when a deprivation is induced by dieting or by exercise.

According to the “incentive salience hypothesis” (Berridge, et al., 2009), there is a change at the neurological level that describes perceptual (*e.g.* cognitive) and motivational (*e.g.* unconscious) components that accompany the shift from a stimulus being neutral to something that is attractive and can energize and motivate behavior. One method that has been designed to examine explicit “liking”/ “wanting” and implicit “wanting” of food is the recent development of the Leeds Food Preference Questionnaire (LFPQ) (Finlayson, et al., 2007b). This LFPQ task presents photographic food stimuli of 20 different items varying along two dimensions—fat (high or low) and taste (savoury or sweet). Implicit “wanting” is measured by the speed with which one stimulus is chosen in preference to an alternative and is additionally measured by relative preference (*e.g.* fat vs. sweet). Laboratory choice paradigms can also be employed to measure the relative-reinforcing value (RRV) of various foods by discriminating the amount of work done to obtain food when offered a choice between other food or non-food items (Lappalainen & Epstein, 1990). Thus under experimental conditions which potentially alter the motivation to eat it can be determined if a food stimulus becomes more or less reinforcing—the more work done to acquire the item, the more reinforcing it is said to be (Epstein & Leddy, 2006). Taken together, examining the wanting and liking and the reinforcing efficacy of foods—collectively operationalized henceforth as food reward—may provide a framework to understand factors influencing food choice and *ad libitum* EI, in the context of different modalities of energy deprivation.

A recent study employing the LFPQ task examined the impact of energy deprivation by means of exercise on food reward and *ad libitum* EI with two sessions, one at 50 minutes of high intensity exercise and then other without exercise (Finlayson, et al., 2009). Although there was no significant difference in the amount of food eaten by test day, it was found that after the exercise session there was a subgroup of “compensators” who ate more after exercise and also displayed a significant increase in “wanting”; specifically, after exercise there was an unconscious *implicit* desire for the

"compensators" to eat high-fat sweet foods (Finlayson, et al., 2009). The main findings from a separate study noted that in a hungry state (3-4 hours acute energy deprivation) subjects "wanted" fat (particularly high fat) and savoury food more so than fat and sweet food, but this trend was reversed and subjects "wanted" low fat sweet after the completion of an *ad libitum* pizza lunch (Finlayson, et al., 2007b). Although to our knowledge there are no studies looking at RRV and exercise, results from an acute energy deprivation ranging in the time of ~13-20 hours in lean individuals indicated that in this relatively short period of deprivation the RRV of a palatable snack food (versus a sedentary activity) significantly increased from the fed state (Raynor & Epstein, 2003), and a positive relationship between the reinforcing value of food and energy intake has also been established (Epstein, Temple, et al., 2007; Epstein, Wright, Paluch, Leddy, Hawk, Jaroni, Saad, Crystal-Mansour, & Lerman, 2004).

The objectives of the current study were to examine, using a psychobiological systems approach, how modality of an acute 3 day energy deprivation differently impacts, olfaction, food reward, *ad libitum* feeding, and appetite hormones. It was hypothesized that independent of modality of deprivation that relative to the control there would be, improvements in smell performance, increased food reward and *ad libitum* feeding, and a decline in leptin and increase in ghrelin. It was also hypothesized that the increased food reward would prove to be a predictor of *ad libitum* EI and that relative to the deprivation by aerobic exercise alone, the deprivation by diet alone would produce the largest changes in appetite and *ad libitum* EI.

Methods

3.1 Subjects

Ten male volunteers aged 23.7±5.1yrs with initial body weight (BW) of 83.1±11.6kg, and BMI 25.1±2.4kg/m^2 participated in this study. Subjects were free from any illnesses that could have influenced the outcome of the experiment and met the following inclusion criteria: non-diabetic, non-smoker, weight stable for ≥6 months (±2 kg), no medications, and aged between 18 and 40 years. Characteristics of subjects after each of the study arms (CON4, DER4, and DEX4) are presented in **Table 1**. This study was conducted according to the guidelines laid down in the Declaration of Helsinki

and all procedures involving human subjects were approved by the University of Ottawa Research Ethics Committee. Written informed consent was obtained from all subjects.

3.2. Design and Procedure

3.2.1 Screening visit and Start of Interventions

This was a crossover study consisting of 3 arms: first there was a control arm (CON) after which subjects were randomly assigned to one of the following: deprivation by diet alone (DER) arm, or the deprivation by aerobic exercise alone (DEX) arm. The study began during the initial (screening) visit to the laboratory, where informed consent was obtained and then height and body weight (BW) were measured. Each subject was then asked to complete a questionnaire to indicate his or her favorite snack food and favorite fruit/or vegetable. For this decision, they were asked to circle five choices each from a comprehensive list of common products (chips, candies, cakes, and chocolate bars) and common fruits/vegetables, or if the choice was not on the list, they were asked to indicate what their favourite food items were. In this manner each subject would have his single favourite snack food and fruit/or vegetable as reinforcers for the "relative-reinforcing value of food paradigm" (RRV). Subjects were presented with photocopies of the food images to be used in the Wanting and Liking (LFPQ) computer task and required to rate, on a five point likert scale, how much they like each of the 20 foods used in the test. A score below 3 would lead to the replacement of the food item with another self-selected item (this did not occur in this sample). Also, subjects were required to complete the Three Factor Eating Questionnaire (TFEQ) and the Sensitivity to Reward questionnaire (SR). Subjects were then scheduled for the V02max test which was required to determine the work rate that would be performed during DEX in order to produce an individualized negative energy balance of 25%. Following the V02max test, subjects were booked to return to the lab for the first of three arms of the study (CON, DEX or DER).

The first arm of the study was CON, consisting of a day 1 visit to the lab (i.e. CON1) and then returning to the lab for the repeated measure on day 4 (i.e. CON4; see **Figure 1**) after 3 consecutive days of eating *ad libitum* from food supplied from our validated food menu (McNeil, et al., 2012) and having worn an accelerometer to

measure components of energy expenditure. Upon arrival for CON1, at approximately 0800 BW and a blood draw were taken while fasted, and subjects were then fed the standardized breakfast. Approximately one hour later smell function was assessed, followed by the RRV and LFPQ computer tasks. Subjects were sent home at approximately 1030 with three large coolers, each filled with one day's worth of self-selected food and drink, and given instruction to eat as they normally would on an average day—but exclusively from the coolers. Along with the indirect calorimetry measure of resting energy expenditure taken at CON4, the accelerometry data from CON was used to calculate total energy expenditure (REE+PAEE*10%) from which the individual -25% deprivation for the DER and DEX arms were then calculated. Depending on the randomization, the second arm of the study was either the DER or DEX intervention.

Day 1 of the DER and DEX interventions each started at approximately 0800 and upon arrival BW and a blood draw were taken. Similar to CON1, subjects were then fed the standardized breakfast, and after an hour, the assessments of smell function, RRV, and LFPQ were conducted. The completion of these tests marked the end of testing for DER1 and subjects were sent home with their three coolers representing the foods to be eaten outside of the lab until the return on day 4. For DEX1, however, subjects remained in the laboratory to further perform the aerobic exercise equivalent of a 25% daily deprivation at 50% of their V02max value measured during the screening visit. Subjects were required to perform this supervised physical activity for three consecutive days in the laboratory during the DEX arm. Briefly, all subjects were required to walk on a treadmill at 50% of their V02max—each had a different pre-determined time that represented the work interval required to produce the 25% energy deficit. The 25% deprivation is relative to the amount of energy intake (EI) and energy expenditure (EE) that would otherwise keep each individual in relative energy balance. After the DEX1 exercise session, the following 2 days of exercise sessions were booked and subjects were sent home with three coolers as previously described. In order to control for the weight loss between the 2 experimental conditions (DER and DEX), a wash out period

of a minimum of 2 weeks was scheduled; to control for the possible carry-over effects of the two experimental sessions.

3.2.2. Experimental conditions: CON4, DER4 and DEX4

For day 4 of each of the three repeated measures days subjects arrived at 0800 fasted from 1900 the prior evening. Body weight was taken, immediately followed by a fasted blood sample. Following the blood draw body composition was measured by a DXA scan. At approximately 0900 the standardized (calorically-clamped) breakfast was served which was required to be completed in 15 minutes. Appetite measures (VAS) were taken fasted, and then every hour after completing the breakfast meal. Similarly, food palatability was measured immediately after the standardized breakfast and lunch, and after the food reinforcers for the RRV computer task, and after completing the *ad libitum* dessert. Nasal chemosensory performance was measured at 1000 and this was followed by the computer task measuring RRV at 1100 (see **Figure 1**). The second computer task—the LFPQ task—measured the implicit/explicit wanting and liking of foods and was employed once at 1145, prior to eating lunch, and then once again immediately following lunch. A standardized (calorically-clamped) lunch was served at ~1200. At approximately 1230, after consuming lunch and after performing the post lunch LFPQ computer task, subjects were offered an *ad libitum* dessert buffet with a 30 minute time limit to eat as much or as little as desired. All sessions ended immediately after completing the buffet and the VAS.

3.3 Measurements

3.3.1. Anthropometric measures

Height (HR-100 Height Rod; Tanita Corporation of America Inc. Arlington Heights, IL) and BW (HR-100; BWB-800AS, Tanita Corporation, Arlington Heights, IL., USA) were measured after voiding and while wearing a standard hospital gown. Body composition was determined using dual-photon x-ray absorptiometry (DXA) (Lunar Prodigy, General Electric, Madison, WI, USA). Coefficient of variation and correlation for DXA was 1.8% (R = 0.99) as determined in 12 healthy subjects in our laboratory.

3.3.2. Questionnaires: TFEQ and SR

The TFEQ was administered during the initial screening process to determine the subject's individual attitude towards eating. This 51 question questionnaire is an instrument that quantitatively assesses three important attitudes to food: chronic dietary restraint, disinhibition (the vulnerability to lose control and over-consume), and susceptibility to hunger (Stunkard & Messick, 1985). The SR was assessed by one scale of the previously validated Sensitivity to Punishment and Sensitivity to Reward Questionnaire (Torrubia, et al., 2001). The SR scale comprises 24 forced-choice items reflecting the respondent's approach responses under various conditions of reward and is proposed to measure individual differences in impulsivity. The scale items reflect both the anticipation of reward (*e.g.* Does the good prospect of obtaining money motivate you strongly to do some things?) and pleasure experienced from rewarding activities (*e.g.* Does your attention easily stray from your work in the presence of an attractive stranger?).

3.3.3. V02max and treadmill exercise for DEX

In order to precisely determine the length of the exercise session to produce the 25% daily energy deprivation for DEX, an aerobic capacity test was performed. The test followed a "ramped high" protocol with a consistent and continuous increase in speed and grade, from 2.6km/hr at 5% grade, to a maximum of 9.6km/hr at a 22% grade. Heart rate was recorded continuously during the test and blood pressure was measured at the end of each stage of the test. The Borg scale (Borg, 1982) was used to measure perceived exertion across all stages of the test. Expired gases were continuously collected and oxygen and carbon dioxide concentrations were determined by using the Vmax system (Sensormedics, Loma Linda, CA). We used specific criteria to determine whether subjects had achieved V02max: 1) predicted maximal heart rate was reached; 2) oxygen consumption remained stable or decreased with an increase in workload; 3) the Borg scale reached 19 or 20. For the three consecutive days of supervised treadmill walking required for the DEX arm, subjects were outfitted with a heart rate monitor (POLAR; Polar Electro, Kempele, Finland) to ensure that the exercise was performed at a fixed intensity. To accomplish this, the monitor was set to ±10 beats of the heart rate measured at 50% of each subject's V02max; when the heart rate fell outside if this

range an alarm would sound to notify then need to accordingly adjust the treadmill speed either up or down. Note that the average V02max for the sample was 52.5±5.6 ml/kg/min, while the average energy equivalent to produce the 25% deprivation was 702±86kcal (2939kJ) and the average time walking on the treadmill of 63.5±8.4mins.

3.3.2. Blood sampling: leptin and ghrelin measurement

Upon arrival at the laboratory at day 1 and at day 4 under all three testing conditions (CON, DER and DEX) a single blood sample (for a total of 6 blood samples) was obtained from the antecubital vein of the non-dominant arm into a tube containing EDTA. Blood samples were centrifuged at 3500 rpm at 4^0C immediately after each session. Samples were stored at -80^0C until assayed. Leptin and total ghrelin (includes both acyl and des-acyl ghrelin) were assayed in duplicates with commercially available ELISA (Total leptin (catalogue # EZHL-80SK) and Total ghrelin (catalogue # EZGRT-89K) ELISA Kits, Millipore, Billerica, Massachusetts, USA). In our laboratory, the intra-kit coefficient of variation for leptin and total ghrelin used in this study was 7.5% and 3.8%, respectively. It is important to note that all the subjects had their samples assayed in the same kit so as to avoid inter-kit variability.

3.3.3. Energy expenditure

For the CON arm subjects wore an accelerometer (Actical-Mini Mitter Co. Inc., Bend, OR, USA) on their lower back level in order to measure activity-related energy expenditure; this location was chosen because it has been evaluated as the best predictor (r 0.92-0.97), compared to lower or upper leg, lower and upper arm, and head and trunk (Brouten, et al., 1997). Subjects were instructed to wear the accelerometer upon waking and to remove it just prior to going to bed, for a total of three weekdays; previous work has shown that this timeframe can achieve an 80% reliability for the measurement of physical activity (Matthews, et al., 2002). The accelerometry values for the mean EE of each day were used to determine the mean three day EE; this value was then used, along with REE, to determine total energy expenditure (TEE) and the kcal (kJ) equivalent required to prescribe the 25% deprivation. During CON, subjects were instructed to carry on with normal routines of physical activity, but for DER and

DEX were instructed not to perform any vigorous activity such as sports or training in the gym.

Upon arrival for CON4, following a 12-hour overnight fast and after a 30-minute resting period in the supine position, a measurement of REE was performed. This measurement was the final measurement for the TEE calculation required to prescribe the amount of individual energy deprivation. Oxygen consumption and carbon dioxide production were assessed by indirect calorimetry with an open-circuit ventilated hood system (Vmax Sensormedics, Loma Linda, CA). Coefficient of variation and correlation for the Vmax metabolic cart was 2.3% (R = 0.98) as determined in 12 healthy subjects in our laboratory. The metabolic cart was calibrated against 95% O_2 / 5% CO_2 reference gas prior to each measure. A plexiglass hood was placed over the subject's head through which fresh air was drawn for 30 minutes. The first and last 5 minutes of measurement were discarded, and the values of VO2 and VCO2 for the middle 20 minutes were averaged for the calculation of the rate of REE.

3.3.3. Olfaction: nasal chemosensory performance

All measures of nasal chemosensory performance were taken in the same well-ventilated room, devoid of outside smells or distractions. Chemosensory performance was measured with Sniffin' Sticks (Burghart Instruments, Wedel, Germany), a 3-test battery of odourized pens that measure odour detection threshold, odour discrimination, and odour identification, each on 16 point scales and added together to form an aggregate total odour score (threshold, discrimination, identification: TDI), as previously described (Hummel, Sekinger, et al., 1997). Briefly, the pens for the threshold test contained 16 concentrations (1-16, strongest to weakest) of n-butanol and subjects were presented with a triplet of pens, the other two being blanks (aqua-conservans). Subjects were required to identify the pen with n-butanol and the threshold score was determined by a single up-down staircase method, as previously described (Hummel, Sekinger, et al., 1997). In the discrimination task, subjects were again asked to identify the pen that smells different when presented with 16 triplets, the difference from threshold being that 2 pens have the same odour and one has a unique odour. The identification test consists of a forced-choice booklet of 16 pages, each with a choice of

4 odours. The subject is presented with 16 different pens (odours) and is required to circle the odour in the booklet that they believe identifies the pen.

3.3.4. Food reward: RRV and LFPQ computer tasks

The reinforcing value of a stimulus refers to how much behaviour the stimulus will support. This can be objectively observed as the increased (or decreased) willingness—a quantitative measure—to work at obtaining food points via a progressive ratio computer task to obtain a desirable food stimulus (vs. some alternative). Specifically, for this study, computer software was used to set up a split screen that alternated between two different choices (a healthy food and palatable snack food) and was navigated with a mouse/pad (Epstein, et al., 2012). Food points were earned by selectively working for the food item of choice. This so-called work was the action and attention allotted to button presses required in order to complete the "slot machine" game; a button press started the movement of geometric shapes and when all three shapes matched, a single food point for that item was earned. There were two "slot machines" on a single screen: one at the top of a computer screen representing a favourite "snack" food and one at the bottom of the screen representing a favourite fruit or vegetable. The top or bottom position of the reinforcer was counterbalanced across the 3 arms of the study. The reinforcement schedule was set at a progressive linear ratio with response requirements across five trials as follows: 4, 6,8,10, and 12; thus, for example, at VR8 (3rd of 5 trials) the subject performed 8 button presses to obtain one fruit/vegetable point. For the snack foods the schedule was also set at a progressive ratio that doubled at each session as follows: 4, 8, 16, 32, and 64. Subjects were be told that it would be harder to obtain snack food points as trials progressed, but were not informed of the exact rate of change of the reinforcement schedule. Subjects were told to work more for the item they would like to have more of and that they would be offered the food reinforcers at the lunch interval. Following the completion of each individual 10-point trial and upon completion of computer task, the total points for each reinforcer were displayed at the bottom of the screen. Therefore the game ended after the subject navigated past all 5 trials and earned a total of 50 points. Note that 1 point was equivalent to 1 gram of the snack or fruit/vegetable. Prior to performing the RRV task

subjects were required to ingest a “primer” of the foods they picked as their favourite—approximately a 50kcal portion of the snack and FV reinforcers. Each subject was then shown what each food portion would look like if they worked entirely for the snack food or entirely for the fruit/ or vegetable. This was done because many of the snack foods were energy dense, and a visual representation of food points translated into food items intended to demonstrate how small some of the snack portions could be; additionally, this was intended to help control for reward expectation (Robbins & Everitt, 1996) and previous research has shown that a primer can increase the ability to show differences in food reinforcement (Reiss & Havercamp, 1996). The RRV computer task was explained at CON1 and a single practice trial was administered so subjects could familiarize themselves with the controls and program operation.

To examine the significance of dual “liking” and “wanting” components of food reward, the use of a previously developed computer-based procedure (Finlayson, et al., 2008b; Finlayson, et al., 2007b) to allow the separate and concurrent assessments of *explicit* and *implicit* “liking” and “wanting” for the same target stimuli was applied. This was be achieved by using a forced choice behavioural measure (a computer task)—the LFPQ task—of implicit wanting in addition to explicit subjective measures of “liking” and “wanting” for photographic food stimuli varying in fat and sugar content. Briefly, this task presents, on a computer screen, photographic food stimuli of twenty different items varying along two dimensions—fat (high or low) and taste (savoury or sweet). These dimensions can be separated into four categories: high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW), and low fat sweet (LFSW). Each of the four categories is represented by five food items for a total of twenty different food stimuli. For the “implicit wanting” task a food stimulus from one of the 4 food categories is paired with one stimulus from the remaining categories to form a series of 150 trials in which the subjects will be given the instruction to select the food they “most want to eat now”. Each choice is made by a key-press on the keyboard, and each choice triggers the program to continue until all possible pairs have been presented (n=150). In addition to recording the frequency of selections made in each category (with a possible range of 0–75) which may reveal a relative preference (*e.g.* fat vs. sweet), reaction time

(in milliseconds) of each choice was also measured. By covertly recording reaction time, subjects remained unaware of implicit changes in their behaviour on the task, while remaining free to determine the direction of their choices. In this measure, the motivated behavioural response independent of the explicit awareness of its incentive value was the key variable. The “explicit liking” task recorded subjective hedonic ratings for each food stimulus using VAS. The trials consisted of the twenty food stimuli presented one at a time and rated according to a 100-mmVAS anchored at each end by the statements “not at all” and “extremely”. Subjects were prompted with the statement “How pleasant would it be to taste some of this food now?” The VAS was presented on-screen beneath each food stimulus and subjects used the mouse to move a centred cursor along the line to indicate their response. When a rating was made, the procedure automatically cycled to the next stimulus trial. Mean ratings for each food category (HFSA, LFSA, etc.) were automatically computed. Similarly, the “explicit wanting” task was measured by rating 20 randomized pictures with a VAS measure on the bottom of the computer screen that asked “How much do you want some of this food now?” (Not at all-Extremely). The subject used a mouse to move a cursor that is centered along the 100mm VAS line and the results are automatically tabulated by the software.

3.3.5. In lab meals and out of lab food and drink

For all testing performed on day 1 and day 4 under each of the three arms of study subjects were presented with a standardized breakfast which had to be consumed in 15 min. The meal consisted of 2 pieces of whole wheat toast (***D’Italiano***®, 147 kcal), 17 grams of peanut butter (*Kraft* Smooth Peanut Butter®, 101 kcal), 15 grams of raspberry jam (*Smuckers*®, 50 kcal), and 250 ml of water for a total of 298 kcal. At ~1200 in all three repeated measures sessions (CON4, DER4, DEX4), subjects were presented with a calorically-clamped lunch, which had to be consumed in 30 min. The meal consisted of 2 slices of cheese pizza (*Michelina’s* Zap ‘Ems Gourmet®, 781 kcal). The *ad libitum* buffet consisted of 5 snack food items and 4 fruit/or vegetable items that were individualized—that is, the buffet items were previously indicated as being favourite food items on the food questionnaire from the RRV task. All items were presented on white foam plates and in standardized quantities (*e.g.* 70g potato chip

serving, 100g apple serving, 100g candy bar serving, etc.). Subjects were allotted 30 minutes to eat as much or as little of the buffet as they wanted and were told they could request more servings of any item. In order to measure total energy and macronutrient intakes and in order to prescribe the caloric equivalents to achieve a daily 25% energy deprivation, subjects' food was self-selected (but calorically clamped) from a sixty-two item food menu; this menu consisted of hot meals, breakfast items, snacks, fruits, vegetables, and beverages to be consumed outside of the laboratory, as previously described (McNeil, et al., 2012). The food and beverage items were packed into plastic containers, which were then stored into a portable cooler for the subject to take home. All food items were weighed to the nearest 0.1 gram (Scout Pro SP2001; Ohaus Corporation) and the difference calculated when the foods were re-weighed upon return to the lab and subsequently analyzed with Food Processor SQL software (version 9.6.2.; ESHA Research).

3.3.6. Appetite and palatability: visual analogue scales (VAS)

Appetite ratings were measured using a pen and paper on a 150-mm visual analogue scale (VAS) adapted from Hill and Blundell (Hill, et al., 1984). Desire to eat, hunger, fullness and prospective food consumption (PFC) were rated using the following questions: 1) "How strong is your desire to eat?" (Very weak- Very strong); 2) "How hungry do you feel?" (Not hungry at all- As hungry as I have ever felt); 3) "How full do you feel?" (Not full at all- Very full), and 4) "How much food do you think you could eat?" (Nothing at all- A large amount). Palatability was similarly measureds immediately following the ingestion of the personalized snack and vegetable reinforcers (~1045), and also immediately following each slice of pizza (~1200), and finally after the *ad libitum* dessert (~1245). The trapezoid method was employed to measure the incremental area under the curve (iAUC) for all appetite-related variables, as previously described (Drapeau, et al., 2007).

3.4 Statistical Analysis

To test for differences in, olfaction, RRV, LFPQ, and plasma hormone concentrations across CON, DER, and DEX, 3 (condition: CON vs DER vs DEX) x 2 (time: day 1 vs day 4) repeated measures of Analysis of Variance (ANOVAs) were

employed. Significant effects were followed up with paired-samples T-tests. T-tests were used to test for differences in anthropometric variables, appetite, palatability, and *ad libitum* EI. Bivariate correlations were used to determine the strength of the relationship between the changes in anthropemetric variables and the change in plasma hormones, smell performance, food reward, appetite, *ad libitum* EI, and palatability. Similarly, bivariate correlations were used to determine the strength of the relationship between plasma hormones, olfaction, food reward, appetite, *ad libitum* EI, and palatability. Statistical analyses were performed using SPSS version 17 (Chicago, SPSS Inc.). All variables were normally distributed and unless otherwise stated, significant results presented are at $p<0.05$.

Results

For anthropometric variables there were no significant differences in body weight or composition upon commencing each of the three arms of this study (CON1, DER1, and DEX1). Mean scores (max and mins in parentheses) for Three Factor Eating Questionnaire scores were 5.8 (±2.7) (2-11), 4.4 (±1.8) (1-7), and 8.2 (±3.7) (4-13), for dietary restraint, dietary disinhibition, and susceptibility to hunger, respectively; the mean Sensitivity to Reward score was 15.4 (±2.7) (11-20). The average energy deprivation was -709±86 kcal and the average time to perform the exercise equivalent was 64±8 minutes at 50% of V02max. Average V02max was 52.9±9.8 ml/kg/min.

There were no significant condition or time effects for changes in mean plasma concentrations of leptin, but there was a trend ($p=0.06$) for a time effect in the decrease in leptin from DEX1 to DEX4 (see **Figure 1**, panel **A**). Also, there were no significant condition or time effects for changes in mean ghrelin concentrations (see **Figure 1**, panel **B**). There were also no significant condition or time effects for differences in any of the deltas for mean fasting concentrations (for values and percent changes see **Table 2**).

There were DER increases in mean *ad libitum* EI (654±216 vs. 1186±313 kcal) and in iAUC for palatability (402.6±56.9 vs. 467.1±61.1mm) when compared to CON; similarly relative to CON, the iAUC for palatability increased under DEX (402.6±56.9 vs. 482.0±46.0mm). Mean *ad libitum* EI was higher for DER compared to DEX (1186±313

vs. 713±176 kcal) and compared to DEX the energy density (average kcal divided by average weight in grams) was also significant higher under DER (p=0.02).There were no significant differences in appetite scores measured prior to consuming the food reinforcers for the RRV task, nor prior to consuming the *ad libitum* portion of the lunch. There were significant differences in AUC scores across the three conditions: desire to eat was greater in DER (vs. DEX), hunger was greater in DER (vs. CON and DEX), and PFC was higher in DER (vs. CON) (see **Table 1**).

There were no significant time effects for any measures of olfaction for the CON arm. Mean scores for nasal chemosensory performance over the CON arm, from day 1 to day 4, were as follows: odour threshold (7.8±.99 vs. 7.6±1.2), odour discrimination (13.3±.67 vs. 12.9±1.3), odour identification (13.5±1.2 vs. 13.5±.95), and TDI score (34.6±2.5 vs. 33.8±2.7). Relative to CON4, there were significant improvements in odour threshold after both modalities of acute energy deprivation (see **Figure 2**, panel **A**); furthermore, relative to CON4 there were significant improvements in TDI score only after the DER intervention (see **Figure 2**, panel **B**).

There were no significant time effects for the CON arm for any measures of the RRV of palatable snack foods compared to preferred fruits/or vegetables. From day 1 to day 4 mean values for snack points earned were (17.6±5.7 vs. 15.5±4.3) while mean values for responses (button presses) for the palatable snack were (377.7±83.1 vs. 347.0±165.5). Relative to CON4, there were significant increases in the RRV of snack foods after both modalities of acute deprivation (see **Figure 3**, panel **A**).

There was a significant time effect for the CON arm on explicit liking and wanting for all food images. From day 1 to day 4 mean values decreased (liking: 43.0±2.5 vs. 32.4±4.0; wanting: 40.9±2.8 vs. 29.0±3.4). There was also a significant interaction between time and food category in the task on explicit wanting, with scores for sweet foods decreasing less than non-sweet foods (change for sweet compared to non-sweet: -8.0 vs. -15.8). For implicit wanting, there was a time and food category interaction with scores for high fat foods decreasing between day 1 and 4 while scores for low fat foods were relatively similar. There were no significant differences in liking or wanting scores after either deprivation conditions compared to CON4. However for explicit liking and

wanting, the trends were in line with RRV and palatability data with an increase in scores at DER4 and DEX4 relative to CON4 (see **Figure 5**). For implicit wanting scores there were no differences between conditions at day 4.

Correlations indicated that the improvements in TDI from CON4 to DER4 were related to increased *ad libitum* EI (r=0.67). There were no significant relationships between either of the peptide hormones and appetite, nasal chemosensory performance, nor with either measures of food reward. There was a positive correlation noted between mean palatability rating for the snack food and mean RRV snack points (r=0.71, p=0.02). Neither TFEQ nor SR scores demonstrated any correlation with any of the above-measured variables.

Discussion:

The objective of this study was to determine if there are measurable differences in the response of psychobiological characteristics to a theoretically comparable energy deprivation of 25% of that which would otherwise be considered energy balance, either imposed by diet alone or aerobic exercise alone. Independent of deprivation modality there were significant condition effects defined by improvements in odour threshold scores, significant increases food reward and palatability, and significantly greater *ad libitum* EI relative to control. Relative to the exercise condition, deprivation by diet alone imposed a greater challenge to appetite as indicated by significantly greater hunger, desire to eat, and prospective food consumption, along with a corresponding increase in *ad libitum* EI. The increase in total odor score (TDI) was positively correlated with the noted increase in EI under the diet alone condition.

Though plasma leptin levels are proportional to fat mass in times of relative weight stability, it is still unclear what drives the up- or down-regulation of leptin production during periods of energy imbalance (*e.g.* insulin-mediated glucose transport by adipocites) (Anubhuti, et al., 2008). Several investigators have reported the most pronounced declines in leptin occur in early stages of energy restriction (Doucet, et al., 2004b; Keim, et al., 1998; Rosenbaum, et al., 1997; Wadden, et al., 1998), often without significant changes in adipose tissue stores. Similarly, our results demonstrated that without significant changes in fat mass, fasting leptin concentrations were lowered by

67.2% from DER1 to DER4, and lowered by 89.3% from CON4 to DER4 (see **Table 2**). What is interesting is that the deprivation by exercise produced the most pronounced changes in leptin, with reductions of 101.6% from DEX1 to DEX4, and 118.8% from CON4 to DEX4. Similar decreases in leptin have been noted after acute exercise in relative energy balance (van Aggel-Leijssen, et al., 1999). Regarding ghrelin, the expression and secretion is decreased in situations of feeding and positive energy balance (*e.g.* overfeeding and obesity) (Hagobian, et al., 2008; Horvath, et al., 2001), and increased in situations of weight loss by caloric restriction (Hansen, et al., 2002; Soriano-Guillen, et al., 2004; Toshinai, et al., 2001). Data remain inconclusive, however, regarding the ghrelin response to short term deprivation without weight loss. There are conflicting results of the response of total ghrelin to exercise: some have shown no decrease in total ghrelin with a single treadmill session (Burns, et al., 2007) or during acute exercise (Dall, et al., 2002), while other acute overfeeding data has shown the addition of exercise to significantly decrease total ghrelin, suggesting independent effects from energy balance (Hagobian, et al., 2008). Also another group found no change in total ghrelin in weight-stable exercisers, but showed that total ghrelin increased in exercisers who lost weight after 3 month intervention (Leidy, et al., 2004). After three consecutive days of expending an average of 702kcal (2939kJ) per day on a treadmill and no significant change in body weight we also found no significant change in fasting total ghrelin concentrations (see **Figure 2**). What is interesting is that, although non-significant, ghrelin was lowered by 10.3% from day 1 to day 4 of the diet alone condition and also lowered by 9.7% from CON4 to DER4, whereas, ghrelin increased 3.2% from day 1 to day 4 of the exercise alone condition and also increased 6.8% from CON4 to DEX4 (see **Table 2**). Recent studies have focused on measuring acylated ghrelin (total ghrelin is acylated+deacyl ghrelin) under the notion that unlike deacyl ghrelin (DG), acylated ghrelin (AG) is known to have downstream action of hypothalamic structures (Cummings, 2006). Although some have demonstrated no change in AG with a prolonged treadmill exercise for 90 min at 68% V02max (King, et al.) or with 60 min of brisk walking (King, Wasse, et al., 2010), others have found a decrease in AG with intense exercise sessions (Broom, et al., 2009; Mackelvie, et al.,

2007). Only a few groups, however, have demonstrated a positive association between exercise induced decreases in AG with decreased hunger (Broom, et al., 2007; Mackelvie, et al., 2007).

Following diet induced weight loss, an increase in orexigenic drive in the fasted state has been reported (Doucet, et al., 2000; Keim, et al., 1998) and similar findings have been shown with aerobic exercise (King, et al., 2009; Whybrow, et al., 2008). Furthermore, evidence suggests that an increased hunger-state can increase food palatability and changes in peripheral leptin can account for heightened hedonics. For example, leptin-deficient individuals report higher liking rating for food, and leptin replacement therapy has been shown to normalize liking ratings even before weight loss was achieved (Farooqi, et al., 2007). In the current study, although palatability increased independent of modality of energy deprivation, there was no evidence of an association with changes in fasting leptin, even if leptin did not significantly change under either condition. Also demonstrating a similar lack of association of leptin and hedonics, a study consisting of a chronic energy deprivation (8 week weight loss trial) and a repeated measure looking at food hedonics pre- and post weight loss, also found no significant relationship between serum leptin and rated pleasantness (Cameron, et al., 2008). Although there were no relationships noted with any appetite related variables and fasting levels of plasma leptin and ghrelin (nor with corresponding deltas), examining the appetite response to the modality of energy deprivation offered a clear picture that the dieting alone arm imposed a greater challenge to appetite than exercise alone. The current study differs from most studies examining the acute impact of exercise in that appetite and EI were measured after three days of structured energy deprivation, and not on the same day that the exercise was performed. This is one of the strengths of our design in that it is not necessarily the influence of exercise *per se (e.g.* exercise-induced anorexia), but the overall effect of energy deprivation by different modality that were measured. Other groups have found no change in appetite or EI within hours after exercise (King, et al., 1994; King, et al., 1996) or on the day after a single session of vigorous aerobic exercise (King, Miyashita, et al., 2010; King, et al., 1997) but no group reported a proxy of absolute levels of daily total energy deprivation.

In contrast, after three days of clamped energy deprivation there were significantly higher integrated scores for desire to eat, hunger, and PFC under DER compared to DEX (see **Table 1**). Furthermore, under DER we noted significant increases in *ad libitum* EI (66.2%) and in fat intake as percent of calories (38%) in the same feeding session when compared to DEX. This is not necessarily in contradiction to previous data showing that acute bouts of exercise (<1week) are often not enough to bring about compensatory changes in appetite and feeding (Stubbs, et al., 2004) and adds credence to the notion that exercise may heighten the sensitivity of short-term appetite control (Martins, et al., 2007)—even if subjects still ate more after DEX compared to CON.

The role that smell function has in feeding and body weight regulation has been studied, sometimes with conflicting results (Koelega, 1994). Studies in the past have shown improved pre-prandial odour sensitivity to food (Schneider, et al., 1955) and non food (Hammer, 1951) odours when compared to post-prandial measures of the same test odourant. Using a standardized and well validated tool (Hummel, et al., 2007), our results confirmed the intuitive role that energy deprivation may have in nasal chemosensory performance by the measured increase (improvement) in mean threshold scores after both energy deprivation conditions (see **Figure 3**). The reliability of this tool, and our findings, is strengthened by the fact that there were no differences in smell scores under relative weight stability (*i.e*. between CON1 and CON4). Our results are in agreement with a recent study using Sniffin Sticks test smell test and found that higher hunger state resulted in improved threshold scores for n-butanol, but not for a food odor (Stafford & Welbeck, 2011). A novel finding presented in our data is the fact thatthe sum of all three odour measures—TDI score—significantly increased only after the deprivation by diet alone. What is interesting is that this increase in TDI score from CON4 to DER4 was positively correlated (r=0.67) with the noted ~81% increase in *ad libitum* EI. To our knowledge this is the first study to show a link between energy-deprivation induced changes in nasal chemosensory performance and EI and highlights the need to further study the dynamic role of olfaction in appetite and body weight regulation (Cameron, et al., 2012a).

Though acute food deprivation (hours) has been shown to influence the motivation to eat food, *i.e.* “wanting”, by increasing the RRV in humans (Epstein, et al., 2003; Raynor & Epstein, 2003), the current study is the first to our knowledge to examine how modality of deprivation impacts RRV. First it is important to note that there were no significant differences in RRV of snack foods under control conditions (CON1 to CON4), which in effect speaks to the reliability of the following findings. Independent of modality of energy deprivation, there were significant increases in both the amount of snack points earned and the amount of responses made, but there was no corresponding relationship with RRV and *ad libitum* EI. Our results are in line with a previous study with a ~13 hour deprivation by diet alone that demonstrated an increase in reinforcing value of a palatable snack food, but do not fit with others who have shown a relationship with increased RRV and increased *ad libitum* EI (Epstein, et al., 2011; Epstein, Temple, et al., 2007; Saelens & Epstein, 1996). A novel finding, however, was that contrary to previous reports of dissociations of wanting and liking of the same target stimuli (Cameron, et al., 2008; Epstein, et al., 2011; Epstein, Temple, et al., 2007), we show that under DER a positive association between liking of the snack reinforcer and the amount of points ultimately earned (*i.e.* wanting) in the RRV task (see **Figure 4**). It is interesting to note that relative to DEX, subjects not only went on to eat more *ad libitum* food under DER, but also consumed food that was significantly more energy dense (see **Table 1**) .

Regarding the forced-choice computer task that was developed in an attempt to measure *implicit* “wanting” and *explicit* “wanting” and “liking”, there exists the potential to test the impact of various forms of energy deprivation on these reward-related variables. A recent study examined the impact of energy deprivation by means of exercise on food reward and *ad libitum* energy intake with two counterbalanced sessions, one at 50 minutes of high intensity exercise and then other without exercise (Finlayson, et al., 2009). Although there was no significant difference in the amount of food eaten by test day for this group of lean women, it was found that after the exercise session there was a subgroup of “compensators” who ate more after exercise and also displayed a significant increase in *implicit* “wanting”. After exercise there was an unconscious

implicit desire for the “compensators” to eat high-fat sweet foods. What is interesting is that a separate study with similar exercise intensity (but longer duration) also demonstrated that lean female subjects overcompensated for the exercise session (vs. an equicaloric lower intensity exercise) when given access to an *ad libitum* for the remainder of the day (Doucet, et al., 2004a). In both studies there was evidence that performing intense aerobic exercise results in increased energy intake, thereby acutely promoting *positive* energy balance. This study had some limitations. Along with the small sample size, the sample itself was composed of young, relatively fit males which may not necessarily be generalized to clinical populations. The results also cannot be extrapolated to predict longer term energy deprivation. Also, that there was only a single fasting value of leptin and ghrelin limited the detection of significant effects on appetite on food reward. Strengths are in external validity—the aerobic exercise was roughly equivalent to one hour of walking at a brisk pace, which is a realistic goal for activity levels for weight contol. Similarly this level of energy deprivation is realistic for goal setting and is sustainable. Also, most research in feeding relies on self-reporting of foods and the utilization of a validated food menu with a relatively high in- and out-lab correlation adds to the strength of our findings.

This study showed that an equicaloric energy deficit by diet alone was a greater challenge to appetite regulation and resulted in greater compensatory increases in EI than deprivation by exercise alone. Independent of deprivation modality there were significant improvements in odour threshold scores, whereas TDI score increased only under DER. The noted increase in TDI scores was positively related to increased *ad libitum* EI. Palatability increased independent of deprivation modality. There was a positive relationship noted between liking and wanting (RRV) of the same palatable reinforcer. Although dieting alone produces more weight loss than exercise alone (Miller, et al., 1997), this study highlights that the acute responses to a diet alone challenge may initially dissuade potential success in body weigh regulation.

Table and Figure Legends

Table 1. Subjects' characteristics (mean±SD) for each of the repeated measures test days: at the end of the control arm (CON 4), at the end of the deprivation by diet alone arm (DER 4) and at the end of the aerobic exercise alone arm (DEX4). As determined with paired samples t-tests, means labeled with different letters are significantly different at $p<0.05$.

Table 2. Mean changes (±SD) and percent change [%] in plasma peptides over the time course for each of three arms (day 1 to day 4) and across the repeated measures conditions. Negative values represent decreases and positive represent increases in concentration of leptin (ng/ml) and ghrelin (pg/ml). There were no significant changes by time or condition.

Figure 1. Protocol for the crossover design.

Figure 2. Mean (±SD) fasting plasma leptin and ghrelin concentrations (panels **A** and **B**, respectively), taken in AM after an overnight fast. There were no significant time or condition effects for changes in plasma concentrations of either appetite-related peptide hormone.

Figure 3. Demonstrating mean Sniffin' Sticks scores measuring nasal chemosensory performance between the three conditions: control (CON4), deprivation by diet alone (DER4) and deprivation by aerobic exercise alone (DEX4). The odour detection threshold significantly improved after both deprivation conditions (panel **A**), indicating that relative to CON4, subjects were able to detect the test odourant (n-butanol) at smaller concentrations. Relative to the CON4 score, the combined odour score (TDI: Threshold, Discrimination, Identification) also significantly improved under the DER4 condition (panel **B**). Means (±SD) labeled with different letters are significantly different at $p<0.05$.

Figure 4. Illustrating the co-varying of the heightened reinforcing value of palatable snack foods (see panel **A**) and increased liking (panel **B**) for the same foods under control (CON 4) and experimental conditions (DER and DEX). A significant positive relationship between liking and wanting of the snack reinforcer was noted under DER only ($r=0.71$, $p=0.02$). Means (±SD) labeled with different letters are significantly different at $p<0.05$.

Figure 5. Illustrating the trends for an increase in explicit liking (see panel **A**) and explicit wanting (panel **B**) for the same food images under experimental conditions (DER and DEX) compared to control (CON).

Fig. 1.

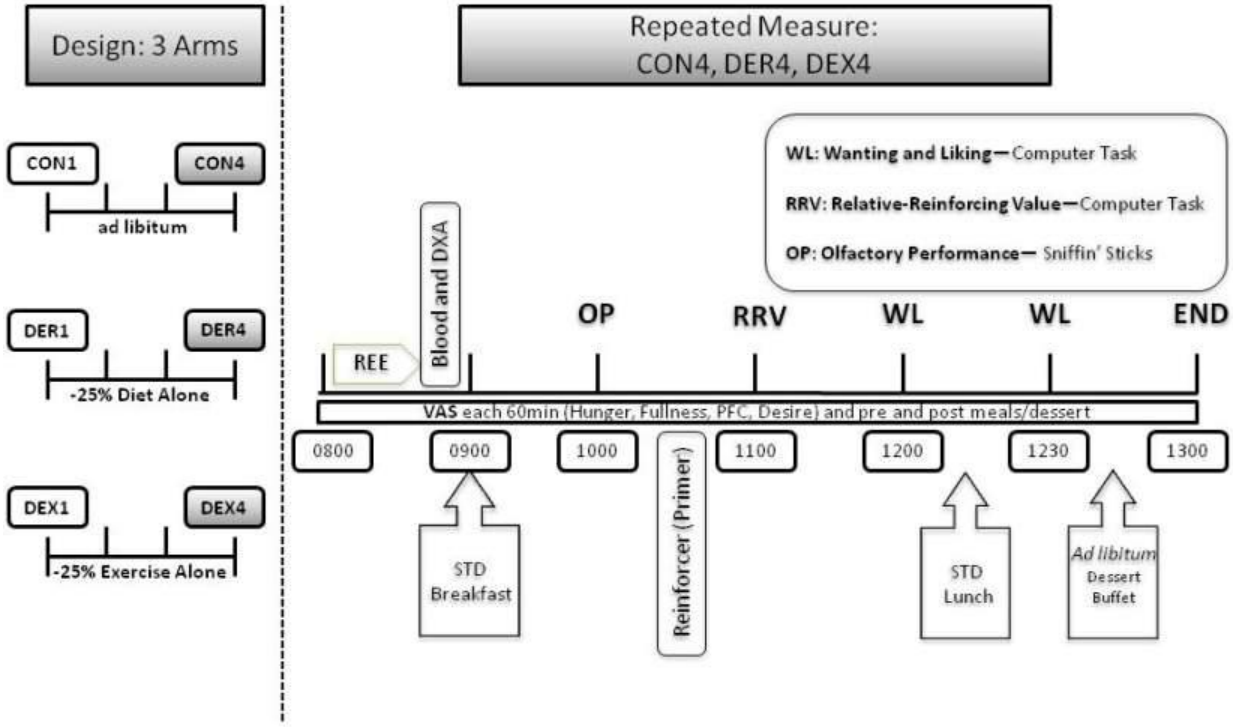
Design: 3 Arms
Repeated Measure:
CON4, DER4, DEX4
CON1
CON4
ad libitum
DER1
DER4
-25% Diet Alone
DEX1
DEX4
-25% Exercise Alone
WL: Wanting and Liking—Computer Task
RRV: Relative-Reinforcing Value—Computer Task
OP: Olfactory Performance— Sniffin' Sticks
Blood and DXA
REE
OP
RRV
WL
WL
END
VAS each 60min (Hunger, Fullness, PFC, Desire) and pre and post meals/dessert
0800
0900
1000
1100
1200
1230
1300
STD Breakfast
Reinforcer (Primer)
STD Lunch
Ad libitum Dessert Buffet

Fig. 2.

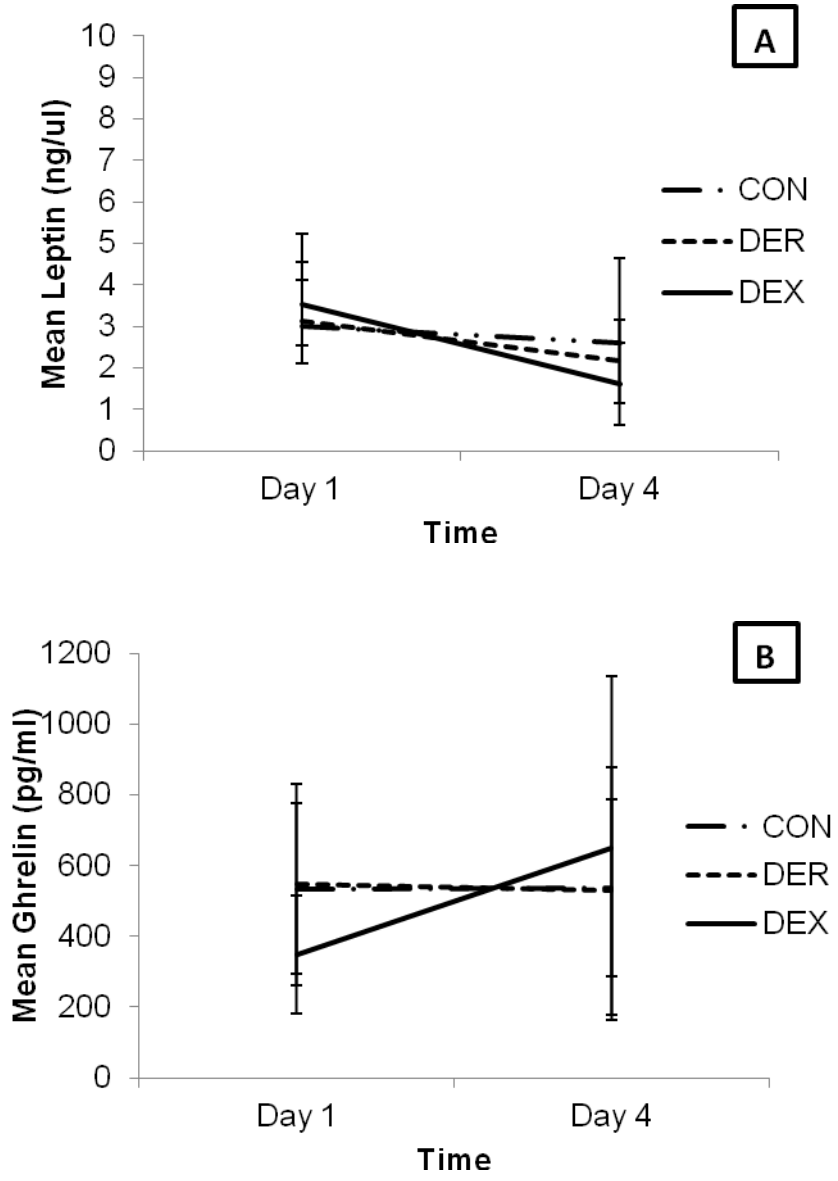
A
Mean Leptin (ng/ul)
10
9
8
7
6
5
4
3
2
1
0
CON
DER
DEX
Day 1
Day 4
Time
B
Mean Ghrelin (pg/ml)
1200
1000
800
600
400
200
0
CON
DER
DEX
Day 1
Day 4
Time

Fig. 3.

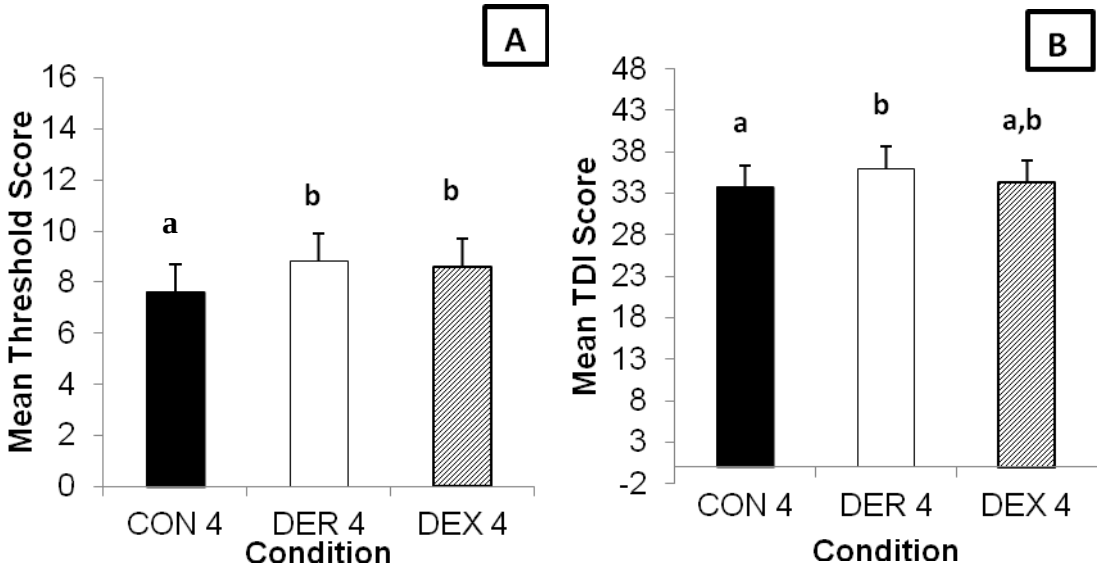
A
Mean Threshold Score
16
14
12
10
8
6
4
2
0
a
b
b
CON 4
DER 4
DEX 4
Condition
B
Mean TDI Score
48
43
38
33
28
23
18
13
8
3
-2
a
b
a,b
CON 4
DER 4
DEX 4
Condition

Fig. 4.

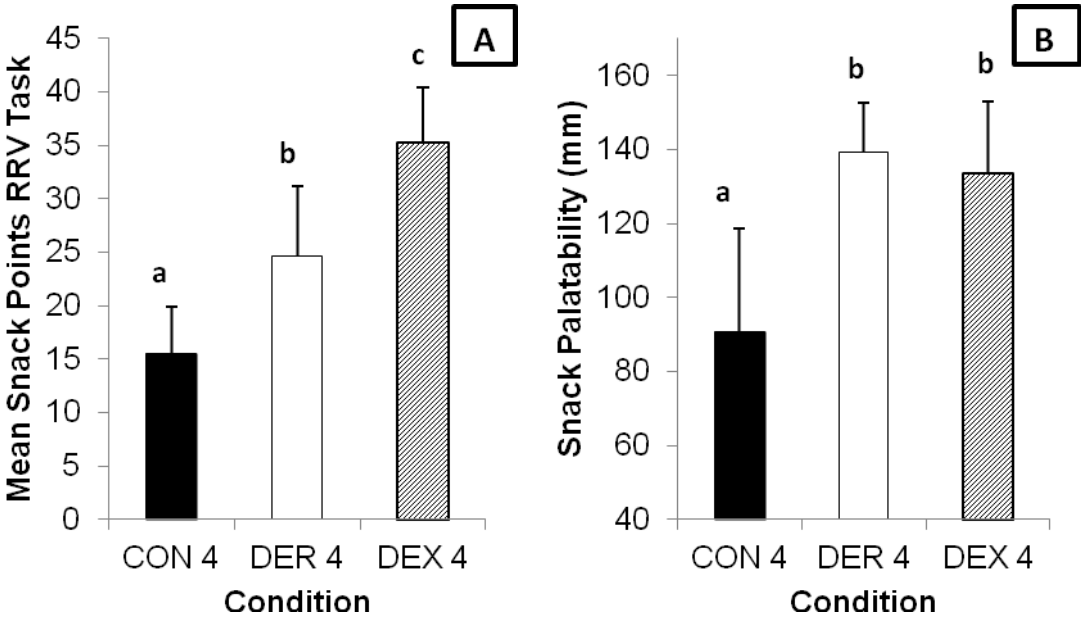
A
Mean Snack Points RRV Task
45
40
35
30
25
20
15
10
5
0
a
b
c
CON 4
DER 4
DEX 4
Condition
B
Snack Palatability (mm)
160
140
120
100
80
60
40
a
b
b
CON 4
DER 4
DEX 4
Condition

Fig. 5.

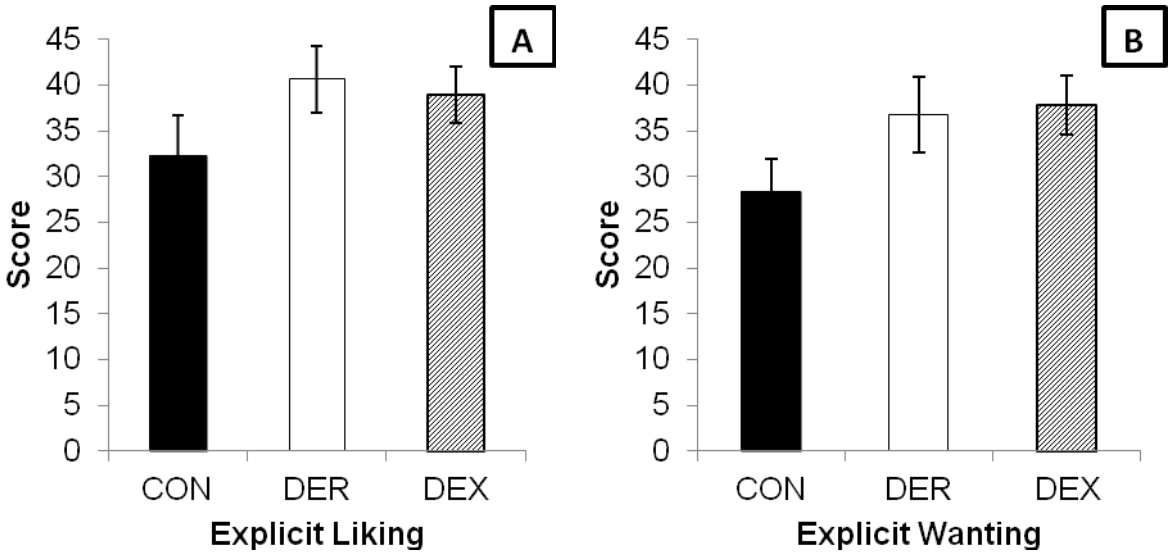
A
45
40
35
30
25
20
15
10
5
0
Score
CON
DER
DEX
Explicit Liking
B
45
40
35
30
25
20
15
10
5
0
Score
CON
DER
DEX
Explicit Wanting

Table 1.

Characteristic	*Condition*		
	CON Day 4	***DER Day 4***	***DEX Day 4***
Anthropometric			
Body Weight	83.2±11.5[a]	81.9±11.5[b]	81.6±11.5[a,b]
Fat Mass	17.6±7.8	17.6±7.8	17.0±7.8
%Fat	20.4±6.6	20.6±7.0	20.3±7.0
FFM	62.8±7.8[a]	61.5±7.3[b]	61.6±7.7 [a,b]
Appetite (iAUC)			
Desire to Eat	338.6±114.1[a,b]	432.5±58.5[a]	389.1±48.5[b]
Hunger	325.8±105.6[a]	418.7±51.3[b]	372.2±41.0[a]
Fullness	349.1±87.4	363.3±45.7	377.2±76.1
PFC	340.4±102.4[a]	446.1±39.4[b]	395.8±66.1[a]
Hedonics (VAS)			
Snack	90.9±27.6[a]	139.2±13.5[b]	133.4±19.6[b]
Fruit/Vegetable	102.9±26.2[a]	133.3±16.9[b]	121.4±18.5[a,b]
Pizza 1	105.4±14.8	99.9±28.9	115.8±19.1
Pizza 2	94.9±30.4	101.8±23.8	113.7±23.3
Dessert	107.9±18.7[a]	124.9±22.5[a,b]	128.8±18.3[b]
iAUC	402.6±58.9[a]	467.1±61.1[b]	482.0±14.6[b]
Ad Libitum EI			
Total kcal	653.9±215.8[a]	1185.7±312.8 [b]	713.2±175.5[a]
Total grams	351.8±118.3[a]	561.6±160.6[b]	433.3±111.9[a]
Energy density	1.9[a,b]	2.2[a]	1.7[b]
%CHO	25.4±6.3	30.6±7.0	26.1±8.6
%Sugar	15.1±7.9	18.2±6.4	15.9±8.7
%Fat	6.6±4.1[a]	8.8±3.5[b]	6.4±2.5[a]

Table 2.

Peptide	Time			Condition		
	CON1_CON4	DER1_DER4	DEX1_DEX4	CON4_DER4	CON4_DEX4	DER4_DEX4
Leptin	-0.2 (±2.3) [38.2%]	-0.47 (±1.6) [67.2%]	-2.1 (±3.7) [101.6%]	-0.5 (±1.9) [89.3%]	-1.9 (±2.1) [118.8%]	-1.5 (±2.7) [94.4%]
Ghrelin	1.5 (±163.2) [5.2%]	-18.8 (±127.9) [10.3%]	201.8 (±615.3) [3.2%]	-8.3 (±168.9) [9.7%]	113.5 (±326.9) [6.8%]	121.7 (±389.5) [11.9%]

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PART THREE: CONCLUSIONS AND RECOMMENDATIONS

CHAPTER VIII: CONCLUSIONS AND RECOMMENDATIONS

In an effort to concisely summarize year's worth of research, **Table 1** below shows data from Articles II, III, and IV; in order to increase the strength of the findings sex was eliminated as a variable and only males were included from Articles II and III (same data set). The picture that emerges is that, acutely, a complete fast has more pronounced effects than dieting alone, which in turn had greater effects on appetite than exercise alone or to controls. It is interesting to note that overall smell function (TDI) improved under all three methods of energy deprivation, but moreso under conditions of deprivation by diet alone, *i.e.*, diet alone (DER)>exercise alone (DEX) and FASTED>DEX. What is more, although non-significant, is the finding that participants consumed approximately 12% more energy under DER than after a 24 hour fast, indicating a larger degree of compensation.

Table 1. Summary of the response of the key variables measured and the changes due to the degree and the modality of energy deprivation. There were no significant differences in age, body weight, or BMI between the two samples of normal weight males. The 25% deprivation represents the sample from Chapter VII and the 100% deprivation represents the sample from Chapters V and VI. All groups experienced the same protocol on the repeated measures test day, i.e. results presented herein.

	25% Deprivation			100% Deprivation		T-Test (p<0.05)
Measure	CON4	DER4	DEX4	FED	FASTED	
Snack Points	15.8±4.5	24.3±6.8	36.2±4.7	15.9±9.6	27.1±11.1	DEX>FASTED
Snack Resp	360.4±169.6	608.9±295.7	936.0±248.4	327.1±179.9	666.5±346.6	n.s.
Threshold	7.3±0.96	8.6±1.1	8.0±1.5	8.6±1.4	8.0±1.1	n.s.
TDI	33.4±2.4	35.8±2.4	34.1±3.1	35.6±2.2	36.9±1.6	DER>DEX; FASTED>DEX
Pal Snack	90.8±29.3	138.4±14.1	131.6±19.9	118.3±35.8	133.1±22.1	n.s.
Pal iAUC	398.9±59.1	462.9±63.3	480.7±48.6	434.7±55.7	487.6±53.5	n.s.
EI kcal	625.9±208.7	1201.7±327.4	715.2±186.1	624.9±448.9	1059.3±318.5	DER>DEX; FASTED>DEX
Desire iAUC	321.2±106.0	439.7±57.2	393.7±49.1	604.7±372.8	752.2±94.9	DER>DEX; FASTED>DER,DEX
Hunger iAUC	309.4±97.6	426.1±50.0	378.9±37.2	457.1±155.5	738.6±88.0	DER>DEX;FASTED>DER,DEX
Fullness iAUC	358.1±87.6	364.7±48.2	383.9±77.4	484.1±124.7	258.1±76.5	FASTED>DER, DEX
PFC iAUC	326.6±98.3	452.8±35.1	384.7±59.5	537.1±144.9	777.7±100.1	DER>DEX;FASTED>DER,DEX

To be sure, more research with larger sample sizes is needed to identify potential metabolic changes that may account for these differences. Contrary to my Article IV secondary hypothesis that the appetite-related peptides ghrelin and leptin would play a role in reward and feeding, fasting concentrations (or deltas) were not associated with a wide-range of heightened responses to reward and feeding. As a speculative note, the amino-static theory of feeding was recently re-visited with data showing quite strong correlations between FFM (and not FM nor BMI) and *ad libitum* EI and also meal size (Blundell, et al., 2012). The authors hypothesized that lean mass sets a lower limit for self-determined meal size. This is in agreement with the 12% increase in single meal intake between DER and FASTED: although body composition was not acquired under FASTED and although quite possibly the changes in FFM were an artifact of measurement error due to shifts in body water, it was indeed found that FFM significantly decreased only under DER. Returning to the macro-level of energy deprivation, reward and feeding, several of our findings agree with **Figure 1**. Albeit, there was no evidence of a peripheral involvement of peptides in changes in reward and feeding, results from Article IV demonstrated inceased 'wanting' *and* increased 'liking'.

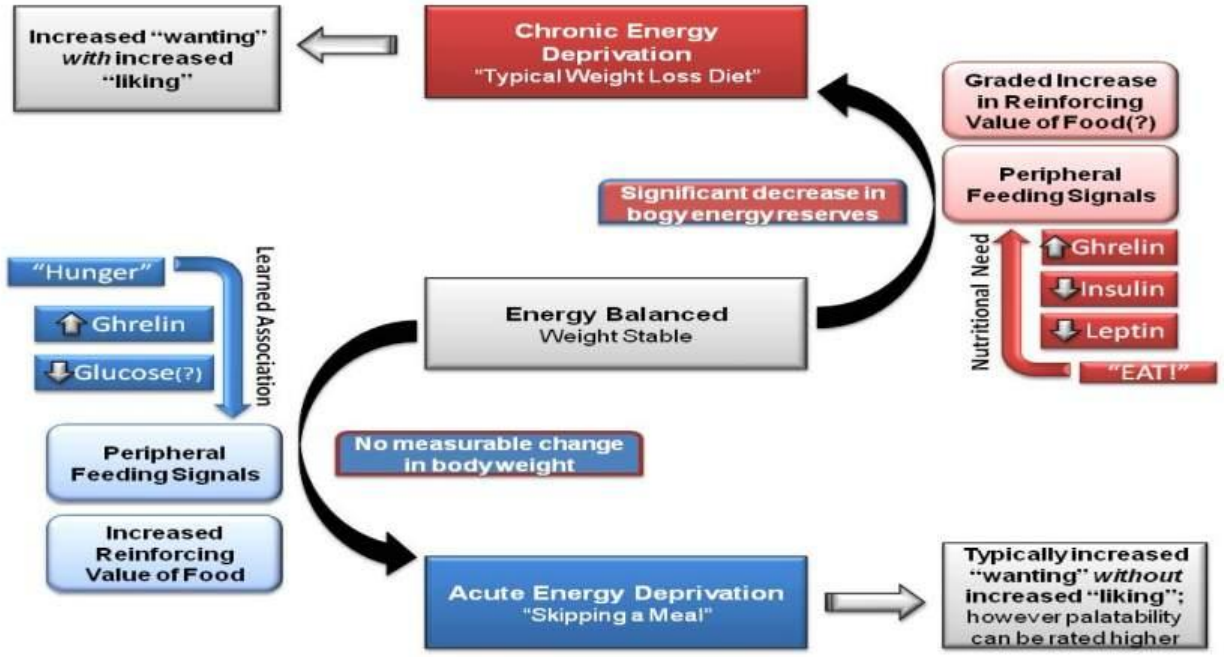

Figure 1. Representing the Ying and Yang of food reward as a function of energy deprivation. When there is no measurable change in body weight, it is believed that peripheral signals act as *cues* to feed, but chronic energy deprivation results in

exaggerated responses from peripheral signals. It may be that significant changes in body energy reserves changes mere cues into powerful signals that motivate appetitive and consommatory feeding behavior.

Finally, that the DEX condition produced that greatest increases—significantly greater than FASTED—in snack food reinforcement may potentially explain why some individuals respond poorly to exercise interventions, compared to similar deprivations induced by diet alone. Thus in the context of body weight manangement, the results from Article IV suggest that, in combination with previous findings linking increased reinforcement with increased EI (Epstein, et al., 2012), it would be beneficial to identify those who are high in reinforcement as they may be resistant to the beneficial effects of exercise in the context of weight control. Similarly pointing the need for more individualized advice for the management of body energy reserves, higher palatability and olfaction scores were noted under the deprivation by diet alone. Taken together, the sum of our findings highlight the need for a more integrated approach in understanding and maping the physiological and behavioural differences in the response to a challenge of negative energy balance.

Recommendations and Future Perspectives

Food intake is the result of a multi-modal representation of the sensory information about the food stimulus: in the brain there is a continuum consisting of the visual exteroreception of the sight of food, to the interoreception of primary and secondary taste corticies' evaluation of taste, temperature, texture, and viscosity. While energy deprivation can have an impact at several of these levels, humans also eat for reasons other than nutritional needs. For most of the developed countries meals are entrained to a schedule thereby eliminating what might be real signals of energy deprivation—this fact has raised to attention that such feeding may anticipate and prevent the development of significant metabolic changes (Woods, 1991). The study of feeding behavior, and in particular the study of subjective hedonic experience and objective measures of motivation, are central to understanding how appetite regulation can be compromised in certain individuals. Furthermore, with an integrated picture of physiological and behavioral changes—particularly with regard to food palatability and reinforcement—that can occur as a result of sustained negative energy balance what

emerges is a better understanding of how palatable food can disrupt attempts at regulating body weight at lower levels of body energy.

Epidemiological data suggests that some people are more susceptible to weight gain (Ravussin, et al., 2004); this propensity to gain, maintain, or lose weight under similar environments has been attributed to various susceptibility levels such as metabolic, behavioral, psychological, physiologic, and genetic (Blundell, et al., 2005). The fact that food intake is not merely based on nutritional or homeostatic requirements but is also very much influenced by its reinforcing properties presupposes a modulating role for dopamine in everyday feeding (Carr, 2007) alongside with leptin and ghrelin (Figlewicz, 2003). If it can be shown with more fecundity that a biological basis exists for differences in the reward experienced in feeding, then as an example, individualized treatment and prevention programs for weight loss could differentially help those who are high in food reinforcement versus someone low in food reinforcement (Epstein, Leddy, et al., 2007). Furthermore, there needs to be more data regarding the change in orosensory reward (olfactive and taste stimuli) that may occur with chronic energy deprivation. In order to help those trying to lose- or maintain a specific level of body energy reserves, there needs to be a better understanding of the processes behind the rewarding qualities of food, both implicit and explicit. In a continual effort to inhibit weight gain in this modern obesogenic environment behavioral strategies promoting dietary restraint—thereby shifting inhibitory control over appetitive motivation—may be what need to be adopted in non-clinical populations, e.g. no identified eating pathology. Of course, changing the food landscape through regulation standards that are at the benefit of our health and not to “harmonize trade” would be a solution that better attacks the problem.

Dissociating a psychological concept such as a reward into separable components of hedonic evaluation and motivation to obtain and consume does, however, have ecological validity. In particular, the RRV tool can offer valuable information regarding the determinants of food choice. There are a lot of data showing that if access to a preferred food (such as cheesecake) is reduced, then people will choose a less preferred—although still enjoyable—alternative (such as yogurt). Applied in light of behavioral economics theory, if the price of a good that is typically purchased

is increased, then as cost increases there will be a certain point where the consumer will *substitute* with something similar, though less expensive.

When considering the millions of people try to lose weight each year, the fact that food reinforcement increases with energy deprivation has obvious concerns. The very thing that is supposed to help with weight loss (*e.g.* decreased energy intake or increased energy expenditure) can act to increase the motivation to eat energy dense food. Although there are no definitive data to show that increased meal frequency (independent of energy intake) promotes weight loss, it may still be a good approach to prescribe such a diet in an attempt to control for overeating due to increased saliency of palatable food. To be sure, irregular meal patterns such as skipping meals can reinstate abhorrent binge-eating behavior in individuals recovering from bulimia nervosa. Nonetheless, a reduced-calorie diet imposed with a greater frequency of meals may also leave individuals to choose a non-food activity that is reinforcing (*e.g.* walking a 5km with a peer group) in the place of impulsively eating due to a perceived deprivation in energy and increased food reinforcement. The increased food reinforcement—and its predicting power of *ad libitum* feeding—may also highlight the importance of limiting access to food in a strategic manner.

Future research should be directed at examining the interplay of genes, nutritional status, food reward and peptide signals in order to have a better understanding of how each of these levels may differentially affect feeding behaviour and obesity at large. A starting point, however ambitious, would be to genotype individuals for specific polymorphisms of both the DAT and DRD2 (there is a much larger list of potential candidates, *e.g.*, FTO) genes and also measure the respective densities, protein availability, and binding potentials using PET technology and receptor-specific radioligands. Upon taking these measures, subjects could be exposed to a controlled energy deprivation by diet alone, exercise alone, diet+exercise, and control. PET measures taken again at the mid-point and immediately following the energy deprivation period. Also measured at the 3 time points (basline, mid, end) would be the RRV and LFPQ computer tasks to assess changes in food reward, along with sampling of leptin, insulin, and ghrelin. With such a design most of the issues raised in this manuscript could be explored: Is there an effect of the environment on the differential

densities of DAT and DRD2 polymorphisms e.g. will there be significant differences in the PET measures of the respective gene products over time? Will changes in long-term feeding signals such as leptin and insulin or the short-term signal ghrelin correlate with changes in PET measured densities or binding potentials? Will food reinforcement change differently depending on modality of energy deprivation? Will carriers of DAT and DRD2 polymorphisms work differently to obtain food reinforcers? Will changes in leptin, insulin and ghrelin correlate with changes in food reinforcement, palatability, or *ad libitum* EI? All of these questions are important avenues to reach a full understanding of how humans are motivated to feed and how the physiology behind these actions interact with or result in human obesity.

PART FOUR: CONTRIBUTION OF COLLABORATORS

CHAPTER IX: STATEMENT OF CONTRIBUTION OF COLLABORATORS

The collaborators involved in the conception and writing of this thesis (J.C. and E.D.) contributed as follows: J.C and E.D. interpreted all findings and collaborated to write each of the four articles presented herein in article format and also collaborated to write a published book chapter titled Reinforcement and food hedonics: a look at how energy deprivation impacts food reward (Cameron, J., & Doucet, E. 2011. Reinforcement & Food Hedonics: A Look at How Energy Deprivation Impacts Food Reward. In C. R. Martin, V. R. Preedy & R. R. Watson (Eds.), *Handbook of Behavior, Food and Nutrition* (Vol. 1, pp. 3667). New York: Springer-Verlag New York Inc.), which was modified and included as my Review of Literature. For this manuscript used as the Review of Literature, J.C. wrote the paper and designed all the original tables and figures.E.D. edited the final draft.

For Article I, titled "The TaqIA RFLP is associated with attenuated intervention-induced body weight loss and increased carbohydrate intake in post-menopausal obese women", J.C. performed the DNA extraction and PCR analysis from blood previously collected. J.C wrote the manuscript, and M-E.R., F.T., G.G, R.R-L., M.B., and E.D. all contributed in the editing of the manuscript.

For Article II, titled "Fasting for 24 hours improves nasal chemosensory performance and food palatability in a related manner," J.C. performed all data collection and wrote the manuscript, and G.G., and E.D. were involved in the elaboration of the experimental design and collaborated in editing the manuscript.

For Article III, titled "The Impact of a 24 hour fast on food reward and intake during *ad libitum* feeding: evidence of increased reward from food and food-related cues," J.C. performed all the data collection and wrote the manuscript, and G.G, G.F., J.B., and E.D. were involved in the elaboration of the experimental design and collaborated to edit the manuscript.

For Article IV, titled "Deprivation by diet alone or by aerobic exercise alone: how modality of an acute intervention can differently impact olfaction, food reward, and ad libitum feeding, and appetite hormones," J.C. performed all the data collection and wrote the manuscript, and M-E.R., G.G., G.F, J.B. and E.D. were involved in the

elaboration of the experimental design and collaborated to edit the manuscript. Claudia Casimiro and J-F Mauger performed the assays for leptin and ghrelin.

PART FIVE: REFERENCES AND APPENDICES

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APPENDIX A

Consent Forms

Chapters V and VI

Université d'Ottawa · University of Ottawa

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

Faculty of Health Sciences
School of Human Kinetics

CONSENT FORM

THE EFFECTS OF ACUTE CALORIC DEPRIVATION ON ODOUR IDENTIFICATION AND FOOD REWARD

Principal Investigator: Éric Doucet (Ph.D)
Research Coordinator: Jameason D. Cameron, M.Sc., Ph.D (candidate)
Faculty of Health Sciences, University of Ottawa
School of Human Kinetics

1. INVITATION TO PARTICIPATE:

You are invited to participate in the above named research study conducted by Éric Doucet Ph.D, and Jameason D. Cameron Ph.D. (candidate).

2. PURPOSE OF THE STUDY:

The purpose of this study will be to investigate whether acute caloric deprivation impacts 1) the ability to identify various familiar odours, and 2) the reward-related qualities of feeding. Measurements will be minimally invasive and will include anthropometry, appetite, odour identification, computer tasks, energy intake, and psychosocial questionnaires. Therefore, if you wish to take part in this study, the intended duration of your participation will be approximately 2 weeks, including 3 visits to the research unit.

3. BACKGROUND:

Few studies have concentrated on the acute effects of fasting (e.g. skipping a meal) on olfactory (smell) acuity and the reinforcing and rewarding value of food. Studies across many species of mammals indicate that even acute caloric deprivation can change the normal feeding response, either making the animal less selective on food choices, or making food items more salient. There are, however, methods to objectively record (alongside subjective reports with human subjects) these changes in the palatability ("liking") and the attractiveness ("wanting") of food items. Measurements during this protocol will include body composition, smell testing, appetite, and psychological and behavioural assessments. Results obtained from this study will enable us to better understand the physiological and behavioural changes that occur in conjunction with acute periods of fasting and to help elucidate why some individuals may be more susceptible to rewarding food cues, and possibly to overeating and lapses in appetite control. Also during this study, data will be collected via computer "games" in order to further understand how

acute caloric deprivation impacts the willingness to perform simple button-presses to obtain food and also how food selection is impacted (e.g. “wanting” of various food items). We aim to eventually be able to better describe the physiological and behavioural differences in those who are more susceptible to food cues (vs. those who for example demonstrate higher dietary restraint or perhaps diminished olfactory acuity) in an attempt to explain individual differences in the feeding response to conditions similar to the first days of dieting to lose weight.

4. DESCRIPTION OF THE STUDY:

Initial visit: You will be asked to visit the research unit for an initial visit of approximately 30 minutes during which the study as well as the consent form will be explained to you. You can then bring the consent form home so further reading and discussion with family members is made possible. Other questionnaires will also have to be filled out for screening purposes and your height and weight will also be measured. Results from these measurements will then be analyzed in order to determine if you correspond to the inclusion criteria of this present study. If you do correspond and agree to participate in this study, you will be asked to come to the research unit for 2 subsequent sessions, each lasting approximately 5 hours. These sessions of testing are described in detail below.

Session A: No Breakfast

A. Arrival at the laboratory (0800-0815)—You will arrive at the laboratory from an overnight fast from 1230 the previous day. Your height and body weight will be recorded and a single fasting blood sample will be taken by a qualified nurse in a sterilized environment.

B. Resting Energy Expenditure (0815-0900)—After a 20 minute resting period in the supine position a measurement of resting energy expenditure will be done. The measurement of resting metabolic rate takes place early in the morning after an overnight fast. A plexiglass hood will be placed over your head through which fresh air will be drawn. The expired air will be sampled for analysis and percentages of oxygen and carbon dioxide determined for 30 minutes. By measuring the flow rate, we will be able to determine the amount of oxygen that is consumed and derive energy expenditure. This test requires that you lie quietly and relaxed in bed for around 30 minutes. There are no risks associated with this procedure.

C. Appetite Ratings (0800-1300)—Appetite ratings will be measured every hour from time 0 (arrival) to time 300 (post lunch). This will be done using a pen and paper on a visual analogue scale. Briefly, desire to eat, hunger, fullness and prospective food consumption (PFC) and food appreciation will be rated. Questions will be asked as follows: 1) “How strong is your desire to eat?” (Very weak- Very strong); 2)“How hungry do you feel?” (Not hungry at all- As hungry as I have ever felt); 3) “How full do you feel?” (Not full at all- Very full), 4) “How much food do you think you could eat?” (Nothing at all- A large amount), and 5) “How did you appreciate this meal?” (Not at all-Very much).

D. Computer Task (i) (1030-1100)—You will be required to complete a computer task to assess the amount of work done for a particular reinforcer (snack or fruit/vegetable). The computer task is as follows. You will sit in front of a laptop with a joystick which allows you to switch from two separate screens, each representing an opportunity to work for food points: one screen is for your favourite snack food, and the other for your favourite fruit or vegetable. To earn the points you must work for them by pressing a button on the joystick, which in turns starts a slot machine. Points are earned when all three objects on the screen match.

E. Computer Task (ii) (1100-1130)—You will be required to complete a computer task to asses food selection and subjective ratings of various food items. The computer task is as follows. You will sit in front of a laptop with a mouse and mousepad. A series of food pictures will be presented to you and you will be required to answer a progression of questions asked by the computer program regarding these foods. All answers are recorded by selecting and clicking with the mouse.

F. Standardized Lunch (1140-1215)—You will be required to eat 2 slices of pepperoni pizza.

G. Computer Task (ii) (1215-1245)—You will be required to complete a second round of the computer task that assesses food selection and subjective ratings of various food items.

H. Smell Test (1245-1315)—You will be required to complete an odour identification test that is composed of 4 booklets, each with 10 microencapsulated odourants. You will rate the odourants on irritation, warmness/coolness, intensity, familiarity, and pleasantness/unpleasantness. Capsules are scratched by pencil tip to release the odour, and examples of some of the 40 odourants are tomato, honey, pizza, and peanut.

I. End of Session 1315.

Session B: Breakfast and Lunch

A. Arrival at the laboratory (0800-0900)—You will arrive at the laboratory from an overnight fast from 1230 the previous day. Your height and body weight will be recorded prior to the resting energy expenditure measure (as indicated in Part **B** in **Session A**).

B. Breakfast (0900-0930)—Breakfast will be composed of 2 pieces of toast, peanut butter, strawberry jam, and orange juice. All items must be consumed within 30 minutes,

C. Appetite Ratings 0800-1300—Appetite ratings will be measured every hour from time 0 (arrival) to time 300 (post lunch), and immediately before and after each meal. This will be done using a pen and paper on a visual analogue scale as indicated in Part **C** in **Session A**.

D. Computer Task (i) (1030-1100)—You will be required to complete a computer task to assess the amount of work done for a particular reinforcer (snack or fruit/vegetable) as indicated in Part **D** in **Session A**.

E. Smell Test (1130-1200)—You will be required to complete an odour identification test that is composed of 4 booklets, each with 10 microencapsulated odourants, as indicated in Part **H** in **Section A**.

F. Computer Task (ii) (1200-1230)—You will be required to complete a computer task to asses food selection and subjective ratings of various food items as indicated in Part **E** in **Session A**.

G. Standardized Lunch (1230-1250)—You will be required to eat 2 slices of pepperoni pizza.

H. Computer Game (ii) (1250-1315)—You will be required to complete a second round of the computer task that assesses food selection and subjective ratings of various food items as indicated in Part **E** in **Session A**).

I. End of Session 1315.

5. POSSIBLE RISKS/DISCOMFORTS:

The single sample of blood required during each of the 2 testing sessions presents very few risks. However, a small local hematoma (a bruise at the venal puncture) could develop during the few days following the blood sampling. Extending an overnight fast until the lunch hour may present some mild feelings of lightheadedness and mild discomfort, possibly due to nausea. It must be noted that each person responds differently to a fasting period, and that there is no risk associated with a fast other than discomfort.

6. BENEFITS:

Your participation in this study will allow you to gather information on your general smell performance and be able to indicate if you should seek further testing due to impaired olfactory functioning. Also, you will gain insight into what your resting energy expenditure is. With this information one could individually tailor a diet for performance or weight loss, for example.

7. MONETARY COMPENSATION:

Parking at the research center is free for participants, as are all scientific tests. Therefore you will be reimbursed any money paid for parking.

8. CONFIDENTIALITY AND ANONYMITY:

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

-Only the following persons will have access to the material: Principal Investigator, and Research Coordinator. Any other individuals involved in the study will not have access to participant's personal information and results.

-The names of participants will not appear on any reports. A number code will be used to identify participants on all research documents.

-All material and information which can be linked to participants will not be made public and will be kept under the strictest confidentiality.

-Participants will not be identified in any way in publications or reports.

-The data collected will be kept in a locked cabinet in the Behavioral and Metabolic Research Unit with restricted access where all participant's folders will be kept. In addition, the computer files will be protected by a password.

-Blood samples will be kept in the research unit's laboratory freezer. Blood samples will be identified by a number code which will not be retraced.

-Data will be destroyed and any blood samples eliminated five years after publication of study results.

9. VOLUNTARY PARTICIPATION:

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

Any information about your rights as a research participant may be addressed to: Protocol officer for ethics in research, University of Ottawa, 550 Cumberland, Tabaret Hall, room 159, Ottawa, Ontario, K1N 6N5; Phone: (613) 562-5841, email: ethics@uottawa.ca.

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

Please choose one of the following options:

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

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

RESEARCHER' SIGNATURE

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

RESEARCH COORDINATORS' SIGNATURE

Jameason Cameron, Ph.D.(candidate): _____ Date: _____

PARTICIPANT'S SIGNATURE:

I agree to participate in this study,

_____ _____ Date: _____

Printed Name Signature

Université d'Ottawa · University of Ottawa
Faculté des sciences de la santé | Faculty of Health Sciences
École des sciences de l'activité physique | School of Human Kinetics

FORMULAIRE DE CONSENTEMENT

LES EFFETS DE lLA DÉPRIVATION CALORIQUE SUR L'IDENTIFICATION DES ODEURS ET SUR LA GRATIFICATION PAR LA NOURRITURE

Chercheur principal: Éric Doucet (Ph.D)
Coordonnateur de recherche: Jameason D. Cameron, M.Sc., Ph.D. (candidat)
Fcaulté des sciences de l'activité physique, Université d'Ottawa
École des Sciences de l'activité physique

1. VOTRE PARTICIPATION:

Vous êtes invités à participer à cette étude. Les chercheurs impliqués dans cette étude sont: Éric Doucet, Ph.D, et Jameason Cameron
Ph.D.(candidat).

2. OBJECTIF DE L'ETUDE:

L'objectif de cette étude est d'examiner l'impact d'une privation calorique importante sur 1) la capacité à identifier différentes odeurs familières, et 2) à identifier les mécanismes de gratification par la nourriture. Les mesures seront minimalement invasive et comprendront des mesures anthropométriques, des mesures de l'apport énergétique ainsi que des mesures de l'appétit et d'identification d'odeurs. Des jeux sur ordinateur et des questionnaires psychosociaux seront également utilisés. Par conséquent, si vous souhaitez prendre part à cette étude, la durée approximative de votre participation s'échelonnera sur une période de 2 semaines, et comprendra 3 visites à l'unité de recherche.

3. HISTORIQUE:

Peu d'études ont investiguées les effets aigus du jeûne, sur la capacité olfactive,et sur le renforcement et la récompense de la valeur des aliments. Des études chez de nombreuses espèces de mammifères indiquent que même une privation calorique aiguë peut changer la réponse alimentation normale, et rendre l'animal moins sélectif sur les choix alimentaires et/ou rendre les aliment plus attrayant. Il y a, toutefois, des méthodes pour enregistrer de manière objective (avec des rapports subjectifs effectués chez des sujets humains) ces variations de palatabilité et d'attraction envers certains aliments. Les mesures effectuées pendant ce protocole comprennent des mesures de

poids corporelle, des tests d'odeur et d'appétit ainsi que des évaluations psychologiques et comportementales. Les résultats obtenus nous permettront de mieux comprendre les changements physiologiques et comportementaux qui surviennent parallèlement avec des périodes de jeûne aigus. Ainsi, nous serons en mesure d'élucider pourquoi certains individus sont plus sensibles aux signaux alimentaires de plaisir, qui éventuellement mènent à la suralimentation et à des lacunes en ce qui attrait au contrôle de l'appétit . Également au cours de cette étude, des données seront collectées via un ordinateur «jeux». Ceci permettra de mieux comprendre l'impacts de la privation calorique aiguë sur la volonté d'accomplir une tâche simple. Cette tâche concistera à presser un bouton afin de choisir un aliment et permettra de déterminer l'impact de la déprivation sur ce choix (par exemple, changement de préférence quant aux choix alimentaires). Notre objectif est d'être capables de mieux décrire les différences physiologiques et comportementales chez ceux qui sont plus sensibles aux signaux alimentaires par rapport à ceux qui, par exemple, se sont montrer supérieur quand à la restriction alimentaire ou quand à la diminution de capacité olfactive. Ainsi, nous tenterons d'expliquer les différences individuelles, utilisée lors des premiers jours de perte de poids, face à la restriction alimentaire.

4. DESCRIPTION DE L'ETUDE:

Visite initiale: Vous serez invité à visiter l'unité de recherche pour une visite initiale d'environ 20 minutes pendant laquelle l'étude ainsi que le formulaire de consentement sera expliqué. Vous pourez ensuite lire à la maison le formulaire de consentement et ainsi en discuter avec les membres de votre famille. D'autres questionnaires devront également être remplie pour fins de dépistage. Votre taille et votre poids seront également mesurés. Les résultats de ces mesures seront ensuite analysés afin de déterminer si vous remplissez les critères d'inclusion pour cette étude. Si vous correspondez et acceptez de participer à cette étude, il vous sera demandé de venir à l'unité de recherche pour 2 sessions; chacune d'une durée d'environ 5 heures. Ces session de tests sont décrites en détail ci-dessous :

Séance A: Aucun déjeuner

A. L'arrivée au laboratoire (0800-0815)—En arrivant au laboratoire, vous devrez être a jeun depuis 1230 (jour précédent). Votre taille et votre poids corporel seront mesurés et un échantillon de sang sera pris par une infirmière qualifiée dans un milieu stérile.

B. Dépense énergétique de repos (0815-0900)—Après une période de repos de 30 minutes en position couchée, une mesure de la dépense énergétique de repos sera effectuée. La dépense énergétique de repos à lieu tôt le matin après une nuit de jeûne. Un bulle en plexiglas, perfusés par de l'air frais, sera placée sur votre tête. L'air sera échantillonné pour ensuite être analysé. Les pourcentages d'oxygène et de dioxyde de carbone seront déterminé à chaque 30 minutes. En mesurant le débit, nous serons en mesure de déterminer la quantité d'oxygène consommé pour ensuite mesurer la dépense énergétique de repos. Pour ce test, vous devrez être calme et allongé dans un lit pendant environ 30 minutes. Il n'y a pas de risques associés à cette procédure.

C. Mesure de l'appetite (0800-1300)—Les mesures d'appétit seront analysées à chaque heure en commençant au temps 0 (arrivée) et terminant au temps 300 (déjeuner post). Ce test sera effectué avec un stylo et du papier sur une échelle visuelle analogique. En bref, l'envie de manger, la sensation de faim et la consommation alimentaire prospective (PFC) ainsi que l'appréciation des aliments seront cotés. Les questions serons demandées comme suit: 1) "Dans quelle mesure avez-vous envie de manger?" (Envie très faible- Envie très fort); 2) "Dans quelle mesure avez-vous l'impression d'avoir faim?" (Envie très faible- Envie très forte); 3) "À quel point vous sentez-vous rempli?" (Pas rempli du tout- Très rempli), et 4) " Quelle quantité de nourriture pourriez-vous manger immédiatement?" (Absolument rien- Une grande quantité).

D. Tâche d'ordinateur (i) (1030-1100)— Vous serez demandé de compléter une tâche à l'ordinateur afin de déterminer le montant de travail que vous serez prêt à compléter pour un renforcement en particulier (collation ou fruit/légume), qui sera donné vers la fin de la session. La tâche sera la suivante: vous devrez vous asseoir devant un ordinateur portatif avec une manette qui vous permettra de changer entre deux écrans séparés. Chaque écran vous donnera l'opportunité de travailler pour des points afin de recevoir de la nourriture. Un écran sera pour votre collation favorite et l'autre sera pour votre fruit ou légume favori. Pour gagner les points vous devrez travailler en appuyant sur la touche de la manette, qui par la suite changera à un écran de machine à sous. Les points seront gagnés quand les trois figures à l'écran seront identiques.

E. Tâche d'ordinateur (ii) (1100-1130)—Vous serez tenus de remplir une tâche à l'ordinateur qui nous permettra d'évaluer vos choix des aliments ainsi que d'en faire une évalusation évaluation subjective. La tâche effectuée à l.ordinateur est la suivante. Vous serez assis devant un ordinateur portable avec une souris et un tapis de souris. Une série de photos d'aliments vous sera présenté et vous devrez répondre à une série de questions posées par le programme informatique concernant des aliments. En sélectionnant ces aliments et en cliquant avec la souris, vos réponses seront enregistrées.

F. Déjeuner standardisé(1140-1215)—Il vous sera demandé de manger 2 tranches de pizza au pepperoni.

G. Tâche d'ordinateur(ii) (1215-1245)—Il vous sera demandé de refaire la tâche à l'ordinateur (E) qui évalue la sélection des aliments et l'appréciation subjectives de ces divers aliments.

H. Test d'odorat (1245-1315)—Vous serez tenus de remplir un test d'identification d'odeur qui est composé de 4 livrets, chacun avec 10 odeurs micro-encapsulés. Vous catégoriserez l'odeur en fonction de l'irritation, de la chaleur / du froid, de l'intensité, de la familiarité, et de l'agrément / désagrément. Les capsules seront ouverte en appuyant

avec par pointe de crayon pour libérer les odeurs. Par exemples, quarante de ces odeurs seront la tomate, le miel, la pizza et le beurre d'arachide.

I. Fin de la session 1315.

Session B: Petit-déjeuner et déjeuner

A. L'arrivée au laboratoire (0800-0900)—Lorsque vous arriverez au laboratoire, vous devrez être a jeun depuis 1230 (jour précédent). Votre taille et votre poids corporel seront enregistrée avant la mesure la dépense énergétique de repos (comme indiqué dans la Partie **B** de la **Session A**).

B. Petit déjeuner (0900-0930)—Le petit déjeuner sera composé de 2 tranches de pain grillé, du beurre d'arachide, de la confiture de fraise et d'un verre de jus d'orange. Tous les articles devront être consommés en 30 minutes.

C. Mesure de l'appetite 0800-1300— Les mesures d'appétit seront analysées à chaque heure en commençant au temps 0 (arrivée) et terminant au temps 300 (déjeuner post). Ce test sera effectué avec un stylo et du papier sur une échelle visuelle analogue (comme indiqué dans la Partie **C** de la **Session A**).

D. Tâche d'ordinateur (i) (1030-1100)—Vous serez tenus de remplir une tâche informatiques pour évaluer la quantité de travail effectué pour un renforcement particulier (collation ou de fruits / légumes) (comme indiqué dans la Partie **D** de la **Session A**).

E. Test d'odeur (1130-1200)—Vous serez tenus de remplir un test d'identification d'odeur qui est composé de 4 livrets, chacun avec 10 odeurs micro-encapsulés (comme indiqué dans la Partie **E** de la **Session A**).

F. Tâche d'ordinateur (ii) (1200-1230)— Il vous sera demandé de refaire la tâche à l'ordinateur (E) qui évalue la sélection des aliments et l'appréciation subjectives de ces divers aliments (comme indiqué dans la Partie **F** dans la **Session A**).

G. Déjeuner standardisé(1230-1250)—Il vous sera demandé de manger 2 tranches de pizza au pepperoni.

H. Tâche d'ordinateur (ii) (1250-1315)—Il vous sera demandé de refaire la tâche à

l’ordinateur qui évalue la sélection des aliments et l’appréciation subjectives de ces divers aliments (comme indiqué dans la Partie **F** dans la **Session A**).

I. Fin de la session 1315.

5. QUELS SONT LES RISQUES / MALAISES:

Le seul échantillon de sang nécessaire pendant chacune des 2 sessions de test présente très peu de risque. Toutefois, un hématome local de petite taille (une contusion à la ponction vénale) pourraient se développer dans les jours qui suivent le prélèvement sanguin. Être à jeun peu entraîner quelques vertige, de l'inconfort et quelques nausées. Il faut cependant noter que chaque personne réagit différemment à une période de jeûne, et qu'il n'y a pas de risque associé à un jeûne autre que l'inconfort.

6. AVANTAGES:

Votre participation à cette étude nous permettra de recueillir des informations sur les performances de votre capacité olfactive. Nous serons en mesure d'indiquer si vous devriez demander des essais supplémentaires en raison de fonctionnement olfactive avec facultés affaiblies. Aussi, vous aurez un aperçu de votre dépense énergétique de repos. Avec cette information, il serait, par exemple, possible de vous prescrire individuellement une alimentation afin d’accroître vos performances ou pour augmenter votre perte de poides.

7. COMPENSATION MONETAIRE:

Le stationnement au centre de recherche est gratuit pour les participants, ainsi que les tests scientifiques. Par conséquent, votre stationnement vous sera remboursé.

8. CONFIDENTIALITE et ANONYMAT :

Afin de garantir la confidentialité et l'anonymat des participants, toutes les précautions et mesures nécessaires seront prises pour s'assurer que les résultats et les informations personnelles des participants sont maintenu sous la plus stricte confidentialité.

-Seules les personnes suivantes ont accès au matériel: Chercheur principal et coordonnateur de la recherche. Toutes les autres personnes impliquées dans l'étude n'auront pas accès aux renseignements personnels des participants et des résultats.

-Les noms des participants n‘apparaîtra pas sur les rapports. Un code numérique sera utilisé pour identifier les participants sur tous les documents de recherche.

-Tous les documents et informations qui peuvent être liés à des participants ne seront pas rendus publics et seront tenus, sous la plus stricte confidentialité.

-Les participants ne seront pas identifiés en aucune manière dans les publications ou les rapports.
-Les données collectées seront conservées dans une armoire verrouillée à accès restreint dans l'Unité de recherche sur le métabolisme et le comportement où les dossiers de tous les participants seront conservés. En outre, les fichiers informatiques seront protégés par un mot de passe.

-Des échantillons de sang seront conservé dans le congélateur du laboratoire de l'unité de recherche. Des échantillons de sang seront identifiés par un numéro de code qui ne pourra pas être retracé.

-Les données seront détruites et le prélèvement sanguin sera éliminé cinq ans après la publication des résultats de l'étude.

9. PARTICIPATION VOLONTAIRE :

Vous êtes libre de refuser de participer et si vous décidez de participer, vous êtes libre de vous retirer de l'étude à tout moment pour n'importe quelle raison. À tout moment, au cours de cette étude, le meilleur intérêt des participants se fera toujours prévaloir sur les objectifs de l'étude. De plus, vous serez mis au courant des nouvelles découvertes qui pourraient influencer votre décision de prendre part à la présente étude.

Toute information sur vos droits en tant que participant à la recherche doivent être adressées au: Responsable d'éthique dans la recherche, Université d'Ottawa, 550 Cumberland, Pavillon Tabaret, pièce 159, Ottawa, Ontario, K1N 6N5, téléphone: (613) 562-5841, email: ethics@uottawa.ca.

Il existe deux exemplaires de formulaire de consentement, dont un que vous pouvez conserver.

S'il vous plaît choisir l'une des options suivantes:

Si je décide de me retirer de l'étude, je veux que toutes les données recueillies jusqu'au moment du retrait soient détruites □

Même si je me retire de l'étude, j'accepte que les données □

recueillies soient utilisées pour cette étude

CHERCHEUR 'SIGNATURE
Éric Doucet, Ph.D.: _____ Date: _____

RECHERCHE DES COORDINATEURS DE SIGNATURE

Jameason Cameron, Ph.D. (candidate): _____ Date: _____

SIGNATURE DU PARTICIPANT:
Je suis d'accord pour participer à cette étude,
_____ _____ Date: _____
Nom en lettres moulées Signature

APPENDIX B

Recruitement Posters

Chapters V and VI

uOttawa
L'Université canadienne
Canada's university

URCM
BMRU

Want to participate in research?

Looking for individuals to participate in a study on the effects of energy deprivation on smell identification and food palatability

Benefits Include: Assessment of Smell function

- ✓ Assessment of Resting Energy Expenditure

Selection Criteria:

- ✓ Men and women aged 18 to 35 years old
- ✓ No major health problems
- ✓ Non-smokers
- ✓ Stable body weight for the last 6 months (± 2kg)

For more information, please call Jameason

Behavioural and Metabolic Research Unit (BMRU)
Lees Campus Block E - University of Ottawa

uOttawa
L'Université canadienne
Canada's university

URCM
BMRU

Vous voulez participer à la recherche?

La recherche de personnes de participer à une étude sur les effets de la privation d'énergie sur l'identification odeur et la palatabilité des aliments

Avantages comprennent:

- ✓ La fonction d'évaluation de l'odora
- ✓ Évaluation de dépense énergétique de repos

Critères de sélection:

- ✓ Les hommes et les femmes âgées de 18 à 35 ans
- ✓ Pas de problèmes de santé majeurs
- ✓ Les non-fumeurs
  - ✓ Poids stable pendant les 6 derniers mois (± 2 kg)

Pour de plus amples renseignements, s'il vous plaît veuilled appeler Jameason
Behavioural and Metabolic Research Unit (URCM)

Lees Campus Bloc E - Université d'Ottawa

APPENDIX C

Consent Forms

Chapter VII

Université d'Ottawa · University of Ottawa
Faculté des sciences de la santé
École des sciences de l'activité physique
Faculty of Health Sciences
School of Human Kinetics

CONSENT FORM

THE IMPACT OF MODALITY OF ENERGY DEPRIVATION ON OLFACTION, FOOD REWARD, AND AD LIBITUM ENERGY INTAKE: EXERCISE VS. DIETING IN THE REWARD CONTINUUM

Principal Investigator: Éric Doucet (Ph.D)
Research Coordinator: Jameason D. Cameron, M.Sc., Ph.D (candidate)
Faculty of Health Sciences, University of Ottawa
School of Human Kinetics

1. INVITATION TO PARTICIPATE: You are invited to participate in the above named research study conducted by Éric Doucet Ph.D, and Jameason D. Cameron Ph.D. (candidate).

2. PURPOSE OF THE STUDY: The purpose of this study will be to investigate whether the modality of caloric deprivation impacts 1) olfactory performance, and 2) the reward-related qualities of feeding. Measurements will be minimally invasive and will include anthropometry, fasting blood samples, appetite, olfactory performance, computer game, energy intake, and psychosocial questionnaires. Therefore, if you wish to take part in this study, the intended duration of your participation will be approximately 4 weeks, or a total of approximately 30 hours.

3. BACKGROUND: Few studies have examined if the modality of energy deprivation (i.e. exercising alone vs. dieting alone) can differently impact the rewarding value of food and olfactory performance. Studies across many species of mammals indicate that even acute caloric deprivation can change the normal feeding response, either making the animal less selective on food choices, or making food items more salient. There are, however, methods to objectively record (alongside subjective reports with human subjects) these changes in the palatability ("liking") and the attractiveness ("wanting") of food items. Measurements during this protocol will include body composition, smell testing, appetite, and psychological and behavioural assessments. Results obtained from this study will enable us to better understand the physiological and behavioural changes that occur in conjunction with acute periods of energy deprivation. Furthermore, the anticipated results could help us understand the effects of modality of weight loss on subsequent energy intake (e.g. macronutrient preference and ad libitum feeding), and to examine if weight loss modality, in first several days of energy deprivation, differs in the potential negative impact not only one's food choices (e.g. choosing energy dense snacks vs. healthier alternatives), but also overall energy intake and energy balance.

4. DESCRIPTION OF THE STUDY: Initial visit: You will be asked to visit the research unit for an initial visit of approximately 30 minutes during which the study as well as the

consent form will be explained to you. You can then bring the consent form home so further reading and discussion with family members is made possible. Other questionnaires will also have to be filled out for screening purposes and your height and weight will also be measured. Results from these measurements will then be analyzed in order to determine if you correspond to the inclusion criteria of this present study. If you do correspond and agree to participate in this study, you will be asked to come to the research unit for 3 subsequent half day sessions, and 6 sessions of approximately 1.5 hours. These sessions of testing are described in detail below.

I) Control, Deprivation by Exercise, and Deprivation by Diet Sessions (3 sessions)

A. Arrival at the laboratory (0800)—You will arrive at the laboratory from an overnight fast from 1900 the previous day. Your height and body weight will be recorded and a single fasting blood sample will be taken by a qualified nurse in a sterilized environment.

B. Body Composition (0800-0820)— Body weight and height will be taken with a digital scale and a wall stadiometer, respectively. A method called dual energy x-ray absorptiometry (DXA) will be used to measure body fat percentage and muscle mass. Participants will have to lie on an examination table, clothed in a hospital gown, while a low-intensity x-ray scans the entire body. The measurement will take less than 10 minutes. The radiation associated with this measurement is less than 0.02-0.05 millirem, which corresponds to one day of exposure to sunlight.

C. Resting Energy Expenditure (0830-0900)— After a 30 minute resting period in the supine position a measurement of resting energy expenditure will be done. The measurement of resting metabolic rate takes place early in the morning after an overnight fast. A plexiglass hood will be placed over your head through which fresh air will be drawn. The expired air will be sampled for analysis and percentages of oxygen and carbon dioxide determined for 30 minutes. By measuring the flow rate, we will be able to determine the amount of oxygen that is consumed and derive energy expenditure. This test requires that you lie quietly and relaxed in bed for around 30 minutes. There are no risks associated with this procedure.

D. Appetite Ratings (0800-1300)—Appetite ratings will be measured every hour from time 0 (arrival) to time 300 (post lunch). This will be done using a pen and paper on a visual analogue scale. Briefly, desire to eat, hunger, fullness and prospective food consumption (PFC) and food appreciation will be rated. Questions will be asked as follows: 1) “How strong is your desire to eat?” (Very weak- Very strong); 2)“How hungry do you feel?” (Not hungry at all- As hungry as I have ever felt); 3) “How full do you feel?” (Not full at all- Very full), 4) “How much food do you think you could eat?” (Nothing at all- A large amount), and 5) “How did you appreciate this meal?” (Not at all-Very much).

E. Breakfast (0900-0915) —Breakfast will be composed of 2 pieces of toast, peanut butter, strawberry jam, and orange juice. All items must be consumed.

F. Smell Test (1245-1315)—You will be required to complete an olfactory performance test that is composed of pen-like devices that have various odours which can be smelled. This test is non-invasive and is simply composed of presenting each “Sniffin Stick” in front of your nose for you to smell.

G. Computer Game (i) (1030-1100)—You will be required to complete a computer task to assess the amount of work done for a particular reinforcer (snack or fruit/vegetable). The computer task is as follows. You will sit in front of a laptop with a joystick which allows you to switch from two separate screens, each representing an opportunity to work for food points: one screen is for your favourite snack food, and the other for your favourite fruit or vegetable. To earn the points you must work for them by pressing a button on the joystick, which in turns starts a slot machine. Points are earned when all three objects on the screen match.

H. Computer Game (ii) (1100-1130)—You will be required to complete a computer task to asses food selection and subjective ratings of various food items. The computer task is as follows. You will sit in front of a laptop with a mouse and mousepad. A series of food pictures will be presented to you and you will be required to answer a progression of questions asked by the computer program regarding these foods. All answers are recorded by selecting and clicking with the mouse.

I. Standardized Lunch (1140-1215)—You will be required to eat 2 slices of cheese pizza.

J. Computer Game (ii) (1215-1230)—You will be required to complete a second round of the computer task that assesses food selection and subjective ratings of various food items.

K. Ad Libitum Dessert Buffet (1230-1300)—Participants will be offered an all-you-want-to-eat buffet consisting of individually tailored favourite snack foods and favourite fruits and/or vegetables.

L. End of Session 1300.

II) Baseline Session for Control Arm (BL1):

A. Arrival at the Laboratory (0800)—You will arrive at Lees at 0800 fasted from 1900 the previous evening. While seated comfortably, a nurse will take a resting blood sample.

B. Body Composition (0800-0820)—See part B above (section I).

C. Resting Energy Expenditure (0830-0900)—See part C above (section I).

D. V02MAX (0900-1000)—The test will be performed by the simple act of running on a treadmill; the level of exercise will be increased every three minutes by increasing the incline or speed of the treadmill, which will result in increased difficulty and effort . The test

is stopped when you reach exhaustion; generally exhaustion is achieved within 10-15 minutes and is defined when you cannot maintain the pace of running during a given interval. Research personnel conducting the test will be certified in CPR and will have the necessary certification from the Canadian Society for Exercise Physiology (CSEP).

E. End of Session (1000)

III) Deprivation by Exercise (BL2):

A. Arrival at the Laboratory (0800)—You will arrive at Lees at 0800 fasted from 1900 the previous evening. While seated comfortably, a nurse will take a resting blood sample.

B. Body Weight and Exercise (0820-0930)—Body weight will be measured on a standard scale. Afterwords, the first of the four consecutive exercise sessions will be instructed/monitored by an individual with CSEP certification. Each exercise session will be at a rate proportional to approximately 65-70% of your maximal aerobic capacity (V02MAX) and will be performed by jogging on a treadmill. After exercise completion, you will be reminded that this same exercise program will be administered for the following 3 days, for a total of 4 days of exercise. Note that the 3 exercise sessions after BL2 are flexible partly to help cater to busy schedules.

C. Access to Shower (0930-1000)—The BMRU has several brand new showers and locker rooms where you can freshen up after each of the four exercise sessions.

D. End of BL2 (1000)

IV) Deprivation by Diet (BL3):

A. Arrival at the Laboratory (0800)—You will arrive at Lees at 0800 fasted from 1900 the previous evening. While seated comfortably, a nurse will take a resting blood sample.

B. Body Weight and Diet (0820-0900)—Body weight will be measured on a standard scale. Afterwords, the 4 day diet will be explained to you; the 30% calorically reduced diet will maintain macronutrient specific percentages according to the Canadian Diabetes Association with the intention of increasing the external validity of the energy reduced diet.

C. End of BL3 (0900)

5. POSSIBLE RISKS/DISCOMFORTS:

The 6 fasting blood samples required for the testing sessions presents very few risks. However, a small local hematoma (a bruise at the venal puncture) could develop during the few days following the blood sampling. When performing a maximal treadmill test, you may feel tired and feel muscle soreness, and may experience a feeling of breathlessness. Sore muscles and a feeling of tiredness may last for a few days.

However, these are common effects of exercise, even more common in those who do not engage in regular physical activity. Nevertheless it should be pointed out that these procedures will be performed by a researcher with a CSEP qualification. Also, regarding the risks for the body composition scan, despite the fact that the risks associated to radiation exposure from body composition measurement (DXA scan) is extremely low (equivalent to 1 day in the sun, 0.02-0.05 milirem), it is important to note that the DXA measure will only be performed three times over the course of the study.

6. BENEFITS:

Several of the measures we would employ for this study provide you with advanced tools and scientific information that help promote your own physical and mental health. For example, the V02MAX test is a tool used in professional sports settings and can cost young athletes upwards of $200.00. We will be offering this test as part of the inclusion. Likewise, our DXA tool is the industry gold standard used to measure body composition, and can cost young athletes or anyone interested upwards of $60.00 too. We will also be measuring resting energy expenditure with indirect calorimetry. Combining all three of the above-mentioned tools we are able to offer you a very good idea of the amount of energy intake, and in combination with energy expenditure, a very good picture of how to keep each participant in energy balance (for example, to maintain a healthy body weight).

7. MONETARY COMPENSATION:

Parking at the research center is free for participants, as are all scientific tests. Therefore you will be reimbursed any money paid for parking. Also, you will be compensated a total of $100.00 upon the completion of the study. Anticipating the possibility of recidivism, you will be offered $10.00/per session completed, or a total of $100.00 upon completion.

8. CONFIDENTIALITY AND ANONYMITY:

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

-Only the following persons will have access to the material: Principal Investigator, and Research Coordinator. Any other individuals involved in the study will not have access to participant's personal information and results.

-The names of participants will not appear on any reports. A number code will be used to identify participants on all research documents.

-All material and information which can be linked to participants will not be made public and will be kept under the strictest confidentiality.

-Participants will not be identified in any way in publications or reports.

-The data collected will be kept in a locked cabinet in the Behavioral and Metabolic Research Unit with restricted access where all participants' folders will be kept. In addition, the computer files will be protected by a password.

-Blood samples will be kept in the research unit's laboratory freezer. Blood samples will be identified by a number code which will not be retraced.

-Data will be destroyed and any blood samples eliminated five years after publication of study results.

9. VOLUNTARY PARTICIPATION

You are free to refuse to participate and if you choose to participate, you are free to withdraw from the study at any time for any reason. At any moment during this study, the best interests of participants will always prevail upon the objectives of the study.

Any information about your rights as a research participant may be addressed to: Protocol officer for ethics in research, University of Ottawa, 550 Cumberland, Tabaret Hall, room 159, Ottawa, Ontario, K1N 6N5; Phone: (613) 562-5841, email: ethics@uottawa.ca.

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

Please choose one of the following options:

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

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

Researcher' signature

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

Research coordInators' Signature

Jameason Cameron, Ph.D.(candidate): _____ Date: _____

PARTICIPANT'S SIGNATURE:
I agree to participate in this study,
_____ _____ Date: _____
Printed Name Signature

Université d'Ottawa · University of Ottawa

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

Faculty of Health Sciences
School of Human Kinetics

FORMULAIRE DE CONSENTEMENT

L'IMPACT DES MODALITES DE privation d'énergie sur l'olfaction, la récompense à la nourriture et l'apport énergétique *ad libitum*: EXERCICE VS. REGIME DANS UN CONTINUUM DE RÉCOMPENSE

Chercheur principal: Éric Doucet (Ph.D)
Coordonnateur de recherche: Jameason D. Cameron, M.Sc., Ph.D. (candidate)
Faculty of Health Sciences, Université d'Ottawa
School of Human Kinetics

1. VOTRE PARTICIPATION: Vous êtes invités à participer à l'étude mentionné ci-haut. Les chercheurs impliqués dans cette étude sont: Éric Doucet, Ph.D, et Jameason Cameron Ph.D. (candidats).

2. OBJECTIF DE L'ETUDE: L'objectif de cette étude sera d'examiner si une modalité de déprivation calorique aura un impact sur 1) la performance de l'odorat, et 2) la récompense des qualités liées à l'alimentation. Les mesures seront minimalement invasive et comprendra l'anthropométrie, mesure sanguine, l'appétit, l'identification des odeurs, jeux sur ordinateur, l'apport énergétique, et des questionnaires psychosociaux. Par conséquent, si vous souhaitez prendre part à cette étude, la durée prévue de votre participation sera d'environ 4 semaines, ou d'une durée approximative de 30 heures.

3. HISTORIQUE: Peu d'étude ont examiné si différentes modalités de la déprivation énergétique (*i.e*. exercise seul vs diète seule) pouvaient avoir un impact sur la gratification et sur la performance de l'odorat. Des études chez de nombreuses espèces de mammifères indiquent que même la privation aiguë calorique peut changer la réponse alimentation normale, soit rendre l'animal moins sélectif sur les choix alimentaires, ou de rendre les produits alimentaires les plus frappants. Il ya, toutefois, des méthodes pour enregistrer de manière objective (aux côtés des rapports subjectifs avec des sujets humains), ces variations à la sapidité («goût») et le plus attrayant («vouloir») d'articles alimentaires. Mesures pendant ce protocole comprennent la composition corporelle, les tests odeur, l'appétit et des évaluations psychologiques et comportementales. L'anticipation de ces résultats pourront aidé à comprendre l'impact de ces différentes modalités de perte de poids sur la prise alimentaire subséquente (e.g. préférence macronutritionnelles et prise alimentaire ad libitum). Aussi il nous sera possible d'examiner si les modalités de perte de poids, suivant les quelques jours de déprivation, vont avoir un

impact sur les choix alimentaires (aliments à haute densité vs aliments favorable pour la santé) mais aussi sur la prise alimentaire totale et sur la balance énergétique.

4. DESCRIPTION DE L'ETUDE: Visite initiale: Vous serez invité à visiter l'unité de recherche pour une visite initiale d'environ 30 minutes pendant lesquelles l'étude ainsi que le formulaire de consentement sera expliqué. Vous pouvez ensuite mettre la maison formulaire de consentement pour la lecture et de discussions approfondies avec les membres de la famille est rendue possible. D'autres questionnaires devront également être remplie pour fins de dépistage et de votre taille et votre poids sera également mesurée. Les résultats de ces mesures seront ensuite analysés afin de déterminer si vous correspondez aux critères d'inclusion de cette étude. Si vous correspondez et acceptez de participer à cette étude, il vous sera demandé de venir à l'unité de recherche pour 2 sessions, chacune durant environ 5 heures. Ces sessions de tests sont décrites en détail ci-dessous.

Séance A: Aucun déjeuner

A. L'arrivée au laboratoire (0800)—Vous arriverez au laboratoire à partir d'une nuit rapide à partir de 1900 le jour précédent. Votre taille et votre poids corporel sera enregistré et un seul échantillon de sang à jeun sera prise par une infirmière qualifiée dans un milieu stérilisé.

B. Composition corporelle (0800-0820)—Votre poids corporel et votre taille seront mesurés avec une balance digitale et un indicateur de niveau placé sur le mur, respectivement. La méthode du DXA (Dual Energy X-ray Absorptiometry) sera utilisée afin de mesurer votre densité osseuse, votre pourcentage de graisse et votre masse musculaire. Pour ce faire, vous devrez vous coucher, vêtu d’une jaquette d’hôpital, sur une table d’examen pendant qu’un rayon-x de faible intensité parcourra votre corps de la tête aux pieds. La durée totale de ce test sera de 10 minutes. La radiation associée à cette mesure correspond à moins de 0.02-0.05 millirem, ce qui équivaut à une journée d’exposition au soleil.

C. Dépense énergétique de repos (0830-0900)—Après une période de repos de 30 minutes en position couchée une mesure de la dépense énergétique au repos sera fait. La mesure du taux métabolique au repos a lieu tôt le matin après une nuit de jeûne. Un capot en plexiglas sera placé sur votre tête à travers lequel l'air frais sera tiré. L'air expiré seront échantillonnés pour l'analyse et les pourcentages d'oxygène et de dioxyde de carbone déterminé pour 30 minutes. En mesurant le débit, nous serons en mesure de déterminer la quantité d'oxygène qui est consommé et d'en tirer la dépense énergétique. Ce test nécessite que vous mentez calme et détendu dans son lit pendant environ 30 minutes. Il n'ya pas de risques associés à cette procédure.

D. Notes Appetite (0800-1300)—Cotes appétit sera mesurée à chaque heure du temps

0 (arrivée) au service 300 (déjeuner post). Ce sera fait en utilisant un stylo et un papier sur une échelle analogique visuelle. En bref, l'envie de manger, la faim, la plénitude et la consommation alimentaire prospective (PFC) et l'appréciation des aliments sera coté. Des questions seront posées comme suit: 1) «Quelle est la force de votre désir de manger?" (Très faible-Très fort), 2) «Comment vous sentez-vous faim?" (Pas faim du tout aussi affamé que je n'ai jamais senti), 3) «Comment vous sentez-vous plein?" (pas plein du tout, très complet), 4) «Quelle quantité de nourriture pensez-vous que vous pourriez manger?" (Rien du tout, un grand nombre), et 5) " Comment avez-vous apprécier ce repas? "(Pas du tout, beaucoup).

E. Petit déjeuner (0900-0915)—Le petit déjeuner sera composé de 2 morceaux de pain grillé, beurre d'arachide, confiture de fraise et un jus d'orange. Tous les articles doivent être consommés.

F. Smell Test (1245-1315)—Vous aurez à completer un test de performance olfactive. Des crayons contenants différentes odeurs vous seront présentés. Ce test est non-invasif et l'objectif est seulement de vous faire sentir ces crayons.

G. Computer Game (i) (1030-1100)—Vous serez tenus de remplir une tâche informatiques pour évaluer la quantité de travail effectué pour un renforcement particulier (snack ou de fruits / légumes). La tâche ordinateur est la suivante. Vous serez assis devant un ordinateur portable avec un joystick qui vous permet de passer de deux écrans distincts, dont chacun représente une opportunité de travailler pour les points de l'alimentation: un écran est pour votre grignotine préférée, et l'autre pour votre fruit préféré ou de légumes. Pour gagner des points que vous devez travailler pour eux en appuyant sur un bouton du joystick, qui tourne en démarre une machine à sous. Les points sont obtenus lorsque les trois objets sur le match à l'écran.

H. Computer Game (ii) (1100-1130)—Vous serez tenus de remplir une tâche ordinateur pour évaluer le choix des aliments et évaluations subjectives de divers aliments. La tâche ordinateur est la suivante. Vous serez assis devant un ordinateur portable avec une souris et un tapis de souris. Une série de photos des aliments sera présentée à vous et il vous sera demandé de répondre à une progression de questions posées par le programme informatique concernant ces aliments. Toutes les réponses sont enregistrées en sélectionnant et en cliquant avec la souris.

I. Normalisé Déjeuner (1140-1215)—Il vous sera demandé de manger 2 tranches de pizza au fromage.

J. Computer Game (ii) (1215-1230)—Il vous sera demandé de remplir un second tour de la tâche ordinateur qui évalue la sélection des aliments et des évaluations subjectives de divers aliments.

K. Buffet de dessert *ad libitum* (1230-1300)—Une buffet de type "all you can eat" contenant vos collations ainsi que vos fruits et légumes favoris vous sera offert.

L. Fin de la session 1300.

II) Session préliminaire pour le control des bras (BL1):

A. L'arrivée au laboratoire (0800)—Vous arriverez au laboratoire à partir d'une nuit rapide à partir de 1900 le jour précédent. Votre taille et votre poids corporel sera enregistré et un seul échantillon de sang à jeun sera prise par une infirmière qualifiée dans un milieu stérilisé.

B. Body Composition (0800-0820)—Voir ci-dessus section B (Session I).

C. Resting Energy Expenditure (0830-0900)—Voir ci-dessus section C (Session I).

D. V02MAX (0900-1000)—Ce test sera effectué sur un tapis roulant. L'intensité de l'exercice sera augmentée (vitesse et pente) à chaque trois minute ce qui aura pour conséquence d'augmenter la difficulté et l'effort. Le test se terminera lorsque vous atteignerez l'épuisement, habituellement entre 10 à 15 minutes ou lorsque vous ne pourrez plus maintenir la vitesse durant un interval donné. Le personnel de recherche sera certifié CPR et aura la certification de la Société Canadienne de Physiology en Exercice (CSEP).

B. Poids corporel et exercice (0820-0930)— Votre poids corporel et votre taille seront mesurés avec une balance digitale et un indicateur de niveau placé sur le mur, respectivement. Ensuite, le premier des quatre exercises consécutifs sera monitoré et supervisé par un individu qui détient la certification CSEP. Cette session se fera à 65-70 % de votre capacité maximale à l'effort (Vo2max) et sera exécuté en pulsant sur un tapis roulant. Lorsque l'exercice sera compléte, vous aurez un rappel stipulant que ce programme d'exercise sera administré les 3 jours suivants, et donc pour un total de 4 jours. Notez que les 3 sessions d'exercise après le BL2 sont flexible et facilement adaptable aux horaires charges.

C. Accès au douche (0930-1000)—Vous aurez à votre disposition des douches ainsi que des cases barrés que vous pourrez utiliser suite à votre session d'exercise.

D. Fin de la session (1000)

IV) Déprivation par la diète (BL3):

A. L'arrivée au laboratoire (0800)—Vous arriverez au laboratoire à partir d'une nuit rapide à partir de 1900 le jour précédent. Votre taille et votre poids corporel sera enregistré et un seul échantillon de sang à jeun sera prise par une infirmière qualifiée dans un milieu stérilisé.

B. Poids corporel et diète (0820-0900)— Votre poids corporel et votre taille seront mesurés avec une balance digitale et un indicateur de niveau placé sur le mur, respectivement. Ensuite, une diète qui réduira votre apport alimentaire de 30 % et maintiendra les pourcantages de macronutriments recommandés selon la Canadian Diabetes Association vous sera expliqué.

C. Fin de la session (0900)

5. QUELS SONT LES RISQUES / MALAISES:

Les six échantillons sanguins pris présentent peu de risque. Toutefois, un hématome local de petite taille (une contusion à la ponction vénale) pouvaient se développer dans les jours qui suivent le prélèvement sanguin. Les risques associés à cette mesure sont la dyspnée (difficulté à respirer), la fatigue extrême et des douleurs musculaires. Quoique peu probable, des arythmies cardiaques et même un infarctus peuvent survenir lors de ce genre d'épreuve. Afin de prévenir ces situations, un électrocardiogramme sera inspecté avec minutie tout au long du test et ce dernier sera arrêté si une anormalité se présente. Le matériel nécessaire pour intervenir en situation d'urgence sera disponible dans le local où aura lieu cette mesure. Le personnel de recherche qui effectuera le test sera certifié en RCR et aura la certification adéquate (CSEP) de la Société Canadienne de Physiologie de l'Exercice. En dépit du fait que cette épreuve est très intense, il est peu probable qu'une situation d'urgence se produise. Il importe toutefois de souligner que cet appareil vous exposera à un minimum de radiation (l'équivalent d'une journée au soleil – 0.02-0.05 millirem).

6. AVANTAGES:

Votre participation à cette étude vous permettra de recueillir de l'information quant à votre santé physique et mentale. Le Vo2max est un outil utilisé par les sportifs professionnels et peut coûter jusqu'à 200.00$ à un athlète pour compléter le test. Ce test vous sera offert à l'inclusion. Votre composition corporelle mesuré avec le meilleur outil, jusqu'à présent sur le marché, est aussi un test très couteaux (60$) et privilégié par les athlètes. Votre métabolisme de repos avec calorimétrie indirecte sera aussi mesuré. En combinant ces 3 tests, nous serons en mesure de vous offrir une bonne idée de vos besoin qu'en à votre apport calorique lorsque vous pratiquez ou par une activité physique. Finalement, nous pourrons avoir une bonne idée sur comment vous pouvez maintenir votre balance énergétique (par exemple, maintenir un poids santé).

7. COMPENSATION MONETAIRE:

Parking au centre de recherche est gratuite pour les participants, comme le sont tous les tests scientifiques. Par conséquent, vous serez remboursé de l'argent versé pour le stationnement. Vous aurez une compensation monétaire de 100.00$ lorsque vous aurez

complété le projet. Si vous ne pouvez continuer le projet de recherché, une compensation de 10$ par session vous sera offert.

8. CONFIDENTIALITE et ANONYMAT

Afin de garantir la confidentialité et l'anonymat des participants, toutes les précautions et mesures nécessaires seront prises pour s'assurer que les résultats et les informations personnelles des participants est maintenu sous la plus stricte confidentialité.

-Seules les personnes suivantes ont accès au matériel: Chercheur principal et coordonnateur de la recherche. Toutes les autres personnes impliquées dans l'étude n'auront pas accès aux renseignements personnels des participants et des résultats.

-Les noms des participants ne fera pas apparaître sur tous les rapports. Un code numéro sera utilisé pour identifier les participants sur tous les documents de recherche.

-Tous les documents et informations qui peuvent être liés à des participants ne seront pas rendus publics et seront tenus, sous la plus stricte confidentialité.

-Les participants ne seront pas identifiées en aucune manière dans des publications ou des rapports.

-Les données collectées seront conservées dans une armoire verrouillée dans le Behavioral and Metabolic Research Unit à accès restreint où les dossiers de tous les participants seront conservées. En outre, les fichiers informatiques seront protégées par un mot de passe.

-Des échantillons de sang sera conservé dans le congélateur de laboratoire de l'unité de recherche. Des échantillons de sang seront identifiés par un numéro de code qui ne sera pas retracé.

-Les données seront détruites et un prélèvement sanguin éliminé cinq ans après la publication des résultats de l'étude.

9. PARTICIPATION VOLONTAIRE

Vous êtes libre de refuser de participer et si vous décidez de participer, vous êtes libre de vous retirer de l'étude à tout moment pour n'importe quelle raison. A tout moment au cours de cette étude, l’intérêt des participants se fera toujours prévaloir sur les objectifs de l’etude.

Toute information sur vos droits en tant que participant à la recherche mai être adressées à: Responsable d'éthique dans la recherche, Université d'Ottawa, 550 Cumberland, Pavillon Tabaret, pièce 159, Ottawa, Ontario, K1N 6N5, téléphone: (613)

562-5841, email: ethics@uottawa.ca.

Il existe deux exemplaires du formulaire de consentement, dont un que j'ai mai garder.

S'il vous plaît choisir l'une des options suivantes:

Si je décide de retirer de l'étude, je veux que toutes les données □
recueillies auprès de moi jusqu'au moment du retrait être détruits

Même si je retirer de l'étude, j'accepte que les données □
recueillies auprès de moi d'être utilisées pour cette étude

CHERCHEUR 'SIGNATURE
Éric Doucet, Ph.D.: _____ Date: _____

RECHERCHE DES COORDINATEURS DE SIGNATURE

Jameason Cameron, Ph.D. (candidate): _____ Date: _____

SIGNATURE DU PARTICIPANT:
Je suis d'accord pour participer à cette étude,
_____ _____ Date: _____
Nom en lettres moulées Signature

APPENDIX D

Recruitment Posters

Chapter VII

uOttawa
L'Université canadienne
Canada's university

URCM
BMRU

Want to participate in research?

Looking for individuals to participate in a study on the effects of energy deprivation on smell identification and food palatability.

Benefits Include: Assessment of Smell function

- ✓ Assessment of Resting Energy Expenditure
- ✓ Assessment of Body Composition
- ✓ Assessment of $V0_{2MAX}$

Selection Criteria:

- ✓ Men and women aged 18 to 40 years old
- ✓ No major health problems
- ✓ Non-smokers
- ✓ Stable body weight for the last 6 months (± 2kg)
- ✓ No allergies to cheese
- ✓ Must be able to offer a total of approximately 30 hours of time

In order to accommodate for time lost due to participation, there will be a $100 compensation for each participant who completes the study

For more information, please call Jameason
Behavioural and Metabolic Research Unit (BMRU)
Lees Campus Block E - University of Ottawa

uOttawa
L'Université canadienne
Canada's university

URCM
BMRU

Vous voulez participer à un projet de recherche?

Nous sommes à la recherche de personnes voulant participer à une étude sur les effets de la privation d'énergie sur l'identification des odeurs et sur la palatabilité des aliments

Les avantages comprennent:

- ✓ L'évaluation de l'odorat
- ✓ L'évaluation de dépense énergétique de repos
- ✓ L'évaluation de la composition corporelle
- ✓ L'évaluation de la $V0_{2MAX}$

Critères de sélection:

- ✓ Être un homme ou une femme âgé entre 18 et 40 ans
- ✓ Avoir aucun problème de santé majeur
- ✓ Être non-fumeurs
- ✓ Avoir un poids stable pendant les 6 derniers mois (± 2 kg)
- ✓ Aucunes allergies au fromage
- ✓ Doit pouvoir offrir un total d'approximativement 30 heures de temps

Afin de s'adapter pendant le temps a perdu en raison de la participation, il y aura une compensation $100 pour chaque participant qui achève l'étude

Pour de plus ample renseignements, veuillez contacter Jameason
Unité de Recherche du comportement et du métabolisme (URCM) Lees Campus Bloc E - Université d'Ottawa

APPENDIX E

Food Appreciation Questionnaire

Chapters V, VI and VII

Food Appreciation

1. Circle one (1) of your favourite snack foods out of the following three (A to C) categories, i.e. not one out of each category.

A. Cake & Desserts

Ah Caramels
Chocolate Cheesecake
Hostess Twinkies
Hostess Cup Cakes
Hostess Ding-Dongs
McCain's Chocolate
McCain's Marble
McCain's Banana
Strawberry Cheesecake
Haagen Dasz Chocolate
Haagen Dasz Chocolate chip cookie dough
Haagen Dasz Chocolate peanut butter
Haagen Dasz Cookies and Cream
Haagen Dasz Strawberry
Haagen Dasz Vanilla
Haagen Dasz Vanilla Fudge Brownie
Two bite chocolate brownies
Two bite banana brownies

B. Candy

Baby Ruth

Butterfinger
Chocolate-coated raisins
Chocolate-coated peanuts
Cadburry's Caramilk
Coffee Crisp
Hershey's Milk Chocolate
Hershey's Milk Chocolate with Almonds
Jersey Milk
Junior Mints
Kit Kat
Licorice (black)
Licorice (red)
Mars
M & Ms chocolate
M & Ms peanut
Milky Way
Oh Henry
Reese peanut butter Cups
Reese Pieces
Skittles
Skor
Smarties
Snickers
Three Musketeers
Tootsie Roll
Twix
York Peppermint Pattie
Wonder Bar

C. Chips (specify favourite flavour)

Cheetos
Doritos
Lay's
Miss Vickie's
Pringles

2. Circle one (1) of your favourite health foods out of the following two categories (i.e. not one out of each category).

D. Fruits

Apple (specify favourite type)
Apricot
Banana
Blueberry
Cheery
Grape
Grapefruit
Honeydew melon
Kiwi
Orange
Peach
Pear
Plum
Prunes
Raspberry
Strawberry
Tangerine

E. Vegetables

Broccoli
Carrot
Celery
Cauliflower
Cucumber
Red Pepper
Green Pepper
Tomatoe
Zucchini

1. Encercler un (1) de votre collation favori dans les trois catégories (A à C), i.e. pas une par catégorie.

A. Gâteaux et Desserts

Ah Caramels
Hostess Twinkies
Hostess Quatre-quarts
Hostess Ding-Dongs
Gâteau de chocolat McCain
Gâteau de marbre McCain
Gâteau de banane McCain
Gâteau de fromage au chocolat
Gâteau de fromage aux fraises
Petit gâteau au chocolat Deux mordre
Haagen Dasz Chocolate
Haagen Dasz Chocolate chip cookie dough
Haagen Dasz Chocolate peanut butter
Haagen Dasz Cookies and Cream
Haagen Dasz Strawberry
Haagen Dasz Vanilla
Haagen Dasz Vanilla Fudge Brownie
Two bite Chocolate Brownies
Two bite Banana Brownies

B. Bonbon

Baby Ruth
Butterfinger
Raisins enrobé au chocolat

Arachides enrobé au chocolat
Cadburry's Caramilk
Coffee Crisp
Hershey's chocolat au lait
Hershey's chocolat au lait avec Amandes
Jersey Milk
Menthes Junior
Kit Kat
Réglisse (noir)
Réglisse (rouge)
Mars
Chocolat M & Ms
Arachide M & Ms
Milky Way
Oh Henry
Reese peanut butter Cups
Reese Pieces
Skittles
Skor
Smarties
Snickers
Three Musketeers
Tootsie Roll
Twix
Wonder Bar

C. Frites (spécifiez votre saveur favori)

Cheetos
Doritos
Lay's

Miss Vickie’s
Pringles
2. Encercler un (1) de votre aliment santé favori dans les deux catégories (i.e. pas un dans chaque catégorie).

D. Fruits

Pomme (spécifiez votre saveur favori)
Abricot
Banane
Bluets
Cerises
Raisin
Pamplemousse
Melon d’eau
Kiwi
Orange
Pêche
Poire
Prune
Pruneau
Framboises
Fraises
Mandarine

E. Légumes

Brocoli
Carotte
Céleri
Chou-fleur

Concombre

Poivron rouge

Poivron vert

Tomate

Courgette

APPENDIX F

Sensitivity to Reward (SR) Questionnaire

Chapters V, VI, and VII

Questionnaire: Sensitivity to Punishment and Sensitivity to Reward (SPSRQ)

1. Does the good prospect of obtaining money motivate you strongly to do some things?
   YES NO
2. Do you prefer not to ask for something when you are not sure you will obtain it?
   YES NO
3. Are you often afraid of new or unexpected situations?
   YES NO
4. Is it difficult for you to telephone someone you do not know?
   YES NO
5. Do you often do things to be praised?
   YES NO
6. Do you like being the center of attention at a party or social meeting?
   YES NO
7. In tasks that you are not prepared for, do you attach great importance to the possibility of failure?
   YES NO
8. Do you spend a lot of your time on obtaining a good image?
   YES NO
9. Are you easily discouraged in difficult situations?
   YES NO
10. Are you a shy person?
    YES NO

11. When you are in a group, do you try to make your opinions the most intelligent or the funniest?
    YES NO
12. Whenever possible, do you avoid demonstrating your skills for fear of being embarrassed?
    YES NO
13. Do you often take the opportunity to pick up people you find attractive?
    YES NO
14. When you are with a group, do you have difficulties selecting a good topic to talk about?
    YES NO
15. As a child, did you do a lot of things to get people's approval?
    YES NO
16. Does the possibility of social advancement, move you to action, even if this involves not playing fair?
    YES NO
17. Do you think a lot before complaining in a restaurant if your meal is not well prepared?
    YES NO
18. Do you generally give your preference to those activities that imply an immediate gain?

YES NO

19. Do you often have trouble resisting the temptation of forbidden things?
YES NO
20. Whenever you can, do you avoid going to unknown places?
YES NO
21. Do you like to compete and do everything you can to win?
YES NO
22. Are you often worried by things that you said or did?
YES NO
23. Would it be difficult for you to ask your boss for a raise (salary increase)?
YES NO
24. Do you generally avoid speaking in public?
YES NO
25. Do you, on a regular basis, think that you could do more things if it was not for your insecurity or fear?
YES NO
26. Do you sometimes do things for quick gains?
YES NO
27. Comparing yourself to people you know, are you afraid of many things?
YES NO
28. Does your attention easily stray from your work in the presence of an attractive stranger?
YES NO
29. Do you often find yourself worrying about things to the extent that performance in intellectual abilities is impaired?
YES NO
30. Are you interested in money to the point of being able to do risky jobs?
YES NO
31. Do you often refrain from doing something you like in order not to be rejected or disapproved of by others?
YES NO
32. Do you like to put competitive ingredients in all of your activities?
YES NO
33. Would you like to be a socially powerful person?
YES NO
34. Do you often refrain from doing something because of your fear of being embarrassed?
YES NO
35. Do you like displaying your physical abilities even though this may involve danger?
YES NO

Questionnaire: (SPSRQ)

1. Est-ce que la perspective d'obtenir de l'argent vous motive fortement à faire certaines choses ?
   OUI NON
2. Est-ce que vous préférez ne pas demander quelque chose quand vous n'êtes pas sûr de l'obtenir ?
   OUI NON
3. Avez-vous souvent peur des situations nouvelles ou inattendues ?
   OUI NON
4. Trouvez-vous difficile de téléphoner à quelqu'un que vous ne connaissez pas?
   OUI NON
5. Est-ce que vous faites souvent des choses pour recevoir des compliments ?
   OUI NON
6. Est-ce que vous aimez être le centre d'attention lors d'une fête ou d'un autre événement ?
   OUI NON
7. Pour les tâches pour lesquelles vous n'êtes pas préparé(e), est-ce que vous attachez une grande importance à la possibilité d'échouer ?
   OUI NON
8. Est-ce que vous consacrez beaucoup de votre temps pour acquérir une bonne image ?
   OUI NON
9. Etes-vous facilement découragé(e) face aux situations difficiles ?
   OUI NON
10. Etes-vous timide ?
    OUI NON
11. Quand vous êtes en groupe, est-ce que vous essayez de dire les choses les plus intelligentes et / ou les plus drôles ?
    OUI NON
12. Est-ce qu'il vous arrive d'éviter de montrer vos talents par peur d'être embarrassé(e) ?
    OUI NON
13. Est-ce que vous saisissez souvent l'opportunité de vous entourer de gens que vous trouvez attractifs ?
    OUI NON
14. Quand vous êtes en groupe, est-ce que vous avez des difficultés à choisir un bon sujet de conversation ?
    OUI NON

15. Quand vous étiez enfant, est-ce que vous faisiez beaucoup de choses dans le but d'obtenir l'approbation des gens ?
    OUI NON
16. Est-ce que la possibilité de progresser socialement vous incite à l'action, même si cela vous conduit à ne pas agir de manière correcte ?
    OUI NON
17. Réfléchissez-vous beaucoup avant de vous plaindre au restaurant si votre plat n'est pas bien préparé ?
    OUI NON
18. Donnez-vous généralement la préférence aux activités débouchant sur un gain immédiat ?
    OUI NON
19. Avez-vous souvent de la difficulté à résister à la tentation de faire des choses interdites ?
    OUI NON
20. Quand vous le pouvez, est-ce que vous évitez de vous rendre dans des endroits inconnus ?
    OUI NON
21. Est-ce que vous aimez la compétition et faire tout ce que vous pouvez pour gagner ?
    OUI NON
22. Etes-vous souvent inquiet(ète) des choses que vous avez dites ou faites ?
    OUI NON
23. Serait-il difficile pour vous de demander une augmentation de salaire à votre patron ?
    OUI NON
24. Est-ce que généralement vous essayez d'éviter de vous exprimer en public ?
    OUI NON
25. Est-ce que généralement vous pensez que vous pourriez faire plus de choses si vous n'étiez pas retenu(e) par un sentiment de peur ou d'insécurité ?
    OUI NON
26. Est-ce que parfois vous faites des choses dans le but d'obtenir un gain immédiat ?
    OUI NON
27. En vous comparant aux autres, êtes-vous quelqu'un qui a peur de beaucoup de choses ?
    OUI NON

28. Est-ce que vous êtes facilement distrait(e) de votre travail par la présence d'un(e) inconnu(e) attirant(e) ?
    OUI NON
29. Est-ce que vous vous inquiétez souvent au point que vos performances intellectuelles s'en trouvent diminuées ?
    OUI NON
30. Etes-vous intéressé(e) par l'argent au point d'être capable de faire des jobs risqués ?
    OUI NON
31. Vous abstenez-vous souvent de faire quelque chose que vous aimez pour ne pas être réprimandé(e) ou désapprouvé(e) par les autres ?
    OUI NON
32. Est-ce que vous aimez ajouter des éléments compétitifs dans toutes vos activités ?
    OUI NON
33. Voudriez-vous être une personne socialement influente ?
    OUI NON
34. Vous abstenez-vous souvent de faire des choses par peur d'être embarrassé(e) ?
    OUI NON
35. Est-ce que vous aimez prouver vos capacités physiques même si cela pourrait impliquer un danger ?
    OUI NON

APPENDIX G

Three Factor Eating Questionnaire (TFEQ)

Chapters V, VI, and VII

FOOD HABITS QUESTIONNAIRE
(Stunkard et Messick, 1984)

This questionnaire contains a certain number of propositions.

If you agree with the statement or if you feel like it can be applied to you, check the case TRUE who correspond to the statement.

If you disagree with the statement or if you feel like it does not applied to you, check the FALSE case who correspond to the statement.

You have the choice to answer (or not) certain questions.

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

eating, an expert would tell me that I had enough or that I can have something more to eat.

9. When I feel anxious, I find myself eating. ☐ ☐

10. Life is too short to worry about dieting. ☐ ☐ ☐ ☐

11. Since my weight goes up and down, I have gone on ☐ ☐
reducing diets more than once.

12. I often feel so hungry that I just have to eat something. ☐ ☐

TRUE

FALSE

13. When I am with someone who is overeating, I usually ☐ ☐
overeat too.

14. I have a pretty good idea of the number of calories ☐ ☐
in common food.

15. Sometimes when I start eating, I just can't seem to stop. ☐ ☐

16. It is not difficult for me to leave something on my plate. ☐ ☐

17. At certain times of the day, I get hungry because I have ☐ ☐
gotten used to eating them.

18. While on a diet, if I eat food that is not allowed, I consciously ☐ ☐
eat less for a period of time to make up for it.

19. Being with someone who is eating often makes me hungry ☐ ☐
enough to eat also.

20. When I feel "blue", I often overeat. ☐ ☐

21. I enjoy eating too much to spoil it by counting calories ☐ ☐
or watching my weight.

22. When I see a real delicacy, I often get so hungry that ☐ ☐
I have to eat right away.

23. I often stop eating when I am not really full as a conscious ☐ ☐
means of limiting the amount that I eat.

24. I get so hungry that my stomach often seems like a ☐ ☐
bottomless pit.

25. My weight has hardly changed at all in the last 10 years. ☐ ☐

26. I am always hungry so it is hard for me to stop eating ☐ ☐
before I finish the food on my plate.

27. When I feel lonely, I console myself by eating. ☐ ☐

28. I consciously hold back at meals in order not to gain ☐ ☐
weight.

29. I sometimes get very hungry late in the evening or at ☐ ☐
night.

	TRUE	FALSE
30. I eat anything I want, anytime I want.	☐	☐

31. Without even thinking about it, I take a long time to eat. ☐ ☐

32. I count calories as a conscious means of controlling weight. ☐ ☐

33. I do not eat some foods because they make me fat. ☐ ☐

34. I am always hungry enough to eat at any time. ☐ ☐

35. I pay a great deal of attention to changes in my figure. ☐

☐

36. While on a diet, if I eat a food that is not allowed, I often ☐

☐

then splurge and eat other high calorie foods.

PART 2

Please answer the following questions by circling the number that best corresponds to you.

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

Rarely	Sometimes	Usually	Always
1	2	3	4

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

Not at all	Slightly	Moderately	Very much
1	2	3	4

39. How often do you feel hungry ?

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

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

Never	Rarely	Often	Always
1	2	3	4

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

Easy	Slightly Difficult	Moderately Difficult	Very Difficult
1	2	3	4

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

Not at all	Slightly	Moderately	Extremely
1	2	3	4

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

Almost Never	Seldom	Usually	Almost always
1	2	3	4

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

Unlikely	Slightly Unlikely	Moderately likely	Very likely
1	2	3	4

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

Never	Rarely	Often	Always
1	2	3	4

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

Unlikely	Slightly Unlikely	Moderately likely	Very likely
1	2	3	4

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

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

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

Unlikely	Slightly Unlikely	Moderately likely	Very likely
1	2	3	4

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

Never	Rarely	Sometimes	At least Once per week

1 2 3 4

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

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

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

What number would you give yourself?

- **Eat whatever you want, whenever you want it**
  0
- **Usually eat whatever you want, whenever you want it**
  1
- **Often eat whatever you want, whenever you want it**
  2
- **Often limit food intake, but often "give in"**
  3
- **Usually limit food intake, rarely "give in"**
  4
- **Constantly limiting food intake, never "giving in"**
  5

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

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

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

QUESTIONNAIRE SUR LE COMPORTEMENT ALIMENTAIRE
(Stunkard et Messick, 1984)

Ce questionnaire comporte un certain nombre de propositions.

Si vous êtes d'accord avec la proposition ou si vous avez le sentiment qu'elle s'applique à vous, cochez la case VRAI qui lui correspond.

Si vous êtes en désaccord avec la proposition ou si vous avez le sentiment qu'elle ne s'applique pas à vous, cochez la case FAUX qui lui correspond.

Vous avez le choix de ne pas répondre à certaines questions.

		VRAI	FAUX
1.	**Lorsque je sens l'odeur d'un steak en train de cuire ou lorsque je vois un beau morceau de viande, il m'est très difficile de ne pas manger même si je sors de table.**	☐	☐
2.	**En général, je mange trop lorsque je suis en groupe, lors d'un "party" ou d'une "Fête", par exemple.**	☐	☐
3.	**J'ai habituellement tellement faim que je mange plus de trois fois par jour.**	☐	☐
4.	**Une fois que j'ai mangé ma ration de calories, je parviens généralement à ne pas manger plus.**	☐	☐
5.	**Il m'est difficile de faire un régime parce que j'ai trop faim.**	☐	☐
6.	**Je fais exprès de me servir de petites portions pour contrôler mon poids.**	☐	☐
7.	**Parfois, ce que je mange me paraît si bon que je continue à manger même si je n'ai plus faim.**	☐	☐
8.	**Comme j'ai souvent faim, il m'arrive de souhaiter que pendant mon repas, un spécialiste puisse me dire si j'ai assez mangé ou si je peux manger plus.**	☐	☐
9.	**Quand je suis anxieux(se), je me retrouve en train de manger.**	☐	☐

	VRAI	FAUX
10. La vie est trop courte pour se tourmenter avec un régime.	☐	☐
11. Comme mon poids ne fait que monter et descendre, j'ai tenté des régimes amaigrissants plus d'une fois.	☐	☐
12. J'ai souvent si faim qu'il faut absolument que je mange quelque chose.	☐	☐
13. Quand je me trouve avec quelqu'un qui mange trop, habituellement, je mange trop aussi.	☐	☐
14. Je sais assez bien combien il y a de calories dans les aliments courants.	☐	☐
15. Quelquefois, quand je commence à manger, c'est comme si je ne pouvais plus m'arrêter.	☐	☐
16. Je n'ai pas de mal à laisser quelque chose dans mon assiette.	☐	☐
17. À certains moments de la journée, j'ai faim parce que j'ai pris l'habitude de manger à ces moments là.	☐	☐
18. Quand je suis au régime, si je mange quelque chose d'interdit, je fais exprès de manger moins ensuite pendant quelque temps, pour compenser.	☐	☐
19. La compagnie de quelqu'un qui mange me donne souvent assez faim pour manger aussi.	☐	☐
20. Quand j'ai le cafard, souvent je mange trop.	☐	☐
21. J'ai trop de plaisir à manger pour le gâcher en comptant les calories ou en surveillant mon poids.	☐	☐
22. Quand je vois une vraie gourmandise, j'ai souvent si faim que je dois la manger tout de suite.	☐	☐
23. Souvent je m'arrête volontairement de manger avant d'être rassasié(e) afin de limiter ma consommation alimentaire.	☐	☐
24. Je deviens si affamé(e) que souvent mon estomac semble être un puit sans fond.	☐	☐

	VRAI	FAUX
25. Mon poids n'a pratiquement pas changé durant les dix dernières années.	☐	☐
26. Comme j'ai toujours faim, il m'est difficile d'arrêter de manger avant d'avoir vidé mon assiette.	☐	☐
27. Quand je me sens seul(e), je me console en mangeant.	☐	☐
28. Pendant les repas, je me limite volontairement pour ne pas grossir.	☐	☐
29. J'ai quelquefois très faim le soir tard ou la nuit.	☐	☐
30. Je mange ce que je veux, quand je veux.	☐	☐
31. Sans même y penser, je passe beaucoup de temps à manger.	☐	☐
32. Le calcul des calories est pour moi une façon de contrôler volontairement mon poids.	☐	☐
33. Je ne mange pas certains aliments parce qu'ils me font grossir.	☐	☐
34. J'ai toujours assez faim pour manger quelle que soit l'heure.	☐	☐
35. Je fais très attention aux modifications de ma silhouette.	☐	☐
36. Quand je suis au régime, si je mange un aliment interdit, souvent après je "m'empiffre" en consommant d'autres aliments à valeur calorique élevée.	☐	☐

2e PARTIE

Veuillez répondre aux questions suivantes en entourant le chiffre au-dessous de la réponse qui s'applique à vous.

37. Faites-vous un régime pour tenter de contrôler votre poids:

Rarement	Quelquefois	Habituellement	Toujours
1	2	3	4

38. Une fluctuation de poids de 2 kg modifierait-elle votre façon de vivre?

Pas du tout	Un peu	Moyennement	Beaucoup
1	2	3	4

39. Avez-vous faim:

Seulement au repas	Quelque fois entre les repas	Souvent entre les repas	Presque toujours
1	2	3	4

40. Vous sentir coupable de trop manger vous aide-t-il à contrôler vos apports alimentaires?

Jamais	Rarement	Souvent	Toujours
1	2	3	4

41. Pourriez-vous interrompre votre dîner en plein milieu et ne rien manger pendant les quatre heures suivantes?

Facilement	Un peu difficilement	Assez difficilement	Très difficilement
1	2	3	4

42. Êtes-vous conscient(e) de ce que vous mangez?

	Pas du tout	Un peu	Assez	Tout à fait
	1	2	3	4

43. Vous arrive-t-il d'éviter d'avoir chez-vous des "réserves" d'aliments que vous aimez?

Presque jamais	Rarement	Habituellement	Presque toujours
1	2	3	4

44. Êtes-vous susceptible d'acheter des produits allégés?

Pas du tout	Un peu	Assez	Tout à fait
1	2	3	4

45. Vous arrive-t-il de manger raisonnablement devant les autres et de vous "empiffrer" quand vous êtes seul(e)?

Jamais	Rarement	Souvent	Toujours
1	2	3	4

46. Vous arrive-t-il de décider de manger lentement pour limiter la quantité de ce que vous mangez?

Pas du tout	Un peu	Assez	Tout à fait
1	2	3	4

47. Vous arrive-t-il de supprimer le dessert parce que vous n'avez plus faim?

Pratiquement jamais	Rarement	Au moins une fois par semaine	Presque tous les jours
1	2	3	4

48. Avez-vous tendance à faire exprès de manger moins que vous ne le voudriez?

Pas du tout	Un peu	Assez	Tout à fait
1	2	3	4

49. Vous arrive-t-il de manger tout ce qui vous tombe sous la main même si vous n'avez pas faim?

Jamais	Rarement	Quelquefois	Au moins une fois par semaine
1	2	3	4

50. Parmi les énoncés suivants, encerclez le chiffre de celui qui paraît correspondre le mieux à vos habitudes.

- **Vous mangez tout ce que vous voulez, quand vous voulez.**
  0

- **Vous avez l'habitude de manger tout ce que vous voulez, quand vous voulez.**
  1

- **Vous mangez souvent tout ce que vous voulez, quand vous voulez.**
  2

- **Vous limitez souvent vos apports mais avec de nombreux écarts.**
  3

- **Vous avez l'habitude de limiter vos apports avec peu d'écarts.**
  4

- **Vous limitez constamment vos apports sans aucun écart.**
  5

51. La déclaration suivante pourrait-elle décrire votre comportement alimentaire?

"Je commence souvent un régime le matin mais, à cause de tous les évènements qui surviennent dans la journée, le soir j'abandonne et je mange ce que je veux en me promettant de recommencer le régime le lendemain. "

Pas du tout	Un peu	Assez bien	Parfaitement
1	2	3	4