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Association of central nervous activity and peripheral endocrine messenger substances in the context of physiological energy homeostasis

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1.4 Abbreviations

abbreviation	term
AUC	area under the curve
BMI	body-mass-index
BOLD	blood-oxygen-level dependent
CV	coefficient of variation
fMRI	functional magnetic resonance imaging
GLP-1	Glucagon-like peptide-1
ms	milliseconds
Ncl.	nucleus
OFC	orbitofrontal cortex
POMC	proopiomelanocortin
PYY	peptide YY
s	seconds
SI	international system of units
α-MSH	α-melanocyte stimulating hormone

2 General Introduction and Hypothesis

2.1 Background

Overweight, obesity and the associated metabolic syndrome have developed over the past decades into an outstanding global health concern [1]. More than one third of the world population is currently obese [2]. Ongoing increases in the prevalence vary highly depending on the geographical region [3]. This development must be regarded as one of the biggest threats for the well-being of the global population as it is associated with highly relevant growths in type II diabetes, depression, cardiovascular diseases, certain cancers and overall mortality [4]. The most extraordinary fact about this spreading pandemic of obesity is however our lacking knowledge about its exact causality.

There is no lack of clues and evidence concerning which changing factors in our environment and society might have led to the overall weight gain of the population. The positive energy balance of individuals in modern consumer society is fostered my massively increased availability of high-calorie foods, which are additionally offered in larger portion sizes [5,6]. Furthermore, lifestyle in modern societies results in an even larger, overall positive energy balance – this time adding weight from the other side of the equation: Reshaping our working environment and a lack of physically engaging recreational activities results in a lack of energy burning processes [7,8]. Other influential factors that can be hold partially responsible include the usage of medication that leads to weight gain [9], insufficient sleep quantity and quality [10] and increased life span [11]. Adding to the effect of environmental factors, genetic aspects appear to be of certain relevance as well. While monogenetic causes for obesity can be of central interest in terms of unravelling physiological regulatory mechanisms of energy control, they are much rarer than the general occurrence of obesity [12]. Polygenetic influences on body weight seem to be more relevant in terms of their commonness, but are not able to explain the phenomenon to its full extend [13]. However,

observational studies were able to explain large proportions of the variance in BMI with polygenetic factors [14]. Overall, it remains unclear how the combination of all these factors can erode the global health status by mass-weight gain. How do we lose our ability to regulate body weight?

Generally, it is often assumed and reiterated that from an evolutionary standpoint, we are programmed to consume as much as possibly energy rich nutrients during times in which they are available. These can be stored as fat in our body, to give us an advantage in times of famine and shortages. This concept commonly known as "thrifty gene hypothesis" [15] leads to a disadvantage in times of lasting availability of high-calorie foods. This is opposed by evidence that even in times of nutritional abundance regulatory mechanisms are very able to reduce our energy consumption to the physiological necessities. For instance, evidence suggests that even if we give in to an excess supply of foods and overeat, this is often followed by a compensatory period of sub-isocaloric consumption [16]. Furthermore, the question arises, why a majority of the population is still able to sustain a healthy weight while being exposed to food in abundance.

Therefore, another hypothesis explores the possibility of understanding obesity as an evolutionary disadvantage for human ancestors in terms of falling prey to predators and their overall physical fitness. Since these factors have become increasingly irrelevant (humans haven't been subject to being hunted by animals to a relevant degree for a long time and physical fitness plays an increasingly smaller role in gaining wealth and an evolutionary beneficial position), random mutations and traits were able to develop that enable and favour obesity. This also explains why a major proportion of the world population, even though exposed to abundant food supplies, has still weight within normal limits[17]. Overall, there seem to be a lot of clues how obesity has become such a predominant health issue, but no consistent pattern how to put them together.

2.2 Concepts of energy homeostasis, hedonism and food consumption

On the regulatory, physiological level of qualitative and quantitative food ingestion many pieces of knowledge exist, again without an overarching conclusive concept that brings them together. Some

central nervous centres that influence food ingestion in simple orexigenic or anorexigenic manner have been described a long time ago. Ablative procedures in rodents in the mid-20th century led to the discovery of functional nodes in the hypothalamus that are highly influential on the energy homeostasis of the body [18,19]. Further research revealed more details about the interaction and functional relationship as well as relevant signalling molecules such a proopiomelanocortin (POMC) [20], its by-products α- and β-melanocyte stimulating hormone (α-MSH, β -MSH) [21] and oxytocin [22,23]. With the discovery of leptin the potentially most influential endocrine factor on these networks was found [24]. On top of convincing evidence of its central role in regulation fat-tissue quantity [25,26] experiments with fMRI scans found clues that neuronal activity is modulated by leptin [27]. The exploration of such connection was often reduced in the past to simple linear relationships [28]. The complexity and highly dynamic nature of these networks involving the hypothalamus as well as cortical and other subcortical and peripheral structures is increasingly acknowledged.

While further neurotransmitter molecules and their role in energy homeostasis have been identified [29], the role of peripheral signals in the regulation of food intake was increasingly explored. Gastro-intestinal signalling molecules like ghrelin, peptide YY (PYY) and glucagon-like-peptide (GLP) as well as other adipokines have been found to be highly influential factors in these regulatory mechanisms [30,31].

Furthermore, the central network involved in food intake regulation is increasingly unlikely to involve only subcortical, hypothalamic centres. Additionally to these homeostatic systems, higher hedonic- and reward centres seem to play a crucial role in determining what and how much we eat [32,33]. These systems could play a crucial role in circumventing normally regulated homeostatic mechanisms that may lead in turn to the development of obesity [28]. Dopaminergic, limbic regions as well as the prefrontal reward system appear to be crucial components in higher regions of the central nervous system (CNS) processing the urge of food intake. Other involved key regions are the amygdala, hippocampus, insula, striatum and orbitofrontal cortex [34,35]. Many of these areas

seem to be influenced by peripheral hormones of gastrointestinal or non-gastrointestinal origin [36–40] (for further details see part III).

Overall, most of the research that is being conducted and cited here is often aiming at the discovery of the therapeutic approaches. Therefore, less attention is being paid on actual physiological reactions. Hence, to which degree hormones and peripheral signalling molecules play a role in determining hunger and satiety in a physiological context remains widely unanswered.

In Part I of this dissertation the endocrine response to fasting, normal isocaloric nutrition and ingestion of a large glucose load in both fasting and non-fasting individuals will be explored. The food intake after glucose ingestion will be measured and the energy-compensating effect of monosaccharides after fasting and non-fasting examined. Furthermore, associations of hormone concentrations and macronutrient intake will be explored.

In Part II the effect of a fasting intervention and a standardised non-fasting control condition and the endocrine response these interventions will be associated with brain activity of regions involved in control of ingestive behaviour.

In Part III associations will be examined between the endocrine response to glucose ingestion and the brain activity beforehand.

3 Part I: Endocrine responses and food intake in fasted individuals under the influence of glucose ingestion

This part* of the thesis is based on the paper published under the same name in PlosOne [41].

* Publication is slightly adapted to fit format, form and content of this dissertation, but is mostly presented here identical to the original, peer-reviewed published article.

3.1 Introduction

Both obesity and its associated metabolic disorders have become a global health burden in recent years [42]. Excessive calorie intake and macronutrient composition of consumed food are major factors in the development of this obesity pandemic [43–46]. Therefore, investigation into the quantity and food composition an individual consumes and why may assist in developing a more comprehensive understanding of how metabolic disorders develop.

The amount of food we consume is regulated by a balance of orexigenic and anorexigenic influences on areas of the central nervous system [47]. This balance is determined by a large number of interneuronal and endocrine signalling substances [21,22,28]. How these factors are computed in the resulting hunger, satiety and overall energy intake and which factors are the most dominant in this equation is not well understood. Hormones involved in the control of glucose metabolism are likely to be part of these crosslinks, and insulin is understood to be one of the factors that act as an anorexigenic signal [48]. It remains uncertain how large the influence of these regulatory subcomponents like hormones of glucose metabolism is.

Previous studies in rodents found differences in food selection after short fasting periods [49], however it has not yet been investigated whether the same would occur in humans after the acute energy shortage has been partly compensated. Short-term energy status of the body is reflected by hormones like insulin and C-peptide while corticosteroids play a fundamental role in control of glucose metabolism. To which degree the physiological release of insulin as a response to raising

glucose levels in the blood after ingestion of carbohydrates can ameliorate feelings of hunger is unknown.

While the quantity of food consumed appears to be the central factor in the development of obesity [46], the quality of its components seems to wield some influence as well. Studies suggest that the composition of macronutrients an individual chooses before initiating a therapeutic diet has implications for the diet's success [50] and similarly that the composition of the therapeutic diet influences diet outcomes and physiological parameters [51,52]. Furthermore, it has been shown that the variation in macronutrient composition of consumed food results in different hormonal responses [53] which might regulate satiety and hunger signals, as well as the choice of macronutrients one prefers. One of these hormones is adiponectin whose serum levels vary after diets as a function of fat and carbohydrate content of the diet [54,55]. Other hormones associated with glucose metabolism, such as insulin [56] and cortisol [57,58], have also been found to be influenced by different macronutrient compositions.

Nevertheless, the interplay between metabolic conditions, hormonal responses and food intake is not well understood and especially little is known about the interplay of macronutrient intake, endocrine factors and absolute energy intake. Most studies examine the effect of diets and food composition on endocrine parameters, food consumption and weight loss. Essential parts of the puzzle, such as hormones regulating the size of the meal and the specific composition of macronutrients, might therefore be missing [59]. Also, the regulation of macronutrient intake in different metabolic situations and long-term deficits or excesses is largely unknown. It has been shown that there are efficient compensatory mechanisms that are unlikely to be explained by fluctuation of hormones with fast kinetics [16]. Adipokines with slower kinetic characteristics may play a major role in this context [27].

To address the questions of how energy deficits, control our eating behaviour even if energy deficits have been partially removed by glucose ingestion was one aim of this dissertation. Additionally, it

was attempted to find out if adiponectin, one of the emerging major players in the understanding of the metabolic syndrome, plays a role in the regulation of non-acute food intake and its composition under the metabolic conditions that apply in our experiment.

To fill in some of the research gaps, we measured the amount and composition of food consumed after a period of fasting or non-fasting (control); and after acute metabolic energy deficits were at least partly compensated for by oral ingestion of a large load of carbohydrates. It was hypothesised that fasting might still have implications on total food intake after the partial compensation of energy shortage by ingestion of glucose and the resulting alterations of hormones concerned with glucose metabolism towards an anabolic status. To induce insulin and C-peptide levels as high as possible after the glucose administration in the fasting condition to evaluate their role on appetite control as clearly as possible, studies that showed fasting induced glucose intolerance were used to determine the length of the fasting period [60,61]. These studies usually used fasting periods around 70 hours. After consultation with the ethics committee of the University of Luebeck we decided that a fasting period of 42 hours would be ideal in terms of combining the aims of likelihood of producing high insulin and C-peptide levels, safety and practicability. Furthermore, it was hypothesised that an association of the proportions of consumed food with adiponectin levels might exist in this context. Only a limited array of hormones was selected on the basis of their importance to assess the impact of the oral glucose administration, due to reasons of practicability and because of the available means.

3.2 Materials and methods

3.2.1 Participants

24 healthy male participants of normal weight and without metabolic disease were enrolled for the study [age (mean ± SEM): 24.5 ± 0.6 years; body mass index (mean ± SEM): 23.4 ± 0.3 kg/m^2]. The sample size of 24 participants was based on pilot studies of our group as well as previous papers which compared the brain activity in different metabolic states 3,62–65. Participants were not on

any medication and had a regular self-reported sleep-wake cycle for 6 weeks before participating in the experiment. Individuals with acute or chronic diseases, drug abuse including alcohol (> 5 drinks per week) and smoking or exceptional physical activity were excluded. We also excluded individuals with special eating behaviours, e.g. vegetarians and vegans. The night before the experiment, the participants were asked to go to bed at approximately 11 p.m. and to avoid any exhausting physical activity. All examinations abided by the Declaration of Helsinki and was approved by the Ethics Committee of the University of Lübeck. All subjects gave written informed consent.

3.2.2 Experimental setting

A within subject design with two conditions was used. Half of the participants started with the condition of total caloric deprivation (fasting condition = FAST) and the other half with the control condition (non-fasting = EAT). Conditions were spaced exactly 7 days apart.

Experiments started on the first day at 8 a.m. with participants arriving at the sleep laboratory of the Department of Psychiatry which is equipped to host long-term studies. For the fasting condition, participants were instructed to refrain from eating from 11 p.m. the evening before. In the control condition participants were provided with a standardised meal by the study team. Subsequently, an antecubital venous catheter was inserted for blood samples and half an hour later the first blood samples were obtained.

Afterwards, participants in the non-fasting condition had a standardized breakfast (2240 kcal, 14% proteins, 46% fat, 40% carbohydrates) followed by regular standardized meals (on average: 1320 kcal, 17% proteins, 31% fat, 51% carbohydrates); and during these meals, further blood samples were obtained. Overall 20 blood samples were obtained per condition over the two days of the experiment (blood samples were taken at 08:45 a.m., 10:00 a.m., 12:45 p.m., 02:00 p.m., 04:00 p.m., 06:00 p.m., 06:45 p.m., 08:00 p.m. and 10:00 p.m. on the first day and 08:45 a.m., 10:00 a.m., 11:45 a.m., 01:00 p.m., 01:35 p.m., 02:15 p.m., 02:45 p.m., 03:15 p.m., 03:45 p.m., 04:15 p.m. and

04:45 p.m. on the second day in both conditions). The fMRI scan was performed in a 25-minute session at 1:00 p.m. on the second day of the experiment and concluded the study (for details see 4.2.4 fMRI task and 4.2.5 MRI acquisition). Participants were provided with oral glucose (polysaccharides dissolved in 300 mg of water that are broken down to 75g of glucose in the intestines; Accu-Chek Dextrose O.G.-T. 300 ml, Roche Diagnostics, ELISA, Indianapolis, IN, USA) at 1:30 p.m..

A standardized buffet was provided at 05:00 p.m. with a wide variety of offered foods and drinks. The participants were allowed to choose freely for one hour [66]. Food intake was measured and macronutrient composition as well as overall energy intake could be calculated for each individual.

The experiment ended at 6 p.m. with the end of the buffet. The subjects in the fasting condition spent 42 hours in total without any caloric intake. No blood samples were obtained during the night-time to keep disturbance of physiological rhythms as minimal as possible. The subjects stayed at the study venue over the whole time of the experiment in single rooms and spend the night there as well with members of the study team being present over the whole time. Figure 1 visualises the experimental design.

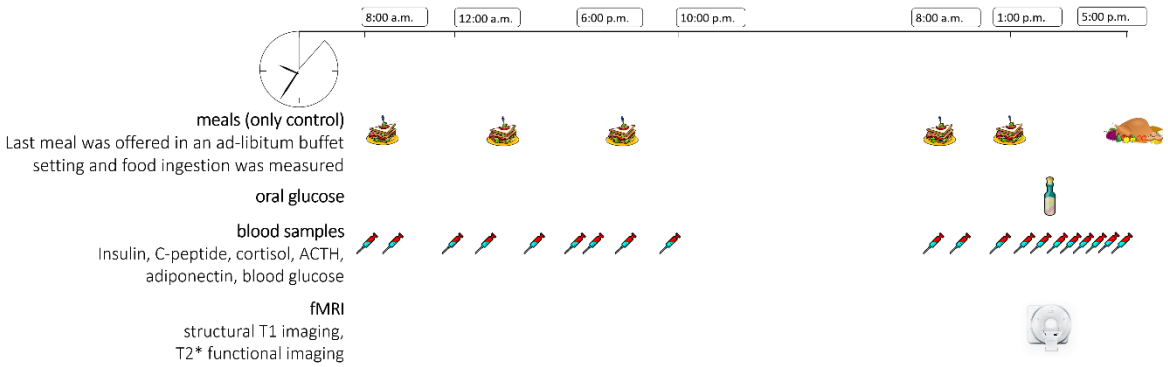

Figure 1: Overview over experimental design.

3.2.3 Assays

Blood samples were centrifuged immediately after they were obtained, and the supernatant was stored at -80° C. The measurement of all hormones took place at the same point of time to avoid inter-assay variabilities. Blood serum and EDTA (Ethylenediaminetetraacetic acid) plasma were used to measure the hormone levels through an immunoassay. The ACTH-assay (Roche Diagnostics, ELCIA, Indianapolis, IN, USA) had a measuring range of 0.220-440 pmol/L, an intra-assay coefficient of variation (CV) of < 2.4% and an inter-assay CV of < 4.2%. The C-peptide-assay had a measuring range of 0.003-13.3 nmol/L, an intra-assay CV of <4.6% and an inter-assay CV of < 5.0. The Insulin-assay had a measuring range of 1.39-6945 pmol/L, an intra-assay CV of < 2.8 % and an inter-assay CV of < 4,9 %. Cortisol had a measuring range of 0.5-1750 nmol/L, an intra-assay CV of < 2.9 % and an inter-assay CV of < 4.7 %. Adiponectin levels were measured with an Adiponectin ELISA (Immundiagnostik AG, Adiponectin total ELISA Kit, Bensheim, Germany) with an intra-assay CV of < 3.4% and inter-assay CV of < 6.3%.

3.2.4 Statistical analysis

MATLAB 2015a, R and SPSS version 23 were used for data preparation and statistical analysis. All data is presented as mean values ± SEM. Comparison of consumed food composition was carried out with paired student t-tests. The area under the curve (AUC) of the time course of the blood concentration of adiponectin was calculated with trapezoidal assumptions for the whole time of the experiment. A multiple stepwise linear regression with the individual AUC-values of adiponectin as a dependent variable and the relative quantities of the consumed macronutrient fat, carbohydrates and protein in the buffet test as independent variables was performed for each condition. For the comparison of the hormonal levels and plasma glucose levels within the different conditions, factorial ANOVAs were performed for all timepoints (time points 1-20) and split into two parts - before the administration of oral glucose (time points 1-13) and afterwards (time points 14-20) to control for differences in the phases of the experiment. Significance was assumed for p-values <0.05.

3.3 Results

3.3.1 Buffet

The total number of kilocalories and the overall amount of each macronutrient consumed differed significantly between the conditions (Figure 2). Absolute food consumption was higher in the fasting group for all measured variables including the total amount of food (EAT: 1442.7±496.7 kcal; FAST: 1841.5±618.7 kcal; $t(23)=5.2$, $p<0.001$), protein (EAT: 235.1±85.2 kcal; FAST: 300.9±103.6 kcal; $t(23)=5.4$, $p<0.001$), fat (EAT: 686.7±262.5 kcal; FAST: 833.5±270.6 kcal; $t(23)=3.8$, $p=0.001$) and carbohydrates(EAT: 521±191.5 kcal; FAST: 707.2±302.5 kcal; $t(23)=3.7$, $p<0.001$). However, the proportion of macronutrients in kilocalories showed no significant statistical difference between both conditions. Both conditions consumed the same percentage of proteins (16%, $t(23)=0.06$, n.s.) and relatively similar percentages of fat (48% in the non-fasting condition and 45% in the fasting condition, $t(23)=1.3$, n.s.) and carbohydrates (36% in the non-fasting condition and 38% in the fasting condition, $t(23)=1.2$, n.s.).

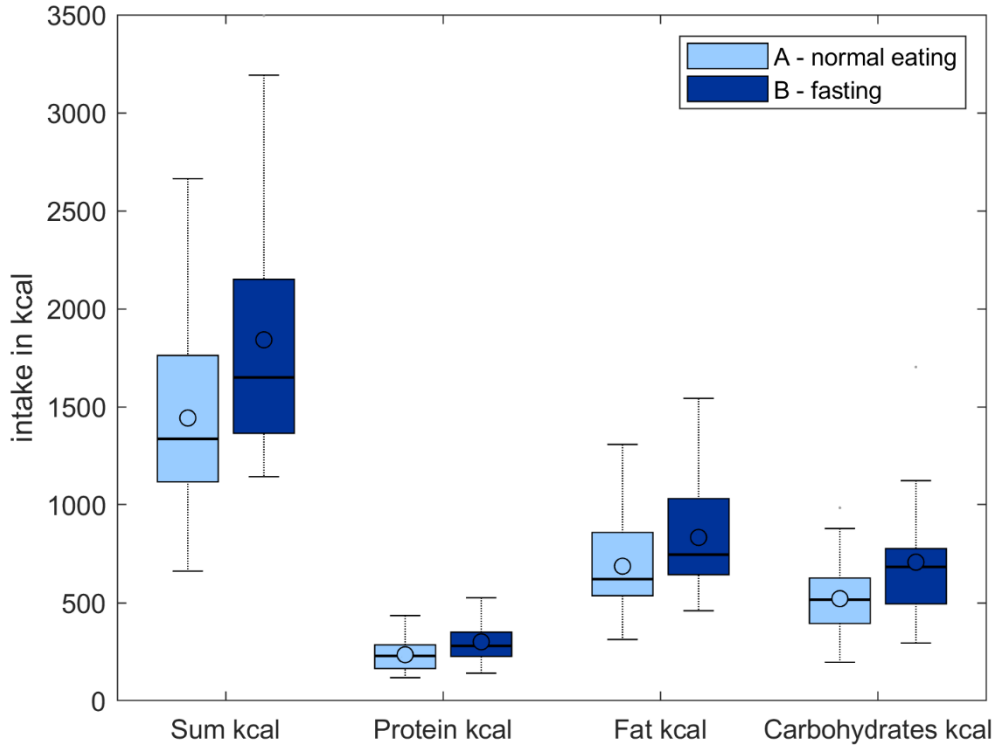

Figure 2: Box Plots of the total food consumption in the buffet scenario for both fasting and normal eating condition. The total amount of kilocalories and the overall amount of each macronutrient consumed differed significantly between the conditions.

3.3.2 Hormones and blood glucose

Repeated measures ANOVAs including all 20 measurement timepoints revealed a main effect of conditions for insulin ($F(1,23)=27.7$, $p<0.001$), C-peptide ($F(1,23)=62.2$, $p<0.001$) and ACTH ($F(1,23)=4.7$, $p=0.042$) but not for cortisol ($F(1,23)=2.8$, n.s.), glucose ($F(1,23)=1.2$, n.s.) and adiponectin ($F(1,23)=3.3$, n.s.). When the timepoints were separated into pre-oral glucose (time points 1-13) and post-oral glucose (time points 14-20), a significant difference was found for insulin (pre: $F(1,23)=371.9$, $p<0.001$; post: $F(1,23)=12.6$, $p=0.002$), C-peptide (pre: $F(1,23)=331.8$, $p<0.001$; post: $F(1,23)=19.2$, $p<0.001$) and glucose (pre: $F(1,23)=30.5$, $p<0.001$; post: $F(1,23)=42.8$, $p<0.001$) in both sectors. Cortisol only showed significant differences in the post-oral glucose sector (pre: $F(1,23)=0.024$, n.s.; post: $F(1,23)=7.6$, $p=0.01$). Both adiponectin (pre: $F(1,23)=2.8$, $p=0.11$; post: $F(1,23)=2.9$ $p=0.1$) and ACTH (pre: $F(1,23)=2.4$ $p=0.14$; post: $F(1,23)=2.9$, $p=0.1$) did not show any

significant differences in either sector. Figure 3 and Figure 4 show the time courses of all measured serum parameters.

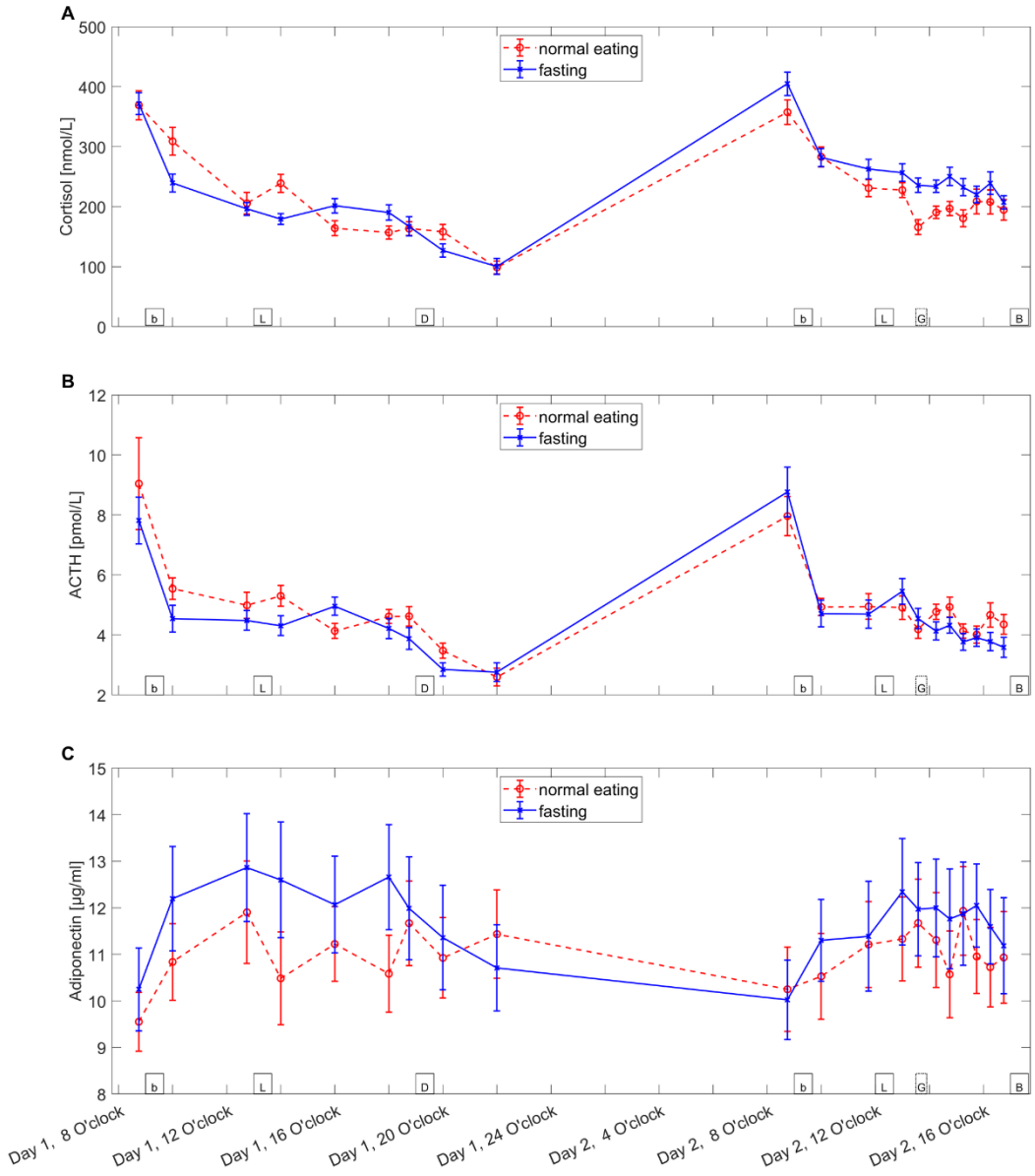

Figure 3: Levels of cortisol (A), ACTH (B) and adiponectin (C). The Figure shows the mean ± standard error of the mean for both fasting (blue line, crosses) and normal eating (red spotted line, circles) condition of the serum parameters during the experiment. Boxes on the bottom of the graph indicate the timepoints of meals (b = breakfast, L = lunch, D = Dinner, G = oral glucose administration, B = Buffet).

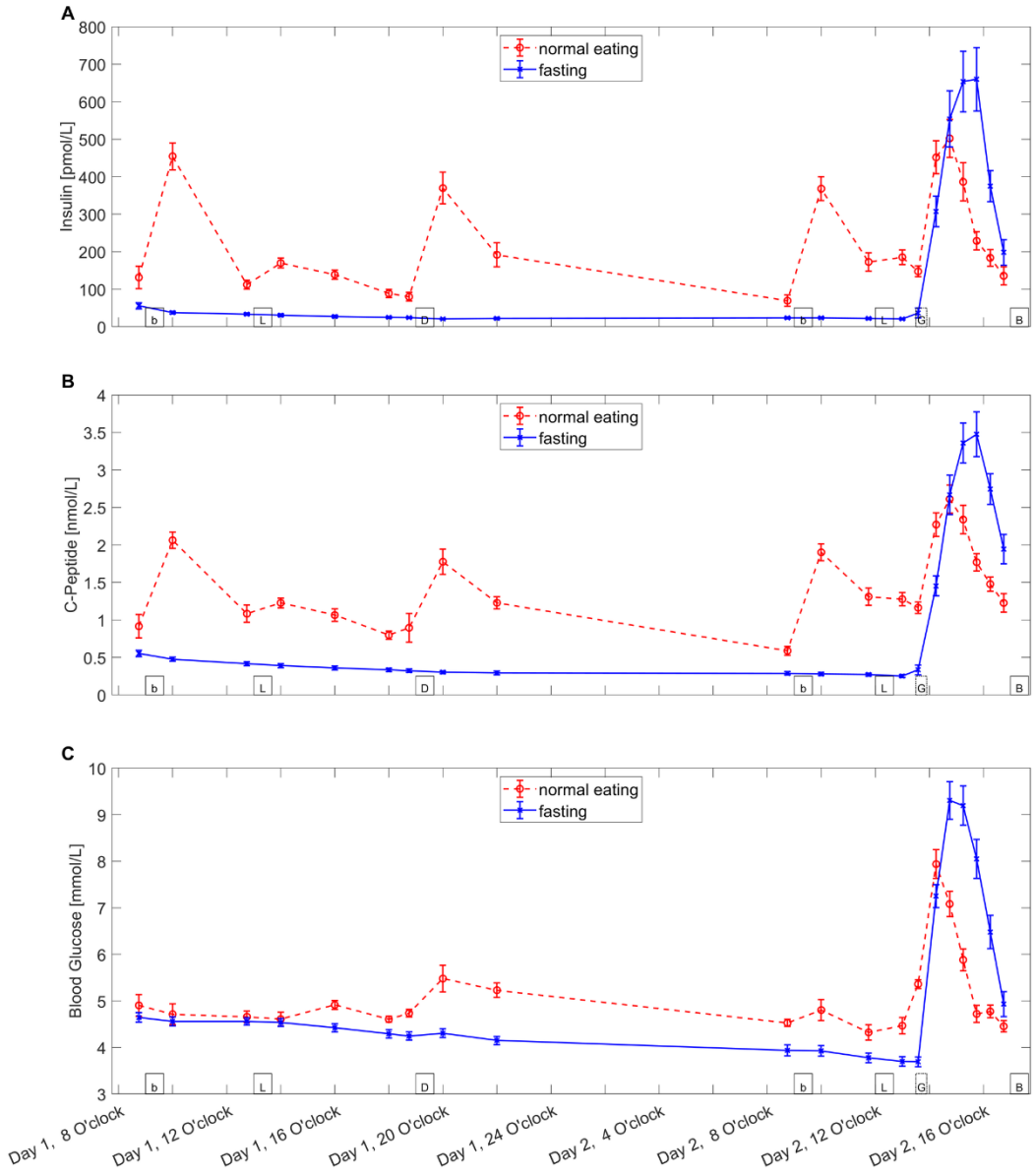

Figure 4: Levels of insulin (A), C-peptide (B), blood glucose (C). The Figure shows the mean ± standard error of the mean for both fasting (blue line, crosses) and normal eating (red spotted line, circles) condition of the serum parameters during the experiment. Boxes on the bottom of the graph indicate the timepoints of meals (b = breakfast, L = lunch, D = Dinner, G = oral glucose administration, B = Buffet).

3.3.3 Correlations of adiponectin and food intake

A significant regression equation was found for the predictor relative protein content in the control condition ($F(1,22)=6.4$, $p=0.019$) with an R^2 of 0.22 when the AUC-values of adiponectin were used as a dependent variable. No significant regression equation was found for the fasting condition.

3.4 Discussion

Part I of this dissertation compared food intake after either fasting or non-fasting periods when an acute caloric deficit was partially removed by oral glucose towards the end of the experiment. Whereas the total quantity of food ingested differed between conditions, the relative proportion of each macronutrient consumed did not. The relative amount of protein intake in the non-fasting condition correlated positively with the AUC-value of adiponectin throughout the experiment.

The observed increase in energy intake following a period of energy restriction has been described before [16,67,68] and appears to be intuitively the expected result. These studies suggest that there is a compensatory increase in overall energy intake after a period of caloric restriction as opposed to when individuals are not fasted. No previous study determined whether similar results would be obtained if the acute caloric deficit was compensated by the means of administering a large quantity of carbohydrates. Also, the question whether the relative proportion of macronutrients consumed changes between fasting and non-fasting conditions has not been tackled previously.

Studies in obese subjects suggested that a compensatory increase in energy intake after a short energy restricted period does not occur [67,69], whereas data obtained in normal weight individuals tend to find an overall compensatory increase in energy intake after energy restriction thus corroborating the current results. This current experiment extended previous results by showing that food ingestion was increased after fasting in spite of application of oral glucose. The overall energy deficit of the body appeared to be driving overall energy consumption, even though insulin, C-peptide and serum glucose had higher levels in the fasting conditions at this time than in the control-condition. These higher levels reflect the well described phenomenon of “post-fasting glucose intolerance” [60,61]. In our case, this phenomenon shows that these parameters are unlikely to be the only factors providing the brain with feedback about the energy status of the body.

The relative macronutrient proportions of the ad libitum consumed food were not significantly different in between the conditions. We stress the fact that the results of the assessment of the

macronutrient composition were only assessed in the context of the pre-meal glucose drink. The administration of the glucose was necessary to test for the main outcome of overall energy intake after a 42 hours caloric restriction after partial compensation with carbohydrates. Since there was no additional non-replenishment control group the influence of the glucose on the composition of food can't be determined by this experiment. Further research is needed to confirm this finding and to determine whether the administered glucose might have affected this measurement as it has been shown that administering carbohydrates before a meal can have an influence on food choice. It has been shown before that carbohydrates can increase subsequent food consumption [70]. Thus, further evaluation of the influence a carbohydrate enriched diet has on food choice is needed. Furthermore, there are other possibilities why the food choice was driven into a certain direction. Some of the food items might have been more appetible than others due to their size or looks. Also, parts of the food were offered in a heated state, which might have directed attention towards these food items. Nevertheless, the stability of the proportions of food consumed under the different metabolic conditions remains noteworthy.

Adiponectin concentration did not differ between the conditions in this study. Due to the short but total caloric restriction, this result is in line with previous work showing that short total caloric restriction for 72 hours does not alter adiponectin levels over time [71]. It has also been shown that low caloric diets do not interfere with adiponectin levels for short [72] and intermediate [73] time periods providing that weight loss is minimal. Usually only long term fasting that results in meaningful weight loss results in rising adiponectin levels [74,75]; and recent work in rodents shows that caloric restriction for four weeks increases adiponectin expression on a cellular level [76]. Of note, declining adiponectin levels during fasting interventions have also been reported [77]. The duration of our study was too short to show alterations in levels of a hormone with slow kinetics such as adiponectin. It therefore supports current literature suggesting a very low diurnal and post-prandial bio variability of adiponectin [78], even when sweetened beverages were offered with normal meals [79].

The proportion of consumed proteins correlated with adiponectin levels only in the non-fasting condition. While existing evidence usually deals with the influence of dietary interventions on adiponectin levels [55,80,81], our finding may hint at a role of adiponectin level in food choice, i.e. macronutrient composition. Indeed, there is evidence adiponectin may take part in appetite regulation in the central nervous system [82,83]. However, whether adiponectin has an appetite stimulating [80] or attenuating role [81] remains controversial. The fact that our experiment did not show a difference in adiponectin levels during the experiment makes it less likely that the detected statistical association is derived from a physiological causal mechanism and overall harder to interpret the finding. No change in both adiponectin levels and macronutrient intake in between conditions while the associations only exists in one of the conditions does not leave any room for causal conclusions. However, different metabolic conditions might also alter the influence of endocrine signals on ingestive behaviour and adiponectin might exercise control differently over central nervous function in a fasting metabolic state than in a normally nourished one. Further research will be needed to evaluate the role of adiponectin in this context. Additionally, with regard to the relationship between adiponectin and protein intake, it has to be stressed that the direction of causality cannot fully determined by the current experiment. Although the adiponectin concentrations were measured before the buffet which might indicate that adiponectin was the cause and the protein intake the effect, as pointed out before, adiponectin has rather slow kinetic characteristics making reverse causality a possible option for the interpretation of the data.

Further limitations of this experiment include that neither women nor people outside an average weight range were included. Thus, these results might not extend to groups other than young, healthy normal weight men. Moreover, the oral glucose administration prior to the administration of the buffet test might have masked effects of fasting on food choice. Future studies might also explore to administer a more tailored quantity of glucose to compensate the energy deficit more precisely, which was not attempted. Only a limited number of hormones directly involved in glucose metabolism and one adipokine were measured which have been shown before to be

influenced by the composition of food. Further research into other endocrine signal parameters such as CKK, glucagon, GLP-1, ghrelin and leptin in this context will be needed to gain a more comprehensive understanding of quantity and quality of food intake.

4 Part II: Modulation of brain activity by hormonal factors in the context of ingestive behaviour

This part* of the thesis is based on the paper published under the same name in Metabolism [84].

* Publication is slightly adapted to fit format, form and content of this dissertation, but is mostly presented here identical to the original, peer-reviewed published article.

4.1 Introduction

On the neural level, energy intake and its regulation is commonly assumed to rely on two main neural systems: A hypothalamic homeostatic system [18,19] and a more extended system including other cortical and subcortical structures that integrates hedonic and reward related processes associated with food intake and obesity [28,32].

The brain is equipped with receptors and sensors for insulin [85], adiponectin [86,87], as well as numerous other hormonal signals [88–90]. Glucose levels seem to be primarily sensed in the portal vein from where neural signals are transmitted to the brain stem and hypothalamus [91–93]. Thus, there are receptors in place that could in principle modulate brain activity as a function of metabolic state. Indeed, previous studies that have externally administered insulin (transnasally) [94–96], leptin [27,97–99] or glucose [100–103] have found marked alterations of the neural processing of food items (see also review by Zanchi et al. [104] who discusses further hormones).

The homeostatic hypothalamic system is influenced by peripheral endocrine signals relaying information about the energy status of the body and food intake [48]. Endocrine signals derived, e.g. from the gut or adipose tissue, may modulate a number of cortical and subcortical sites besides the hypothalamus [36,105,106]. For example, insulin alters the activation of brain regions like the fusiform gyrus, right hippocampus, right superior temporal cortex and mid frontal cortex after intranasal administration [38]. This shows that the networks regulating hunger and satiety interact with other cortical and subcortical brain systems, e.g. those supporting attention processes [28,29]. Glucose, as

the primary source of energy of the brain, has also been found to alter the fMRI signal of cortical regions after being infused in human subjects [103]. Furthermore, the adipokine leptin interferes with the activation of brain regions such as the striatum [27]. Less knowledge is available for other adipokines, such as adiponectin whose role as a beneficial factor for insulin sensitivity and anti-inflammatory acting agent makes it a promising target for research [107–109]. Higher peripheral levels of adiponectin correspond to higher levels in the central nervous system with adiponectin receptors being expressed in the CNS. Intraventricular injection of adiponectin leads to weight loss and higher energy expenditure [110–112]. Of note, most studies have been carried out in rodents and human imaging studies have used external application of substances intravenously, intranasally or in the intraventricular system. Consequently, very little is known about the interaction of endocrine and metabolic parameters with the central nervous system in a physiological context, i.e. during different metabolic conditions such as hunger and satiety. One of the few exceptions is a PET study that did not find an association of between the glucose and physiological insulin levels [113]. Also, electrophysiological experiments in rodents showed an influence of insulin-mediated signals arising from the ventral tegmental area to various sites of the brain that are considered to be part of the central functional reward system [114].

Under this paradigm, the same hormones as in Part I were tested. These included endocrine parameters associated with glucose metabolism (insulin, C-peptide, cortisol, ACTH, serum glucose). Adiponectin was also included as it supports the crosstalk between fat tissue and nervous system in the regulation of food intake.

Peripheral hormones derived from gut and adipose tissue as well as metabolites are one avenue by which the energy status of the body is affecting central brain activation, which differs for example in fasting and normally nourished individuals [115]. The brain regions affected by these metabolic and energy level differences depend on the paradigm of the study. While some areas such as the hippocampus, ventromedial prefrontal cortex, amygdala, parahippocampal gyrus and

fusiform gyrus appear to be more affected by presentation of visual food cues [115,116], regions like the insula appear to be especially responsive when individuals are asked to rate visual cues, again modulated by metabolic condition [34,117].

Here, we attempted to find associations of the mentioned endocrine parameters and central activation in a physiological context of varying metabolic conditions over a longer period of time.

We hypothesised that hormones and serum parameters measured either after 38 hours of fasting or 38 hours of a controlled eating condition (before the fMRI) would be associated with the activity of brain regions involved in ingestive behaviour. Brain regions of interest (ROI) were selected *a priori* from the pertinent literature as being relevant for food intake regulation. Furthermore, it was hypothesised that these ROIs are modulated by the rating of food cues during the fMRI scan, the metabolic condition (fasting vs. non-fasting) or a combination of both.

4.2 Methods

4.2.1 Participants

Details can be found in 3.2.1 Participants. All participants completed the whole experiment, except for the data of one subject had to be discarded due to motion artefacts.

4.2.2 Experimental setting

Details can be found in 3.2.2 Experimental setting. For this part of the dissertation only endocrine measurements before the fMRI were taken into account.

4.2.3 Serum parameters

Details can be found in 3.2.3 Assays.

4.2.4 fMRI task

A slow event-related design was used with 72 pictures presented via monitor goggles one after another in a randomized order for each participant to reduce bias for beginning vs. end of scan

session. The images are part of a high-resolution picture database of the Department of Neurology of the University of Lübeck. The pictures were rated in terms of their caloric content and sweet and savoury qualities by four expert raters and selected to show high and low calorie, sweet and savoury food. A new picture was presented every 20 seconds for two seconds. After the picture had disappeared the subjects had time to rate the depicted food on a scale of 1 to 8 regarding their craving for the particular food item by pressing a button on a keyboard. No time-limit was imposed for the rating; however after 20 seconds the next picture was shown and the next rating iteration was started.

4.2.5 MRI acquisition

A 3 Tesla Philips Achieva MR-scanner equipped with an 8 channel head-coil was used. A structural T1 weighted 3D turbo gradient Echo sequence with SENSE was performed with 180 sagittal slices of 1 mm, a 240 x 240 matrix and a flip angle of 9°. The echo time was 3.04 milliseconds (ms) with a repetition time of 6.72 ms. The functional session followed subsequently and consisted of 366 volumes. T2* weighted images were acquired with an Echo-planar pulse frequency with SENSE factor 2. Sagittal slices of 3 mm in a 64 x 64 matrix and a field of view of 192 mm and a flip angle of 80° were measured. The repetition time was 2 s and the echo time 25 ms.

4.2.6 Statistics and fMRI analysis

Matlab R2015b, SPSS 22 and R 0.99.902 were used for data analysis. For the non-parametric food rating data, the Wilcoxon test was used while t-tests were calculated for reaction times. A 2 x 2 factorial ANOVA was carried out with the factors rating (median split) and study condition for the percent signal change values of the ROI analysis. The percent signal change values of the 10 bilateral ROIs were defined as dependent variables for a multivariate multiple regression, in which the AUC values of the serum parameters of the time before the fMRI (time points 1-13) were used as predictor variables. AUC values were chosen to represent a measure of the overall activity of an

endocrine parameter during the experiment, being applicable due to the high level of standardisation in food intake and timing of the experiment.

Analysis of fMRI data was carried out with SPM 8 (Wellcome Trust Centre for Neuroimaging, UCL, UK). Preprocessing of the functional data comprised slice time correction with Fourier phase shift interpolation, followed by realignment and coregistration of functional and structural T1- to the mean functional image. The DARTEL algorithm was used to adjust T1 images to the Montreal Neurological Institute (MNI) template [118]. Functional images were spatially normalized to MNI space by applying the normalization parameters of the structural DARTEL normalization procedure to the functional data. In a final step functional data were smoothed with an 8 mm full width at half maximum (FWHM) Gaussian kernel and then analysed in a comparative fasting>control paradigm. A general linear model was designed according to the within study design with two conditions with the SPM 8 canonical hemodynamic response function, restricted maximum likelihood and an additional regressors for movement artefacts. An uncorrected factorial design of paired t-tests for the contrast in between the activation parameters was used with a threshold of $p < 0.001$ and a minimal Cluster size of 10 Voxels (270 mm^3). Percent signal change values were calculated for the most significant cluster with the function Rfxplot for SPM8 with matlab 2015b [119].

From a literature review of fMRI studies addressing food intake and fasting, the following regions of interest (ROI) were defined (see Figure 5 for illustration): amygdala, caudate nucleus, insula (3 different regions), nucleus accumbens (NAcc), orbitofrontal cortex (OFC, 2 different regions), pallidum and putamen [62,63,65,120–122]. The OFC and insular regions were defined according to the Jülich histological atlas [123]. The Harvard-Oxford subcortical atlas was used for all other brain regions [124]. The means of the percent signal change values were calculated individually for the subjects for each condition and the grouping according to high and low rating of the pictures.

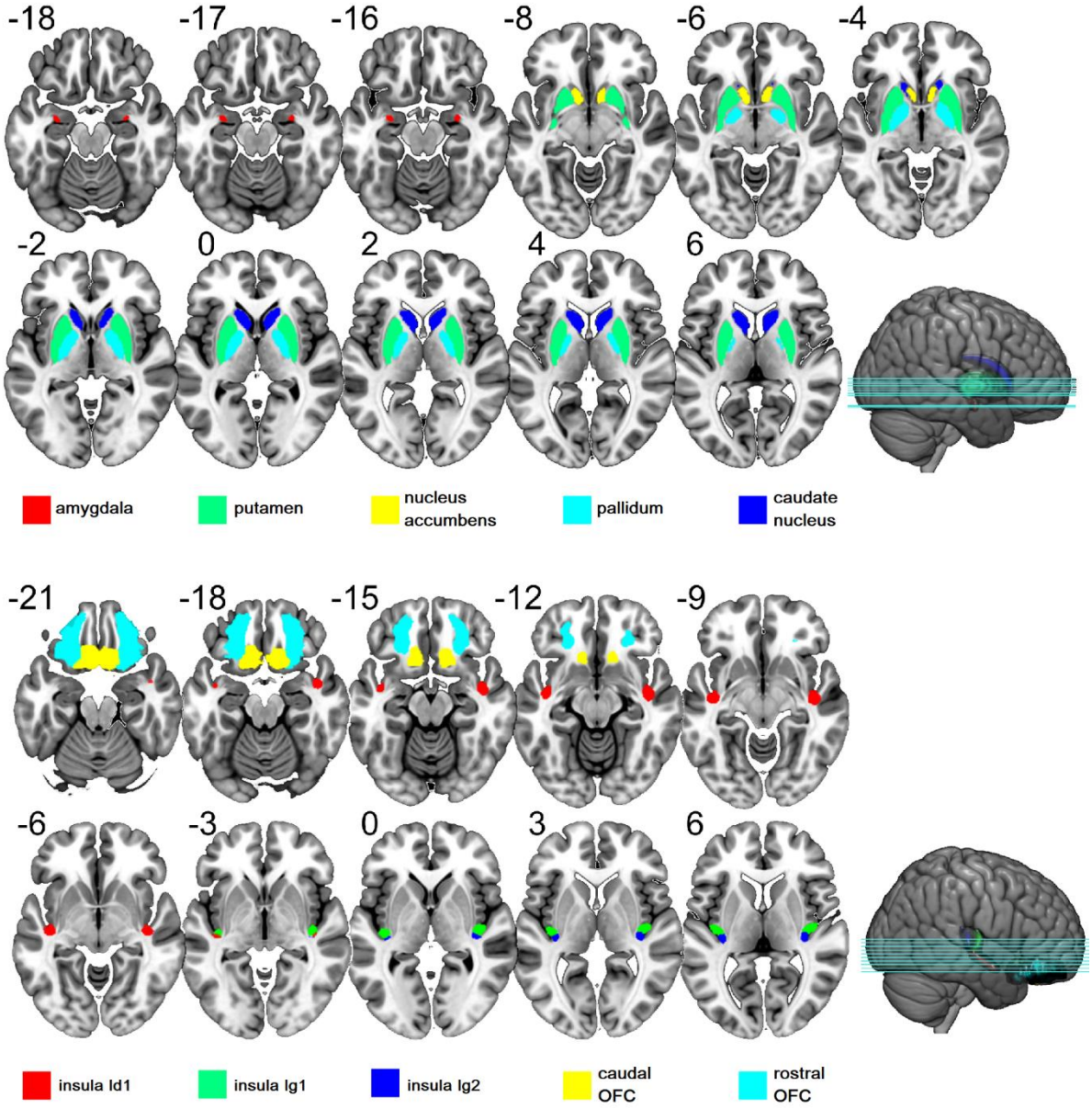

Figure 5: Illustration of the different regions of interest used in the present experiment.

4.3 Results

4.3.1 Rating of food pictures

This analysis was performed to assess the behavioural changes in the participants in response to the fasting manipulation. Ratings differed significantly between the conditions (median fasting: 6; non-fasting: 4 Z=-16.1, p<0.001, see Figure 6 for histogram). Subjects were faster in the fasting condition (fasting 3313 ± 1094 ms; non-fasting 3574 ± 1208 ms, t(22)=2.6, p=0.016). 41 ratings (1.2

%) were missing as subjects failed to respond and these ratings were therefore excluded from analysis.

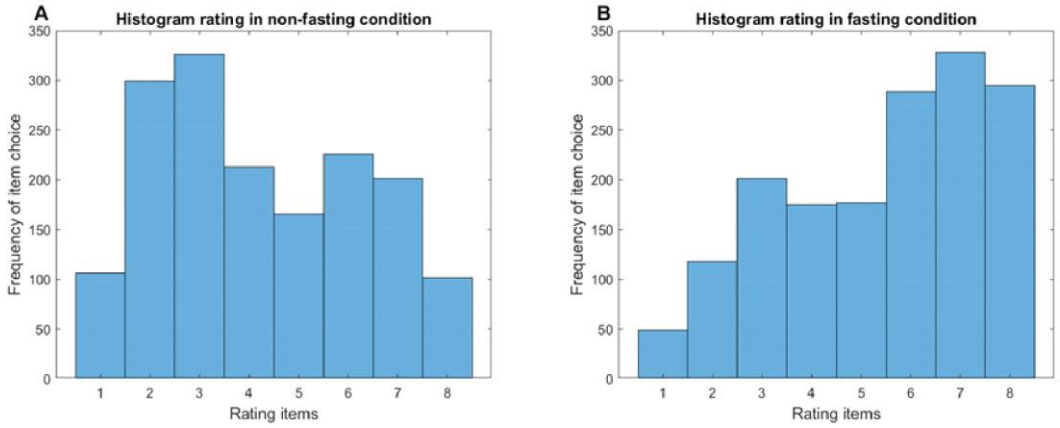

Figure 6: Rating of food images during the fMRI measurement. Histograms of the distribution of the rating are shown in A for the non-fasting condition and B for the fasting condition.

4.3.2 fMRI Region of Interest

Significant clusters are defined with $p<0.001$ (uncorrected) with a minimum cluster size of 10 voxels. The BOLD signal of two clusters differed significantly in between the fasting and the control condition (fasting > eating). These clusters are anatomically located bilaterally in the insula/operculum (Brodmann area 48). Details can be found in Table 1.

Table 1: Statistical values for the fasting > eating analysis. Two clusters were found to be significantly different in between the conditions when applying a threshold of p < 0.001 and a minimal cluster size of 10 Voxels.

Area	Brodmann area	Cluster size [mm³]	p-value cluster uncorrected	peak z-value	uncorrected p-value peak voxel	coordinates x,y,z
left insula/operculum	48	10233	0.001	3.98	< 0.001	-45, -18, 21
right insula/operculum	48	4077	0.023	3.74	< 0.001	42, 9, 15

ROI analysis was performed to assess how brain activation changes in response to food stimuli and to fasting. A 2 (fasting vs. non-fasting) x2 (low vs. high rating) factorial ANOVA was performed with

the percent signal change values for every single ROI (Figure 7). Bonferroni-corrected significant results were found for five ROIs for the main effect rating including the right caudate nucleus (signal change low rating: 5%, high rating: 10.7%; F (1,22) = 22; p < 0.001; η^2_p = 0.5), left NAcc (low rating: 2.6%, high rating: 7.1%; F (1,22) = 12.35; p = 0.002; η^2_p = 0.36), left caudal OFC (low rating: 1.1%, high rating: 8%; F(1,22) = 17.9; p < 0.001; η^2_p = 0.45), left rostral OFC (low rating: 4%, high rating: 11.4%; F(1,22) = 23.2; p < 0.001; η^2_p = 0.513) and left putamen (low rating: 18.5%, high rating: 4.2%; F (1,22) = 16.1; p = 0.001; η^2_p = 0.422). No other main effects or interactions were significant after correction for multiple testing.

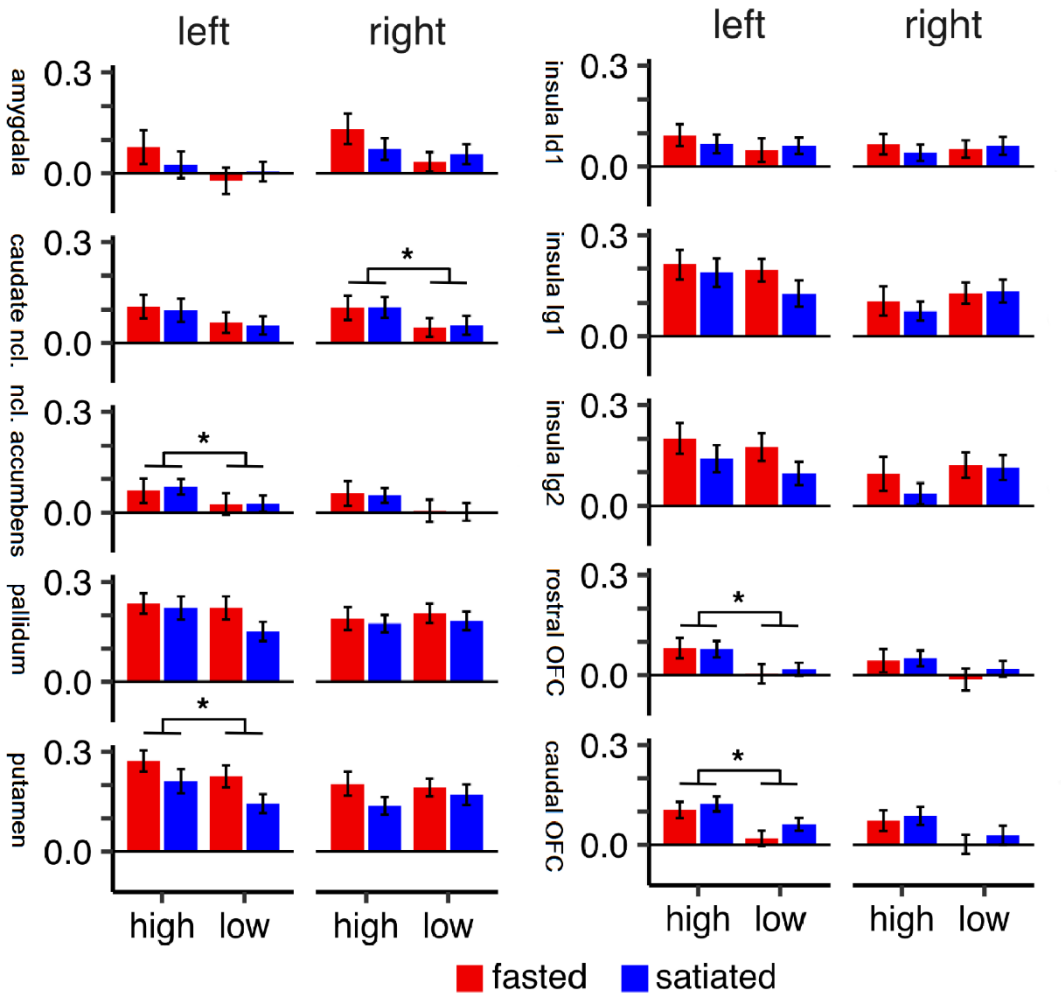

Figure 7: Region of interest analysis. Depicted is the percent signal change in the different conditions per ROI. The significant effects are indicated for each bar graph (p< 0.05, Bonferroni corrected).

4.3.3 Relationship between ROI activations and serum parameters before the fMRI

The following analyses were performed to get insight into the relationship between the fasting-related dynamics of hormonal / metabolic parameters and brain activations. A multivariate multiple linear regression was calculated for each condition with the percent signal change of the ROI as dependent variables and the AUC serum parameter values as independent variables. In the non-fasting condition (Table 2) associations were mainly found between bilateral orbitofrontal regions with the AUC-values of blood glucose measurement before the fMRI. In the fasting condition adiponectin AUC-values were associated bilaterally with the caudate nucleus (Table 3 and Figure 8). AUC-parameters of insulin were also associated bilaterally with orbitofrontal regions and the right orbitofrontal region was associated with the AUC of C-peptide levels during the experiment.

Table 2: Significant results of multivariate multiple regression of the non-fasting condition with the endocrine AUC-values as predictors for the percent signal change activation of the ROIs.

Region of Interest	Endocrine parameter	β-coefficient	Standard error	T-value	p-value
left rostral OFC	blood glucose	2.34E-04	1.10E-04	2.12	0.05
right rostral OFC	blood glucose	3.18E-04	1.50E-04	2.12	0.05

Table 3: Significant results of the multivariate multiple regression of the fasting condition with the endocrine AUC-values as predictors for the percent signal change activation of the ROIs.

Region of Interest	Endocrine parameter	β-coefficient	Standard error	T-value	p-value
left caudate nucleus	adiponectin	7.83E-04	2.85E-04	2.74	0.014
right caudate nucleus	adiponectin	6.47E-04	3.03E-04	2.14	0.049
left caudal OFC	insulin	4.09E-04	1.88E-04	2.17	0.045
right caudal OFC	insulin	5.41E-04	1.91E-04	2.84	0.012
right caudal OFC	C-peptide	-4.67E-02	2.11E-02	-2.22	0.042

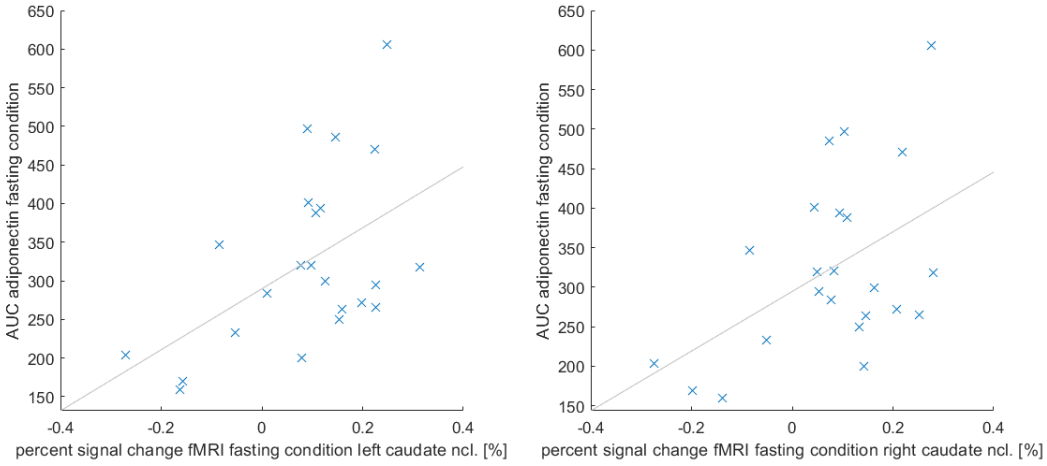

Figure 8: Scatterplots showing the association of adiponectin and the caudate nucleus bilaterally in the fasting condition. The percent signal change is depicted in relation to the AUC of adiponectin and a line of best fit is added (least squares method) for visualising individual results of the regression analysis.

4.4 Discussion

The main purpose of this section was to get first insights into the relationship of hormonal / metabolic parameters and brain activations during the processing of food stimuli. The novel aspect of this experiment is that natural variations of hormonal and metabolic parameters were induced by a fasting period and compared to conditions of regular food intake. Multivariate regression analysis revealed associations between brain activity and hormonal / metabolic parameters for orbitofrontal regions and blood glucose levels in the non-fasting condition. More associations were

found in the fasting condition: insulin levels were associated with fMRI signal change in bilateral orbitofrontal cortex, whereas adiponectin levels were associated with activations in the left and right caudate nucleus. Finally, C-peptide levels were associated with food-related activity in right orbitofrontal regions.

Differences in the BOLD signal of the insula were found in the analysis fasting > control bilaterally in this study. Early studies exploring the activation of brain regions after a fasting intervention pointed out the significance of the insula in this context [125]. Later studies following the paradigm of fasting interventions found that the insula especially plays a role in rating processes of food cues [34]. This might be explained by the insulas role in generating salience [126]. These functions of the insula appear to demand more activity in a hungry state, supporting evidence for is potential role in food evaluation and ingestion. To increase statistical power for the fMRI-analysis we used a ROI based approach instead of a whole brain approach for the exploration of the connection between of hormonal / metabolic parameters and brain activations. Regions of interest were defined on the basis of previous studies that have investigated the processing of food items [62,63,65,120–122]. The statistical analysis revealed statistically significant effects for the factor rating (highly desirable vs. less desirable food items) for the right caudate nucleus, right nucleus accumbens, left putamen and left orbitofrontal cortex, i.e. regions that have been previously associated with the evaluation of items. Of note, the desirability ratings were significantly different between the fasting and non-fasting conditions which is in line with previous results [127]. Subjects rated food pictures also significantly faster in the fasting condition suggesting a heightened attention towards food in this condition [128,129]. For the fMRI analysis we performed a median split to ensure an equal number of stimuli in the two categories. Despite the overall higher desirability ratings of food stimuli, we did not obtain a stimulus category x metabolic state (fasting vs. non-fasting) interaction effect in the ROI analysis. One reason for this failure to find an interaction might be the composition of the stimulus materials. In an earlier study comparing patients with anorexia and healthy controls during the processing of visual stimuli in fasted and satiated states, we had employed pictures of objects

and food items [130]. In this study, clear group x stimulus category x metabolic state interactions were observed. Thus, we argue that non-food control stimuli might have increased our ability to detect stimulus category x metabolic state interactions.

As stated above, the main purpose of Part II of this dissertation was to reveal a relationship between brain activations and hormonal / metabolic parameters. This analysis revealed effects of insulin on the OFC. The OFC is concerned with the valuation of stimuli in general [131–134] and food in particular [135]. Suzuki et al. found that food value is represented in patterns of neural activity in both medial and lateral parts of the OFC [135]. It is interesting that insulin during the fasting condition modulated orbitofrontal brain responses to food items. Obviously, the valuation of food stimuli should be dependent on the metabolic state of an individual [136]. Insulin is a key signal for metabolic status and energy needs. While insulin receptors are abundantly expressed in prefrontal cortex [137–139], it remains unclear whether the modulating effect of insulin on orbitofrontal activity is due to a direct action or to an effect on upstream neural centres, e.g. in the hypothalamus; [139,140], which likewise express insulin receptors.

With regard to the modulating effect of adiponectin on brain activations in the left and right caudate nucleus it is important to note that adiponectin has been shown to bind and influence hypothalamic structures which in turn are functionally connected to other cortical and subcortical structures [34].

While this experiment thus suggests a modulation of brain activation related to ingestive behaviour by a number of hormonal / metabolic factors in a quasi-natural setting, i.e. without external application of hormones, it is important to note its limitations. First, the current approach is correlative. Therefore, further studies are needed to demonstrate a causal influence. Many other factors such as psychological or autonomous parameters may influence relevant brain areas under fasting conditions and take part in controlling their activity. Second, we only included male, healthy, normal weight subjects. Results in obese individuals might be different, as differences in brain

activations to food items [117,141–144] and in hormonal levels [145–148] have been demonstrated repeatedly. Third, we have selected only a few hormones for the current study. As reviewed in Zanchi (2017) multiple further hormones related to ingestive behaviour have been shown to interact with brain function.

5 Part III: Association of endocrine response to oral glucose and preceding central nervous activity in fasting and non-fasting individuals

This part of the thesis is based on the paper in preparation entitled with the same name.

5.1 Introduction

As pointed out in Part I of the thesis, studying the endocrine responses to ingestion of high-energy food, in particular glucose, can be of vital importance in understanding the physiology and pathology of food intake and obesity. In Part II literature review and results of this thesis showed the association of these endocrine signalling pathways – as a response to food intake or in fasting individuals – and central nervous activity. The measurable brain activity – including subcortical and higher, cortical structures – appears to be responsive to peripheral signalling and dependant on the food intake and energy status of the body [33]. If the state of the brain activity before or during energy intake can be associated with the following endocrine response to this intake is so far unexplored. Theories that place the brain in a central regulatory position that controls food intake to cover energy needs by various means are among the most popular scientific models for the explanation of the obesity pandemic [149,150]. An association with the endocrine response to glucose consumption could be understood as an extended arm executing these diverse suggested control mechanisms of the brain. If this method of signalling after food ingestion is part of ingestive control it is furthermore to be expected that this reaction and its associations with central activity depend on the general metabolic situation and energy status of the body.

It was therefore hypothesised that concentrations of peripheral signalling molecules in the blood after glucose ingestions show associations with preceding brain activity in regions associated with control of food intake. These associations are dependent on and differ in between fasting and non-fasting conditions.

5.2 Methods

5.2.1 Participants

Details can be found in 3.2.1 Participants. All participants completed the whole experiment, except for the data of one subject, which had to be discarded due to motion artefacts.

5.2.2 Experimental setting

Details can be found in 3.2.2 Experimental setting.

5.2.3 Serum parameters

Details can be found in 3.2.3 Assays.

5.2.4 fMRI task

Details can be found under 4.2.4 fMRI task.

5.2.5 fMRI acquisition

Details can be found under 4.2.5 MRI acquisition.

5.2.6 Statistics and fMRI analysis

Details about the fMRI analysis, statistics and calculation signal change values of can be found under 4.2.6 Statistics and fMRI analysis. The percent signal change values of the 10 bilateral ROIs were defined as dependent variables for a multivariate multiple regression, in which the AUC values of the serum parameters of the time after the fMRI (time points 14-20) were used as predictor variables.

5.3 Results

5.3.1 Relationship between ROI activations and serum parameters after the fMRI

The multivariate multiple linear regression revealed statistically significant associations between the endocrine responses to oral glucose administration and the preceding brain activity. Detailed results can be found in Table 4 and Table 5 and an exemplarily visualisation as a scatter plot in

Figure 9. In the fasting condition, associations were found mainly of bilateral orbitofrontal regions and the bilateral caudate nucleus with insulin and C-peptide. Additionally, the activity in right amygdala and the left pallidum were found to be associated with the AUC of ACTH. In the non-fasting condition, overall more significant associations were found. Cortisol levels turned out as a significant predictor for the caudate nucleus, insula, nucleus accumbens, orbitofrontal regions, pallidum and putamen. Adiponectin was found to be associated with activity in insula, pallidum and putamen. Activity in the right pallidum and putamen were associated with ACTH levels and the left caudal nucleus with C-peptide levels.

Table 4: Significant results of the multivariate multiple regression of the fasting condition with the endocrine AUC-values after the fMRI and glucose administration as predictors for the percent signal change activation of the ROIs.

Region of Interest	Endocrine parameter	β-coefficient	Standard error	T-value	p-value
right amygdala	ACTH	-3.32E-02	1.25E-02	-2.66	0.017
left caudate nucleus	insulin	2.77E-04	1.08E-04	2.56	0.021
left caudate nucleus	C-peptide	-6.07E-02	2.80E-02	-2.17	0.046
right caudate nucleus	insulin	2.92E-04	1.04E-04	2.80	0.013
left caudal OFC	insulin	2.92E-04	8.13E-05	3.59	0.002
left caudal OFC	C-peptide	-5.46E-02	2.11E-02	-2.58	0.020
right caudal OFC	insulin	3.35E-04	9.85E-05	3.40	0.004
right caudal OFC	C-peptide	-5.63E-02	2.56E-02	-2.20	0.043
left rostral OFC	insulin	1.73E-04	7.55E-05	2.29	0.036
right rostral OFC	insulin	2.01E-04	8.84E-05	2.28	0.037
left pallidum	ACTH	-2.56E-02	1.17E-02	-2.19	0.044

Table 5: Significant results of the multivariate multiple regression of the non-fasting condition with the endocrine AUC-values after the fMRI and glucose administration as predictors for the percent signal change activation of the ROIs.

Region of Interest	Endocrine parameter	β-coefficient	Standard error	T-value	p-value
left caudate nucleus	cortisol	4.58E-04	1.66E-04	2.76	0.014
right caudate nucleus	cortisol	4.32E-04	1.74E-04	2.48	0.025
left insula ld1	adiponectin	5.52E-03	1.97E-03	2.80	0.013
left insula ld1	cortisol	3.62E-04	1.16E-04	3.12	0.007
right insula ld1	cortisol	4.32E-04	1.21E-04	3.58	0.003
left insula lg1	adiponectin	8.96E-03	3.47E-03	2.58	0.020
right insula lg1	adiponectin	7.68E-03	2.50E-03	3.07	0.007
right insula lg1	cortisol	4.85E-04	1.47E-04	3.30	0.005
left insula lg2	adiponectin	8.84E-03	3.06E-03	2.89	0.011
left insula lg2	cortisol	4.86E-04	1.81E-04	2.69	0.016
right insula lg2	adiponectin	7.66E-03	2.87E-03	2.67	0.017
right insula lg2	cortisol	5.65E-04	1.69E-04	3.34	0.004
left ncl. accumbens	cortisol	3.06E-04	1.23E-04	2.48	0.025
right ncl. accumbens	cortisol	3.07E-04	1.26E-04	2.44	0.027
left caudal OFC	cortisol	3.15E-04	1.16E-04	2.72	0.015
left caudal OFC	C-peptide	6.08E-02	2.48E-02	2.45	0.026
right caudal OFC	cortisol	4.08E-04	1.10E-04	3.71	0.002
left rostral OFC	cortisol	3.28E-04	1.05E-04	3.12	0.007
right rostral OFC	cortisol	4.12E-04	1.51E-04	2.72	0.015
right pallidum	adiponectin	5.29E-03	2.27E-03	2.33	0.033
right pallidum	ACTH	-1.73E-02	7.06E-03	-2.45	0.026
right pallidum	cortisol	4.20E-04	1.34E-04	3.15	0.006
left putamen	cortisol	4.65E-04	1.83E-04	2.55	0.022
right putamen	adiponectin	5.65E-03	2.45E-03	2.31	0.035
right putamen	ACTH	-1.84E-02	7.63E-03	-2.41	0.029
right putamen	cortisol	5.30E-04	1.44E-04	3.67	0.002

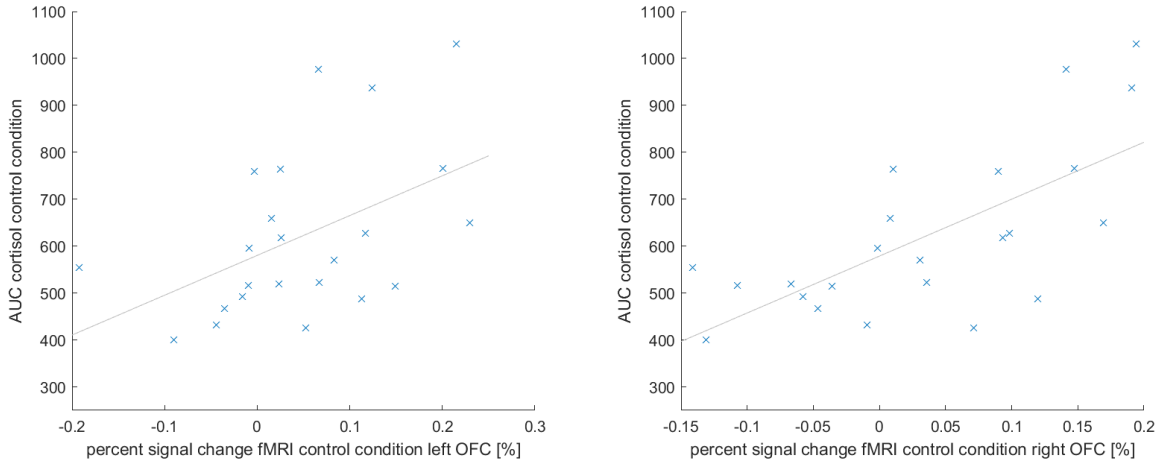

Figure 9: Scatterplots showing the association of cortisol and the OFC bilaterally in the control condition. The percent signal change is depicted in relation to the AUC of cortisol and a line of best fit is added (least squares method) for visualizing individual results of the regression analysis.

5.4 Discussion

In both fasting and non-fasting conditions endocrine factors responding to oral glucose ingestion were found to be significantly associated with brain activation of specific regions before the ingestion. In the fasting condition, pancreatic anabolic hormones were predominant factors of these associations with orbitofrontal regions and the caudate nucleus. Generally, a link between insulin and activity of such brain regions has been described before in non-physiologic settings (with artificial administration of the biologically active substance) [94,95]. However, these data lead to the possibility of a central nervous modulation of insulin release and therefore glucose tolerance depending on metabolic conditions. Earlier studies have found that exposing the CNS directly to insulin does not only modulate brain activity but also the peripheral release of C-peptide and therefore insulin [151]. Since only insulin was nasally administered in these studies, and C-peptide and insulin measured peripherally, direct effect of absorbed insulin into the blood circulation could be differentiated from a potential centrally modulating effect on the peripheral production of C-peptide.

In the non-fasting condition, associations were mainly found for adiponectin and cortisol with a wide variety of brain regions. This pattern of associations differs significantly from the pattern we

measured in the fasting condition. The wide variety of association and partially high statistical significance and at the same time clear difference between the nutritional statuses indicate a dependency of these effects on energy status of the body.

To interpret these results, it has to be emphasised that these correlational models, despite the commonly used naming of the input variables as “predictor” and “dependant” variable, infer no clear causation or directionality of the results, especially considering the low temporal resolution of both fMRI and endocrine parameters. Furthermore, the speed at which hormones might act on the CNS and vice-versa is unknown – making the temporal resolution even more blurry. Regardless of these uncertanties, a correlational relationship seems to become much more likely given the presented data and the possibility of a reciprocal relationship between peripheral blood carried signalling and CNS appears to be worth exploring.

6 Conclusion

In conclusion, food intake control is a complex regulatory system that interlinks cortical, subcortical and peripheral signalling molecules. In Part I of this thesis the response of peripheral signalling molecules was examined during fasting and non-fasting periods and food intake afterwards. The total quantity of ingested food differed after the two study conditions, however the proportion of macronutrients in the food stayed stable. We also found an association in between aggregated adiponectin levels during the experiment and protein intake in the non-fasting conditions.

In Part II brain activity was added as a factor to the experiments and relations to the measured and described peripheral endocrine molecules analysed. Multivariate regression showed associations for orbitofrontal regions and blood glucose levels in the non-fasting condition. In the fasting condition associations were found for insulin levels with fMRI signal change in bilateral orbitofrontal cortex, and for adiponectin levels with activations in the left and right caudate nucleus. Finally, C-peptide levels were associated with food-related activity in right orbitofrontal regions. Whole brain analysis revealed differences in the BOLD signal of the insula for fasting > control bilaterally. While these regions that were found in the whole brain approach to be of relevance and others used for ROI analysis have been described as important for food intake regulation before, the associations found with hormones in a physiological setting without administration of biologically active signalling substances are novel.

In the final Part III, an attempt was made to link the endocrine response to energy intake to the preceding brain state. This proved overall, somewhat unexpectedly, to be the most fertile approach measured by the number and strength of associations. The interpretation of these results must be performed with outmost carefulness, as multiple factors limit inferring a directionality of correlation and forbid inferring causation.

However, connections between peripheral blood carried signalling molecules and central brain activity seem to be present given the presented data, indicating an overall modulating effect of

these factors on different brain regions. These effects furthermore depend on the nutritional status of the body. These modulating effects seem to be of relevance before measuring brain activity and afterwards. If these correlational observations of physiological statuses can be transferred into causational knowledge and knowledge leading to an understanding and remedy for the obesity pandemic will have to be shown by further research.

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9.1 Publications

Publications of relevance for this dissertation:

Janis Marc Nolde, Jana Laupenmühlen, Arkan Al-Zubaidi, Marcus Heldmann, Kamila Jauch-Chara, and Thomas F. Münte. 2019. “Modulation of Brain Activity by Hormonal Factors in the Context of Ingestive Behaviour.” Metabolism 99:11–18.

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Arkan Al-Zubaidi, Sandra Iglesias, Klaas E. Stephan, Macià Buades-Rotger, Marcus Heldmann, **Janis Marc Nolde**, Henriette Kirchner, Alfred Mertins, Kamila Jauch-Chara, Thomas F. Münte. 2020. “Effects of hunger, satiety and oral glucose on effective connectivity between hypothalamus and insular cortex.” Neuroimage, 217:116931.

Janis Marc Nolde, Sophia G Connor, Arkan Al-Zubaidi, Jana Laupenmühlen, Marcus Heldmann, Kamila Jauch-Chara, and Thomas F. Münte. 2019. “Endocrine profile dataset of fasting and normally eating young, healthy men and following activation of brain areas involved in ingestive behaviour.” Data in Brief (Elsevier), 27:104676.

Janis Marc Nolde, Sophia G Connor, Arkan Al-Zubaidi, Jana Laupenmühlen, Marcus Heldmann, Kamila Jauch-Chara, and Thomas F. Münte. “Association of endocrine response to oral glucose and preceding central nervous activity in fasting and non-fasting individuals.”, in preparation.

Other publications:

Omar Azzam , Marcio G. Kiuchi, Jan K. Ho, Vance B. Matthews, Leslie Marisol, Lugo Gavidia, **Janis Marc Nolde**, Revathy Carnagarin, and Markus P. Schlaich. 2019. “New Molecules for Treating Resistant Hypertension : A Clinical Perspective.” Current Hypertension Reports.

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Janis Marc Nolde, Márcio Galindo Kiuchi, Leslie Marisol Lugo-Gavidia, Jan K. Ho, Justine Chan, Vance B. Matthews, Lakshini Herat, Revathy Carnagarin, Omar Azzam, Markus P. Schlaich. 2020 "Nocturnal hypertension: a common phenotype in a tertiary clinical setting associated with increased arterial stiffness and central blood pressure", in press, Journal of Hypertension