ABSTRACT

Title of Dissertation: **THE EFFECTS OF SUGAR INTAKE ON ENERGY CONTROL**

Changhui Zhao, Doctor of Philosophy, 2015

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Long term use of sugars can induce excess caloric intake and/or obesity. To evaluate the effects of sugar intake on different regions of the hypothalamus (the brain's control center for energy homeostasis) we first developed and then evaluated a microscope-assisted dissection method. Because of the small size of the paraventricular nucleus, we validated the samples by measuring several hormones mainly synthesized in the paraventricular nucleus. These include corticotropin-releasing hormone, oxytocin, arginine vasopressin and thyrotropin releasing hormone. We measured the mRNA expression of each of these hormones using quantitative PCR and detected them principally in the paraventricular nucleus. We further evaluated the effects of various sugar solutions on the expression of several important hypothalamic neuropeptides because they play a pivotal role in

energy homeostasis. We provided Sprague Dawley rats 24 hour access to 15% solutions of glucose, fructose, sucrose or high fructose corn syrup. We then measured the expression of several neuropeptides in different hypothalamic regions, all of which were previously shown to be influenced by sugar consumption (mainly based on the results from a series of PCR arrays). Additionally, we measured plasma leptin, known for its close correlation with body fat mass. As expected, rats that had access to sugar solutions consumed less chow. However, rats with free access to sugar solutions maintained a similar amount of energy intake compared with control. Of the four sugars tested, only fructose decreased expression of cholecystokinin significantly, whereas glucose and sucrose significantly increased the expression of tumor necrosis α only in the paraventricular nucleus, not in the ventromedial nucleus or the lateral hypothalamic area. Fructose and sucrose decreased growth hormone expression in the ventromedial nucleus. Glucose increased dopamine receptor D1A expression in the paraventricular nucleus only. We conclude that 24 hour free access to different sugars can influence the expression of several hypothalamic neuropeptides in different ways and these changes are region dependent. Changes in the expression of these neuropeptides do not disrupt the total energy intake immediately but may contribute to the obesity caused by long term intake of sugars.

THE EFFECTS OF SUGAR INTAKE ON ENERGY CONTROL

By

Changhui Zhao

Dissertation submitted to the Faculty of the Graduate School of the
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FOREWORD

Included in this dissertation are two previously published papers as well as one submitted manuscript. In adherence with the policies set forth by the Graduate School of the University of Maryland, College Park, I note here that I am the principal author of all the aforementioned papers.

DEDICATION

To my beloved wife Qi Li, who made it all possible for me to accomplish my PhD degree in University of Maryland-College Park, USA. Thanks for her continued support and understanding. It's her who gave up the school-life in the middle and travel across the Pacific Ocean just to stand by me and help me. It's her who made it easier for me to make the hardest decision and live though the hardest time. It's also her who made me keep positive and optimistic toward our future. I would like to say to her with all my heart: "Thank you! Thank you for all you have done for me, for us and for our future. Without you, I could not complete my PhD program. I love you, forever".

Also to my mother, for her full support and encouragement all the way. Thanks for all her efforts in raising me up, supporting me financially and spiritually and moving me forward whenever I feel puzzled and tired. Thank you!

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LIST OF ABBREVIATIONS

11β-HSD1:	11β-hydroxysteroid dehydrogenase type 1
11β-HSD2:	11β-hydroxysteroid dehydrogenase type 2
ACC:	Acetyl-CoA carboxylase
ACTH:	Adrenocorticotropic hormone
Adr2b:	Alpha 2B adrenergic receptor
AGRP:	Agouti related peptide
AME:	Apparent mineralocorticoid excess
CCK1R:	Cholecystokinin A receptor
AMP:	Adenosine monophosphate
AMPK:	AMP-activated protein kinase α
Apo C3:	Apo lipoprotein C3
ARC:	Arcuate nucleus
ATP:	Adenosine triphosphate
AVP:	Arginine vasopressin
BMI:	Body mass index

CART: Cocaine and amphetamine regulatory peptide

CB1: Endocannabinoid receptor 1

CBG: Corticosteroid-binding globulin

CCK: Cholecystokinin

cDNA: Complementary DNA

CNS: Central neural system

CRH: Corticotropin releasing hormone

DNA: Deoxyribonucleic acid

fMRI: Functional magnet resonance imaging

GLP-1: Glucagon like peptide 1

GLP-2: Glucagon like peptide 2

GLUT: Glucose transporter

GPCR: G protein-coupled receptor

GR: glucocorticoid receptor

GRP: Gastrin-releasing peptide

GRP: Gastrin-releasing peptide

HFCS: High fructose corn syrup

HPA axis: Hypothalamic pituitary adrenal axis

IL-1: Interleukin -1

IL-6: Interleukin -6

Jak: Janus kinase

JNK: c-Jun N-terminal kinase

Ldha: Lactate dehydrogenase A

LH: Lateral hypothalamus

LXR-α: Liver X receptor α

LXR-β: Liver X receptor β

MC4: Melanocortin 4 receptor

MR: Mineralocorticoid receptor

mRNA: Messenger Ribonucleic acid

NADPH: Nicotinamide adenine dinucleotide phosphate-oxidase

NPY: Nucleus peptide Y

NTS: Nucleus of tractus solitarius

OXM: Oxyntomodulin

OXT: Oxytocin

PCR: Polymerase chain reaction

PEPCK: Phosphoenolpyruvate carboxykinase

POMC: Pro-opiomelanocortin

PP: Pancreatic polypeptide

PRL-RL: Prolactin releasing hormone

PRL-RL: Prolactin releasing hormone

PTP1B: Glucose-6-phosphotase, protein tyrosine phosphatase 1B

PVN: Paraventricular nucleus

PYY: Peptide YY

RAMP3: Receptor activity modifying peptide 3

RPLP1: Ribosomal protein, large, P1

STAT: Signal transducer and activator of transcription

Ta: Annealing temperature

TG: Triglycerides

TNF-α: Tumor necrosis factor α

TRH:	Thyrotropin releasing hormone
VLDL:	Very low density lipoprotein
VMH:	Ventral medial hypothalamus
WHO:	World health organization
α-MSH:	α-melanocyte stimulating hormone

CHAPTER 1. SUGARS AND ENERGY CONTROL – A REVIEW

Overview

Obesity is spreading like a plague worldwide. As a result, the research to curb this trend is urgent. HFCS has taken over the food marketplaces as a sweetener for several years. Because the fructose content of HFCS is greater than that of sucrose, several authorities have speculated that it is this higher fructose intake that has caused the current obesity problem [1-3]. Energy balance is achieved only as the result of both central and peripheral regulators working together. As high sugar intake can induce obesity and metabolic symptoms, whether free access to sugar sweetened solutions also have the same consequences is seldom discussed. In fact it's been known for years that long term access to sugar solutions can induce excess energy intake and overweight. We believe that if sugars induce obesity, they must have the ability of disrupting this system. The question is "How?"

This review chapter describes the background about the basic characteristics of obesity as well as its possible causes and mechanisms. The review also summarizes the current evidence that shows how sugars, typically sugar solutions, affect the energy control in both peripheral and central aspects in an attempt to explain sugar induced change of body weight and/or associated metabolic diseases.

Sugar and Obesity

Obesity epidemic

Obesity is a disease that is characterized by excess fat accumulation as well as a shift in metabolism. The body mass index (BMI) has been developed as a tool to help researchers quantify the degree of obesity [see Table 1]. According to World Health Organization (WHO), people with BMIs over 30.0 are classified as obese. By this definition, the obese population has nearly tripled in the US over the last forty years. Obesity has become a public health epidemic in America. Unfortunately, the obese population is still growing and predicted to reach around 50% of total population in 2020 in US [4]. Furthermore, the obesity epidemic is not limited to the US. Modernization has been accompanied by increasing rates of obesity worldwide. The reason that obesity is a concern is not only because obesity can affect mobility and ease of movement, but also due to the close association between obesity with various comorbidities, such as those illustrated below in Figure 1. It should be noted that most of these are leading causes of death in the US. As a result a great deal of effort and money has been spent on developing obesity treatments. Although obesity is one of the most preventable health problems, to date, there is no effective cure. Dieting and physical exercise are still the mainstays

for obesity treatment. Reduced physical activity and increased energy intake are thought to be the main causes of obesity.

Table 1 BMI and obesity

BMI	Classification
< 18.5	underweight
18.5–24.9	normal weight
25.0–29.9	overweight
30.0–34.9	class I obesity
35.0–39.9	class II obesity
≥ 40.0	class III obesity

Note: $BMI = \frac{Mass\ (kg)}{(Height\ (m))^2}$

There are several types of obesity, of which central obesity is the most harmful. Central obesity is characterized by excessive abdominal or ectopic fat accumulated around the stomach and abdomen. The "pot belly" or "beer belly" are terms that are often used to refer to central obesity. It needs to be noted that central obesity is not confined to obese subjects. It is proposed that it is the body fat instead of body weight that is the key to evaluating health risks related to obesity. One human study showed that fructose-sweetened beverages increased visceral adiposity in overweight/obese subjects, but glucose-sweetened beverages did not[5]. Generally speaking, obese population are at a higher risk of motality[6], therefore we will not distinguish different obesity types in the current review.

Figure 1 Obesity related diseases.

Obesity is closely associated with various comorbidities, including cancer, hypertension, cardiovascular diseases, sleep apnea and gall bladder disease, most of which are leading causes of death in the US.

The body has its delicate mechanism to keep energy balance so as to maintain body weight. Normally, the body matches energy expenditure to energy intake. Any undesirable factors that disrupt this balance can lead to changes in body composition [see Figure 2]. These are often accompanied with an increase of risks of diseases. Animals compensate for variations in caloric content by adjusting intake, within limits. Adolph was the first to report that dilutions of food greater than 50 % resulted in weight loss [7]. By comparison, enriched diets (by increasing its caloric density) promote increased caloric intake [8-10]. From the evolutionary viewpoint, protection from starvation by storing fat has a great advantage when food is scarce. When food is abundant, this “advantage” becomes a disadvantage. Although the body has multiple mechanisms to maintain energy balance under normal conditions, the long-term use of high energy containing foods (including high sugar or high fat diets) can disrupt energy balance. However, different from the sugar enriched diet that can be considered as calorie dense diet, whether free access to sugar sweetened solutions also has the similar consequences needs further investigation.

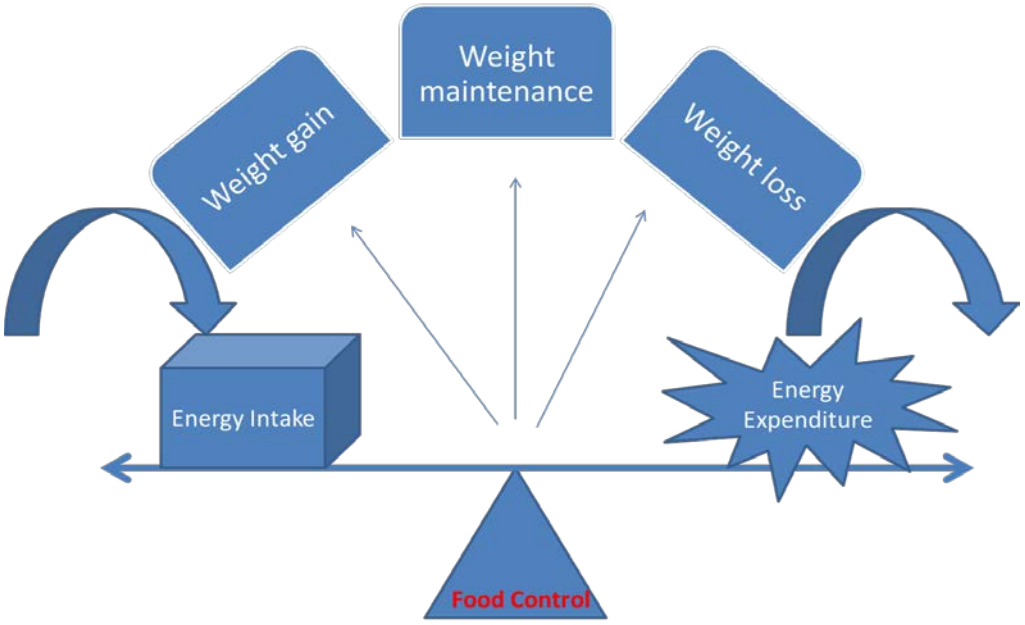


Figure 2 Scheme of energy balance.

The body has its delicate system that matches energy expenditure to energy intake. Any undesirable factors that disrupt this balance can lead to changes in weight gain or weight loss.

Fructose hypothesis debate

There are many factors that can cause obesity. These include some specific diseases like schizophrenia and downs syndrome as well as genetic mutations like FTO gene mutation, leptin gene or leptin receptor gene mutations. Modernized lifestyle including reduced physical activity, sedentary lifestyle or dietary factors especially high sugar diet and high fat intake that can also promote obesity. Because the obesity rate has risen to epidemic proportions it is likely that both genetic and environmental factors are responsible.

It is well known that high fat diet can lead to obesity. However, the fact that years of promotion of low fat or fat free products failed to change the obesity increasing trend shifted people's attention to high sugar diet[2]. Low carbohydrate intake is comparably or even more effective when compared with low fat intake in promoting weight reduction for up to one year [11, 12]. The total consumption of sweeteners has been increasing slowly for years. HFCS has replaced over half of the sucrose over the past few decades. Taken these factors together, the fructose hypothesis was generated: high intake of HFCS or fructose has caused the current obesity epidemic [1-3]. The fructose hypothesis has been bantered about for years. Although the expanding obese population is well correlated with increasing HFCS intake before 1999, HFCS intake has decreased after 2000 whereas the rate of

obesity did not stop or slow down accordingly. Many people argue that HFCS is similar to sucrose and intake of either sugar has little relationship with the obesity epidemic. They have proposed that the obesity epidemic is probably just a result of excess energy intake instead of specific sugar intake [13, 14]. On the other hand, several researchers have continued to propose that fructose or HFCS are responsible for the obesity epidemic [15-17]. They note that even though the total HFCS intake has dropped since 2000, the intake of fructose is still at a high level, which represents approximately 10% on average of total calories consumed [18]. Second, many animal studies have shown that high fructose diets have high potential to induce insulin resistance, hypertriglyceridemia and metabolic syndrome [19-21]. These symptoms are frequently observed in obese people [22]. In fact, several researchers even use high fructose diets to generate obese or insulin resistance models for study [23-25]. Third, fMRI results from human subjects showed that fructose has little effect on the suppression of food intake signals compared with glucose[26].

The data from human studies are limited and inconclusive. Differences in the design of animal studies may also be responsible for inconsistent results. High intakes of sugars by adulterating the diet [21, 27, 28] or by gavage [29] under laboratory conditions are not convincing because they fail to mimic how humans

typically consume sugars voluntarily. On the other hand, some foods with high sugars, many types of fruits for example, are associated with improved metabolic parameters [30, 31]. This is maybe because the natural biological matrix may have the ability of buffering the sugar effects [32]. At this point, studying the free access to sugar solutions are more helpful in understanding the effect of sugars on the current obesity epidemic.

Sugar induced obesity

Recently two questions have been put forward [33]: first, is there evidence that excess energy intake from sugars is more detrimental than excess energy in other forms? Second, is there evidence that specific sugar(s) can promote excess energy intake? Calorie dense foods can increase energy intake and cause obesity, but controlled high fat food can even decrease body weight and improve several metabolic parameters [34]. From this point of view, since excess sugar up-taken can also be stored as fat, high sugar diet induced obesity should also be controlled by restriction or alternate dieting. However, sugar solutions are not considered as calorie dense food if they are freely available. Therefore, in this review we will focus on the evidence that's mainly associated with the effects of free access to

sugar solutions on the energy control, which is helpful to answer the second question.

HFCS intake has increased over 1000% between 1970 and 1990 [1]. Our bodies have several control mechanisms to limit the adverse effects of glucose intake. Different from glucose, fructose (with its limited physiological controls), is much easier to convert to triglycerides. This is probably an evolutionary result due to the ready availability of glucose from traditional foods. Although much evidence has shown that fructose has many adverse effects on our health such as generation of the toxic advanced glycation end-products and causing chronic diarrhea or other functional bowel disorders [35], how sugars affect energy balance needs deeper investigation. One early study showed that short term of high fructose gavage did not show apparent toxic symptoms and the immediate effect of fructose is the glycogenesis in both liver and muscles [36]. Moderate fructose supplementation over 4 weeks increases fasting triglyceride, VLDL-triglyceride, lactate, leptin and glucose without any significant changes in body weight, insulin resistance, hepatocellular lipids and myocellular lipids [37]. However, another research group used high glucose or fructose diet on the overweight and obese human subjects and extended the treatment for up to ten weeks [5]. They found that both sugars induced increased weight gain, however, the visceral adipose volume was only increased in subjects consuming fructose.

Most researches are focusing on the comparison between fructose and glucose. Sucrose is broken down into the monosaccharides glucose and fructose by sucrase in the intestine before absorption. HFCS is a mixture of glucose and fructose as well as some polycose. Since it's easier to regard sucrose and HFCS as just an intermediate between glucose and fructose, few reports include them into research for comparison. Sucrose and fructose are naturally biosynthesized sugars widely found in plants and fruits. Because of economic reasons, HFCS has replaced most of the sucrose as a food additive. It is argued that the health issues caused by HFCS are mainly attributed to its fructose content. In fact, many popular brands of soft solutions use either 65% or 55% fructose containing HFCS [38] and the ratio of fructose to glucose varies from around 1 to 1.5 [39]. HFCS or fructose/glucose mixture is different from sucrose in many ways. For example, HFCS is more preferable than the simple glucose and fructose mixture because of the existence of some polycose [40]. Fructose can facilitate glucose absorption and the fructose in excess of glucose can lead to malabsorption [41]. Subjects with consumption of HFCS-sweetened beverages had higher fructose absorption and glucose bioavailability than those with sucrose-sweetened beverages [42]. Fructose/glucose mixture has more potential to generate hepatic uric acid and triglycerides and cause fatty liver than sucrose. By contrast, sucrose can increase more TNF-α expression in both liver and plasma as well as hepatic MCP-1 expression than fructose/glucose

mixture[43]. The effect of sugars with different chemical structures on gut microbiota may also be different. There is a possibility that different gastrointestinal effects may result from consuming these different sugars. A recent report even showed that non-calorie sweeteners can induce glucose intolerance by changing the gut microbiota[44]. All these suggest that studying the effect of individual sugar forms is necessary. This also points to a follow-up question that whether or not these differences can affect the central neural system (CNS) and lead to changes in energy homeostasis.

It has been known for years that two months' *ad libitum* access to sugar solutions (glucose, sucrose or fructose), can induce excessive caloric intake, weight gain and/or even obesity [45-47]. Further studies showed that long term free access to HFCS (55 % fructose), fructose,sucrose or glucose solutions can all induce overweight and obesity after months' exposure [5, 48-50], though fructose drink seem to be more effective to increase visceral fat. Under normal conditions, energy balance is maintained: the body matches energy expenditure and energy intake [51]. However, this system of energy control seems to have bias under specific "emergent circumstances" which facilitates excess energy intake [52]. Some factors, sugar solutions when freely accessible for example, can apparently disrupt the energy balance so as to induce obesity.

Sugars and peripheral metabolic controls

Cooperating with the hypothalamus, peripheral energy status signals play an important role in energy homeostasis (see Figure 3). A list of well-studied peripheral signals is shown in Table 1. Presented below are several of these that were shown to be affected by sugar intakes based on previous work (for example, Colley et al.'s work [53]).

Table 2 A list of peripheral signals

Signals that arise from peripheral organs	
Catabolic	Anabolic
Leptin	Ghrelin
Insulin	
Amylin	
CCK	
Bombesin family (GRP, neuromedin B, bombesin)	
Glucagon	
Enterostatin	
Apolipoprotein AIV	
Somatostatin	
PYY	
GLP-1	
Signals that act within the hypothalamus	
Catabolic	Anabolic
Leptin	Galanin
Insulin	Corticosterone
Amylin	Cortisol
Urocortin	Dopamine
Urocortin II	Orexins
Neurotensin	Ghrelin
Histamine	Beacon
GLP-1	Cannabinoid
GLP-2	β-Endorphin

TNF-α	Dynorphin
IL-6	Norepinephrine
IL-1	Amino acids
PYY	
PRL-RL	

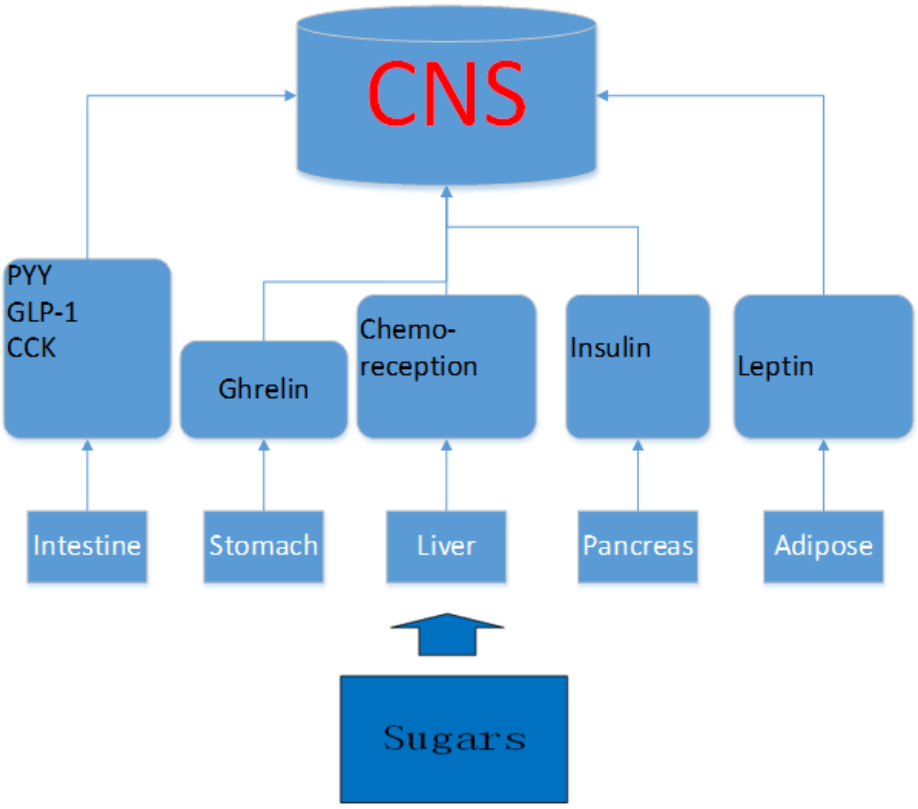


Figure 3 Signals in regulation of food intake.

The dietary sugars affect many peripheral tissues including stomach, intestine, liver, pancreas and adipose tissue, all of which can produce their distinct circulating signals that may further reach the CNS.

Fructose and hepatic glucose metabolism

Dietary fructose is absorbed slower than glucose, making it likely that the effect of the high fructose load to the liver is buffered [36]. Fructose was shown to increase glucose uptake [54], glucose phosphorylation [55, 56] and glycogen synthesis [57] by increasing synthase I activity [36, 58]. Intravenous administration of high fructose can significantly reduce the activity of synthase I and increase phosphorylase a in the liver [59]. However, even high dietary fructose intake cannot reach this level in the bloodstream. Around 38% of absorbed fructose will be stored as glycogen in liver and 37% in the muscle, which is significantly higher than glucose intake [36]. The extracted fructose also facilitates gluconeogenesis. The fructose induced gluconeogenesis is partly due to the increased glucocorticoid receptor activity via the hypothalamic AMPK activation [60]. In contrast, dietary fructose intake seems to contribute little to the circulating glucocorticoids [61]. Therefore, the increased GR activity may be caused by fructose induced 11β HSD1, which is capable of mediating intracellular glucocorticoid [62]. Although fructose and glucose can quickly increase circulating fructose or glucose concentrations [26], one day treatment of these sugars has only a mild effect on circulating glucose levels [63]. All of these show that short term fructose intake is not able of causing obvious health problems. However, as fructose in excess of glucose can lead to

malabsorption [41], it's speculated that long term access to HFCS or sucrose can facilitate sugar absorption and therefore may be more harmful than pure sugar intakes.

Sugars and triglycerides

Fructose and glucose are two of the main sugar sources in the human diet. While glucose can easily pass through liver and be metabolized in nearly every type of cells, fructose metabolism is performed mainly in liver. Fructolysis quickly produces the hepatic trios-phosphates. Most of the trios-phosphates are converted into glucose and lactate. Another part of trios-phosphates are subjected to de novo lipogenesis (see Figure 4). Quick metabolism of a high amount of fructose can lead to an overburdening of the liver, increasing the risk of many hepatic diseases and comorbidities, including non-alcoholic fatty liver disease, steatohepatitis, ectopic obesity and hepatic insulin resistance [64]. Decreased very low density lipoprotein (VLDL) triglyceride clearance, an increased VLDL-triglyceride secretion as well as de novo lipogenesis together contribute to fructose induced metabolic syndrome [64-67]. With fewer metabolic controls, fructose can increase circulating triglycerides quickly before the appearance of the obesity phenotype [67, 68].

Mice lacking fructokinases (isoform A and C) fail to develop fructose-induced metabolic syndrome [49].

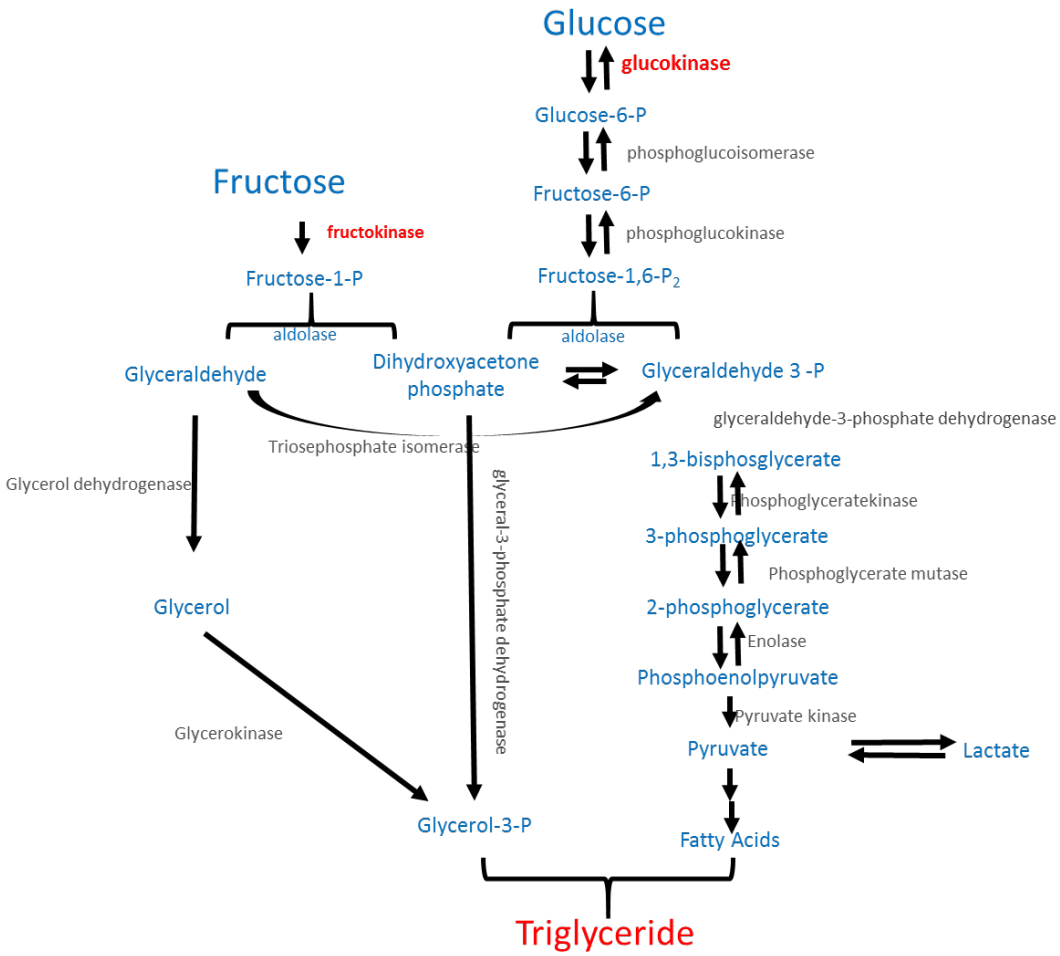


Figure 4 Different hepatic metabolism of fructose and glucose.

Fructokinase is more efficient to metabolize fructose than what glucokinase does for glucose. As a result, glucose can easily pass through liver and be metabolized in nearly every type of cells, whereas fructose metabolism is mainly performed in the liver. Although both share many steps, fructolysis can enter the triglyceride synthesis pathway at a faster fashion.

VLDL carrying triglycerides was thought to cause the fructose induced hypertriglyceridemia[69]. Apo lipoprotein C3 (Apo C3) is an important surface component of VLDL. Because fructose can quickly increase circulating triglycerides, Apo C3 was proposed as a key factor in promoting fructose-induced hypertriglyceridemia. Our lab recently found that hepatic Apo C3 mRNA expression from fructose intake alone did not change significantly compared to the control. On the contrary, hepatic Apo C3 expression was increased by around 250% in glucose, sucrose and HFCS groups [70]. This suggests that Apo C3 expression level is not sufficient to predict the ability of VLDL-TG secretion. Apo B may be another determinant which needs further investigation. A supporting report showed that consumption of fructose-sweetened instead of glucose-sweetened beverages (25% of energy requirement) in addition to *ad libitum* diet could cause postprandial hypertriglyceridemia and increased circulating LDL and Apo B levels in young men and women [71].

Sugars and glucocorticoids

The parvocellular cells of the PVN secrete CRH which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH) into circulation. ACTH activates the adrenal gland to release glucocorticoids, including cortisol in humans

and corticosterone in rodents. The glucocorticoids then suppress the PVN and pituitary to form a feedback loop. In mammals, the adrenal cortex synthesizes mineralocorticoids and glucocorticoids. Circulating levels of glucocorticoids vary widely, from low levels during sleep to relatively high levels during severe stress. These hormones are tightly bound to a high-affinity corticosteroid-binding globulin (CBG) and albumin. As a result only around 5% of glucocorticoid is free, while the inert glucocorticoid level is higher. The glucocorticoids pass through the cell plasma membrane to take effect by binding to the mineralocorticoid receptor (MR) or the glucocorticoid receptor (GR). Once activated, GR or MR translocates to the nucleus and binds to the regulatory regions of target genes to affect downstream pathways. Glucocorticoids can function to increase glucose levels, reduce inflammatory reactions and promote cell differentiation.

Glucocorticoid induced obesity, especially visceral obesity, is well documented. Bilateral adrenalectomy can lead to body weight loss by reducing glucocorticoids [72]. Glucocorticoid induced obesity is clinically manifested as Cushing's syndrome [73]. Surprisingly, circulating plasma glucocorticoids are stable in simple obese patients. As a result local glucocorticoid concentrations are proposed as a key contributor to the obesity development, which is mainly determined by local 11β-HSD1 activity(see Figure 5) [74]. 11β-HSD1 is an enzyme

that catalyzes the inter-conversion of active and inactive glucocorticoids (e.g. cortisol to cortisone in humans and corticosterone to hydroxycorticosterone in rats). The activity and reaction direction of 11β-HSD1 varies by cell types. In intact mature cells, 11β-HSD1 primarily acts as an oxoreductase to facilitate active glucocorticoid generation in both hepatocytes [75] and mature adipocytes [76]. 11β-HSD1 is abundantly expressed in liver, adipose, gonadal and central nervous system tissues. Applying the specific adipose 11β-HSD1 inhibitors has been proposed to be a novel approach to the treatment of central obesity [77]. Both overexpression and knockout of adipose 11β-HSD1 showed that 11β-HSD1 activity is positively associated with metabolic syndrome [78-80]. Similarly, Zucker obese rats that were treated with carbenoxolone (an hepatic 11β-HSD1 inhibitor) had improved lipid profiles [81]. Knockout of hepatic 11β-HSD1 mice resist 1-dehydrocorticosterone induced metabolic syndrome as do the global 11β-HSD1 knockout [82]. Several 11β-HSD1 inhibitors have been and are being tested for use as clinical drugs.

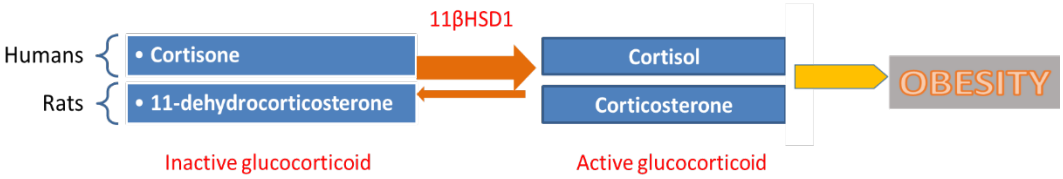


Figure 5 Intracellular glucocorticoids and obesity.

11β-HSD1 is the critical enzyme that can increase the level of active glucocorticoids by regenerating cortisol in humans and corticosterone in rats. The increased intracellular level of glucocorticoid is involved in development of obesity, especially central obesity.

It is well known that obesity is associated with high level of 11β-HSD1 in adipocytes. Fructose can increase 11β-HSD1 in adipocytes [67, 83] and quickly increase hepatic 11β-HSD1 expression after 24 h exposure but decrease its expression after one week [67]. However, the difference between sucrose or HFCS and their controls failed to reach statistical significance. Compared with glucose, HFCS and fructose also slightly reduced 11β-HSD1 mRNA expression in the ventromedial hypothalamus [84]. From this point of view, the fructose component has more potential in causing obesity and/or metabolic syndrome. Consistent with these findings, fructose-sweetened beverages is more effective than glucose to increase visceral adiposity in overweight/obese subjects [5].

Communications between CNS and peripheral tissues

There is a close interaction between CNS and peripheral tissues via circulating signals as well as through the vagus nerve. The hypothalamus can directly sense some peripheral signals including insulin, leptin, glucocorticoid and glucose. The nucleus tractus solitarius (NTS), located at the hindbrain, is another important hub that integrates the peripheral information mainly from the vagus nerve and then relays the signals to the hypothalamus and other brain areas that control feeding behaviors [85].

Effects of sugar intake on CNS

Central control of food intake - Hypothalamus

The hypothalamus, a brain structure that is located below the thalamus, contains a number of nuclei that control a variety of functions. The hypothalamus links the nervous system to the endocrine system via the pituitary gland. In response to various types of stimuli the hypothalamus synthesizes and secretes a series of neuro-hormones which in turn stimulate or inhibit the secretion of pituitary hormones that regulate various endocrine glands and organs. Through this mechanism, the hypothalamus can control body temperature, fatigue, sleep, circadian cycles, hunger and thirst. The hypothalamus can also communicate neuronally with other part of the brain areas to control specific behaviors.

The hypothalamus is the body's energy control center. Several hypothalamic neuropeptides have been identified to play essential roles in energy control. The hypothalamus is composed of various neurons involved in a complicated neuronal network. The arcuate nucleus (ARC) was early proposed as the "first order" control of hypothalamus. It senses peripheral signals in order to control food intake by activating the "secondary control" areas that include the paraventricular nucleus (PVN), ventromedial hypothalamic nucleus (VMH) and lateral hypothalamic area

(LH). This belief is challenged by two phenomena: 1st, diet induced obesity can cause ghrelin resistance in ARC but not PVN [86] ; 2nd, conditional knockout of leptin receptors from POMC [87, 88] and/or agouti related peptide (AGRP) neurons [89] can only cause mild obesity, whereas pan-hypothalamic leptin receptor deletion lead to the severe obesity similar to that with global loss of leptin signaling [52, 90]. Therefore, we think different hypothalamic regions may be able of sensing the circulating signals in various ways. The current study is attempting to find out how different sugars affect energy control by regulating different neuropeptides in these hypothalamic regions.

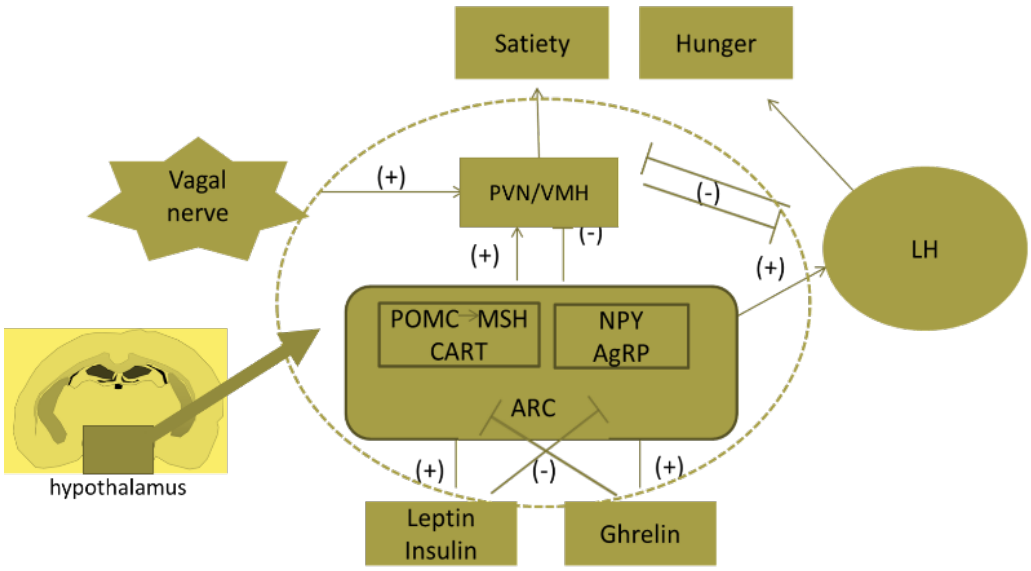


Figure 6 The classic model of hypothalamic control of food intake.

The ARC of the hypothalamus is involved with both anorexic and orexic circuits containing many types of receptors including leptin receptors, insulin receptors and ghrelin receptors. The neurons expressing NPY and AgRP as well as neurons expressing POMC and CART) can receive the signals from the circulating hormones. They work cooperatively to activate PVN and VMH to inhibit food intake and inhibit this process to activate LH to increase food intake or vice versa. PVN, VMH and LH have their own complicated neuro-signals for further feeding behaviors. On the other hand, the hypothalamus can also sense the signals to control food intake via vagus nerve system.

The ARC of the hypothalamus is involved with both anorexic and orexic circuits. The ARC, with its reduced blood brain barrier, can sense signals from the bloodstream quickly. NPY and AgRP are co-localized in ARC neurons, while pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART) are co-localized in a distinct, but adjacent, subset of ARC neurons [91, 92]. Both types of neurons express leptin receptors [93] and insulin receptors [94]. This also explains the critical role of ARC in leptin signaling [95] and insulin signaling [96]. NPY is an orexigenic peptide. Loss of NPY can attenuate the obesity of ob/ob mice [97]. NPY deficiency does not affect feeding behavior[98]. This compensation may be attributed to the activation of other orexic neuropeptides in the absence of NPY. Melanocortin peptides including α-melanocyte stimulating hormone (α-MSH) are cleaved from POMC and functions by binding to a family of melanocortin receptors including MC3 and MC4 receptors [99]. Upon activation, α-MSH and CART act cooperatively to inhibit food intake, whereas NPY and AgRP inhibit this process and increase food intake. However, brief sugar intake did not change these neuropeptides in the ARC [63]. By contrast, different sugars have different effects on the activities of the hypothalamus, where appetite, motivation and reward processing functions are regulated [26, 100]. It has been known for quite some time that different hypothalamic structures and regions influence hunger and satiety. For example, Stellar proposed that the VMH and LH acted together to

control food intake [101]. The “Dual Center Hypothesis” was one of the most studied theses in 20th century neurophysiology. The PVN was added to this mix later, noting that there were differences in metabolic and behavioral controls of hunger [102]. PVN, VMH and LH have their own complicated neuro-signals for further feeding behaviors. For example, PVN and VMH control for satiety whereas LH controls for hunger [Figure 6]. To achieve this, the hypothalamus has complicated neuronal circuits with many redundant neuropeptides [Table 3]. Switching on and off of these neuronal signals is linked with specific feeding behaviors. Through this mechanism, the hypothalamus coordinates energy balance. Once the balance is biased, obesity and/or other energy disorders may occur. The hypothalamus can also sense energy status by communicating with the nucleus of the solitary tract (NTS). The NTS integrates peripheral information via the vagus nerve [85]. The hypothalamus interprets the collection of information and then signals NTS or other brain areas to activate specific feeding behaviors.

Table 3 Neuropeptides in the control of energy homeostasis

Orexigenic	Anorexic
NPY	α-MSH
AGRP	CRH
MCH	TRH
Hypocretin 1 and 2/orexin A and B	GLP-1
Galanin	CART
Galanin like peptide	Urocortin 1,2 and 3
Noradrenaline	IL-1β
Endogenous opioids (β-endorphin, encephalin and dynorphin)	Oxytocin
	Neurotensin
	Serotonin
	CCK
	TNF-α

Although there is ample evidence detailing how sugars metabolize peripherally, the effects of sugars in the CNS are not well understood. Glucose increases the neuronal activities in the brain particularly the hypothalamus where appetite, motivation, and reward processing are regulated. Fructose has the opposing effect [100, 103]. Fructose intake altered hypothalamic liver X receptor (LXR)-α and LXR-β expressions, which play a role in regulation of carbohydrate and lipid metabolism[104]. Fructose can induce hypothalamic AMPK activation by disrupting the glucagon-like peptide-1 (GLP-1) pathway [105]. This induces hepatic gluconeogenesis due to increased corticosterone release [60]. Fructose intake affects the enzymes involved in the synthesis and degradation of hypothalamic endocannabinoids [27]. Fructose can also increase the expression of the endocannabinoid receptor 1 (CB1) [106]. The increased endocannabinoid pathway signaling can stimulate satiety.

In summary, all of the above makes it clear that fructose can affect the CNS, particularly the hypothalamus in a way that facilitates excess energy intake. Under *ab libitum* conditions, the effects of HFCS or sucrose on the CNS remain unknown. Peripheral fructose can quickly reach the hypothalamus. The fructose transporter GLUT5 is found throughout the hypothalamus [26]. The initial ATP-consuming step of fructose metabolism in the hypothalamus is faster than the ATP-consuming

step of glucose metabolism. The rapid depletion of ATP causes a rise of AMP which further leads to ACC inactivation and downregulated malonyl-CoA [107]. Reduced malonyl-CoA causes increased expression of the orexigenic hypothalamic neuropeptides, and in that way stimulate food intake [108]. Surprisingly, the accumulated lactate converted from fructose decreases food intake [109]. However, only a small portion from dietary sugar intake can reach the brain, the effect of dietary sugar intake should not as effective as this kind intracereventricular administration.

Recent advances in the sugars and CNS

To better understand the effect of sugar intakes on the CNS under *ad libitum* conditions, we recently fed Sprague Dawley rats with different sugar solutions. Glucose, fructose, sucrose and high fructose corn syrup (HFCS) solutions were made available for 24 h. We then evaluated the expression of 84 genes in the hypothalamus of these animals using PCR array (see Figure 7). We found several hypothalamic neuropeptides were regulated differently by different sugars [63]. For example, hypothalamic CCK was downregulated in 24 h when rats had access to the fructose solution, whereas CCK was unchanged in 24 h when rats had access to the same concentration of glucose solution. Several other peptides were found to

respond differently to different sugars. These include RAMP3, TNF-α, TRH and CRH [63]. RAMP3 plays an important role in the transport and modulation of G protein-coupled receptors (GPCRs), but its involvement in energy control is not quite clear. Hypothalamic RAMP3 was found to be upregulated by glucose and downregulated by both HFCS and fructose. TRH, regulated by energy homeostasis [110], promotes catabolic metabolism by decreasing food intake and increasing locomotor activity, body temperature and oxygen consumption [111]. TRH expression is upregulated in rats given access to fructose. TNF-α is an important inflammatory chemokine that was also found to regulate food intake. Consumption of glucose, sucrose or fructose all resulted in an upregulation of TNF-α expression in the hypothalamus. CRH is an important hormone involved in the hypothalamic-pituitary-adrenal axis and is also a well-known anorexigenic neuropeptide. We recently found only HFCS can downregulated hypothalamic CRH compared with other sugars. Since the hypothalamus is composed of different functional regions, we set out to determine which region(s) of the hypothalamus is/are affected by different sugars.

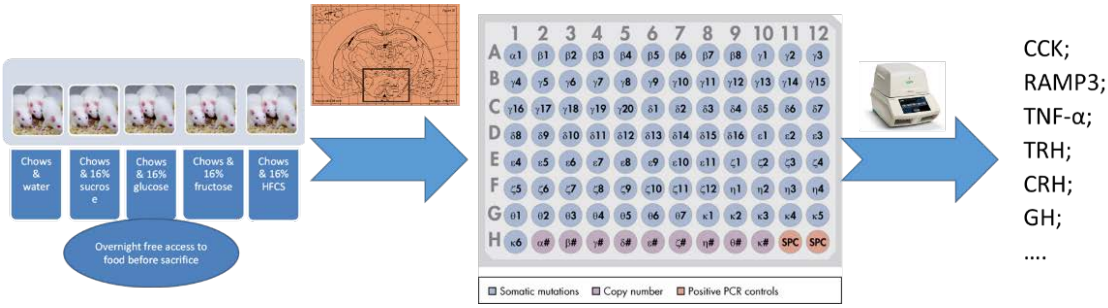


Figure 7 PCR array assay of rat hypothalamus.

We recently fed Sprague Dawley rats with different sugar solutions including glucose, sucrose, HFCS and fructose. The hypothalamus samples from different groups of rats were subjected to PCR array with primed 84 genes. Several hypothalamic neuropeptides were found to be regulated differently by different sugars including CCK, RAMP3, TNF-α. TRH, CRH, GH etc.

Hypothesis

In the current research, we hypothesize that:

Sugar intake can affect these neuropeptides in a way that facilitates energy imbalance. These effects are restricted to specific hypothalamic regions depending on the specific sugar tested.

Objectives

Objective 1: To evaluate a microscope assisted method to accurately sample the specific regions of hypothalamus;

Objective 2: To determine the mRNA distribution of several hypothalamic neuropeptides that are associated with energy control;

Objective 3: To evaluate the varying effects of different sugars on the expression of several neuropeptides in regulation of energy balance;

Objective 4: To understand the relationship between the sugar intake and adiposity hormones- insulin and leptin.

CHAPTER 2. REGIONAL MRNA EXPRESSION OF SEVERAL HYPOTHALAMIC NEUROPEPTIDES USING A FAST AND EASY DISSECTION METHOD [84]

Abstract

The PVN plays an essential role in neuroendocrine regulation. Accurate sampling of the PVN is critical for any successful measurement of many of its unique characteristics. Here we report a microscope-assisted fast and easy method to dissect hypothalamic regions. We applied this method to sample PVN that was validated by measuring several hormones mainly synthesized in the PVN. These include CRH, OXT, AVP and TRH. We measured the mRNA expression of each of these hormones using quantitative PCR in samples dissected from the PVN. We then compared their abundance to that found in samples taken from nearby structures (the VMH and the LH). The expression of CRH, OXT, AVP and TRH in the PVN samples was significantly higher than those in the VMH or the LH. The rat to rat variation found in our PVN samples is a reflection of individual differences as well as sampling error. We believe that this method could dramatically reduce the sampling time needed to characterize small neural targets like the PVN.

We have previously shown that several hypothalamic neuropeptides can be affected after 24 hour sugar exposure. Since the hypothalamus is composed of

different functional regions, how and where these neuropeptides were affected by different sugars is unknown. The distributions of several of these neuropeptides were reported previously whereas most of them were not. The reported distributions of some neuropeptides were mainly based on northern blotting or histochemistry. Neither method is sensitive enough to detect low amounts of gene expression compared with quantitative real time PCR. In this chapter, we analyzed the regional mRNA expression of several neuropeptides in the microdissected hypothalamic samples from Sprague Dawley rats. This step is critical for us to continue measuring these neuropeptides in specific hypothalamic regions of rats fed with different sugars. The neuropeptides analyzed include NPY, AgRP, CCK, RAMP3, TNF-α, Drd1a, Adra2b, GH and 11β-HSD1.

Consistent with previous reports, CCK was mostly abundant in the PVN of the hypothalamus. Interestingly, RAMP3 expression was also much higher compared with that in the other two regions tested. NPY and TNF-α were uniformly expressed in the hypothalamus. AgRP was highly expressed in the VMH. Drd1a and Adra2b had the similar distribution patterns, i.e. both of them were mostly expressed in the PVN followed by VMH and LH. GH was only detected in the VMH and LH. 11β-HSD1 was widely detected in the hypothalamus with its expression in the LH

at the highest level. Understanding the distribution of these neuropeptides is important in determining the hypothalamic targets of dietary sugars.

Introduction

The PVN is an integral part of hypothalamus, located laterally adjacent to the upper part of the third ventricle. The PVN contains several regions, including the magnocellular and parvocellular regions, as well as different types of peptide-containing cells that project to many extra-hypothalamic sites. The PVN plays a critical role in the neuroendocrine system by synthesizing a variety of hormones including CRH [112], OXT [113, 114], AVP [114] and TRH [115, 116]. All of these peptides were previously found to be mainly expressed in the PVN. Specifically, CRH and TRH are mainly concentrated in the parvocellular region of the PVN [116, 117], whereas most of OXT and AVP are expressed in the magnocellular region of the PVN [117].

CRH is an important regulator of the hypothalamus-pituitary-adrenal (HPA) axis. CRH stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH) into circulation. Among other things increased ACTH activates the adrenal gland to release glucocorticoids. The glucocorticoids then suppress further CRH release so as to form a feedback loop often referred to as “the HPA axis”. The operation of the HPA axis is important in adaptive responses to stress [118], food intake control [119] and immunity system [120-122]. OXT and AVP are two other important hormones synthesized and secreted from the PVN. OXT is an important

vasoconstrictor and plays a role in birth delivery and lactation [123, 124]. AVP is critical in body fluid maintenance by promoting water reabsorption, but also increases vasoconstriction [124]. Another PVN secreted hormone TRH is regulated by energy homeostasis [110]. TRH promotes the catabolic metabolism by decreasing food intake and increasing locomotor activity, body temperature and oxygen consumption [111]. Interestingly, all these peptides participate in food intake control [111, 125-127], which support the view that the PVN is a "satiety center" [51, 128].

Because of the physiological importance of the PVN in the hypothalamus, it is often sampled for molecular studies. Several investigators have used micro-puncturing or dissection techniques to sample the PVN or other specific hypothalamic regions [129-134]. However, the pretreatment of the samples including staining or immuno-labeling often takes a long time. That delay may compromise the RNA or protein quality for downstream applications. Here we employed CRH, OXT, AVP and TRH as markers to evaluate a fast and easy method to sample the PVN as well as VMH and LH of Sprague Dawley rats.

After validation of this method, we further used it to measure several hypothalamic neuropeptides that had been reported to be affected by 24 hour sugar exposure based on the PCR array data [63]. These neuropeptides include CCK,

RAMP3, TNF-α, Adr2b Drd1a, GH and 11β-HSD1. We attempted to measure these neuropeptides in specific hypothalamic regions including PVN, VMH and LH. The well-studied ARC was not included in the current study. Because two important orexigenic neuropeptides NPY and AgRP which are mainly synthesized in ARC were not affected by 24 h exposure to sugar solutions [63]. We hypothesize that the neuropeptides will probably be affected where it was originally biosynthesized. In addition to the reported neuropeptides we also include the neuropeptides OXT [125, 126], AVP [135, 136] and 11β-HSD1 [137], which also participate in the energy control based on previous research.

Different hypothalamic regions have differing effects on the feeding behaviors [51]. Specifically PVN and VMH control satiety whereas LH controls hunger [101, 102]. Recent research has identified many neuropeptides in these hypothalamic regions that play important roles in energy control. The current study was performed to determine the mRNA expression pattern of these neuropeptides in the hypothalamus of Sprague Dawley rats.

Materials and Methods

Animal treatment

Eight experimental adult male Sprague-Dawley (CD strain) rats (Charles River Laboratories, Wilmington, MA) weighing approximately 300g were used. All animals were individually housed under a 12h light/dark cycle in a temperature controlled room (22 ±1°C). After 1 week acclimation, the rats were killed by slow replacement of air in a specialized chamber with pure CO_2 followed by rapid decapitation and exsanguination. This method has been approved for use by the Panel on Euthanasia of the American Veterinary Medical Association as well as the UM IACUC. All procedures described herein are in compliance with the University of Maryland's ACUC guidelines. At the time of sacrifice, the rat brain tissues were collected, snap frozen in isopentane/dry ice and stored at -80°C.

Brain slicing

The frozen brains were embedded using M1 embedding matrix (Lipshaw, Pittsburgh, PA). An IEC Minot Custom Microtome (Damon/IEC Division) was used for cryosectioning. The blade and antiroll plate were pretreated with RNaseZap® to remove any possibility of RNase contamination and cleaned with paper towel presoaked in DEPC-treated water. The brain was sliced according to the rat brain atlas in stereotaxic coordinates at -10℃ (see below). The thickness of

each slice was 110 µM. The slices were carefully transferred to pre-cleaned slides (Fisher Scientific, Pittsburgh, PA) and then stored at -80 °C until sampled.

Hypothalamic Dissection

Hypothalamic dissection was carried out within one month after brain slicing. Because brain slice samples after standard staining can lead to RNA loss by 10% in as little as 30 minutes [138], ethanol dehydration was used in our experiment. Preliminary test showed that cresyl violet staining did not help much in distinguishing hypothalamic structures due to the thickness of our samples. As a result we employed three steps of ethanol dehydration without staining, i.e. starting with 95% ethanol for 30 sec, followed by 100% ethanol for 1min and then finally 100% ethanol for at least an additional 1min.

Before the slices were completely dried out, the PVN, the VMH and the LH were sampled with a sterile 23 G X 1" hypodermic needle (B-D PrecisionGlide, Franklin Lakes. NJ) under a light microscope (Figure 1). Six to seven brain slices were used for the PVN dissection. PVN sampling was initiated approximately at -1.30 mm behind Bregma (Paxinos & Watson, The Rat Brain in Stereotaxic Coordinates, Second Edition). VMH and LH samples were dissected starting at around -2.12 mm behind Bregma. VMH samples were approximately 1.5 mm thick while LH samples were approximately 3 mm thick. Captured tissues were carefully

transferred into cold 1.5 ml eppendorf tubes. RLT lysis buffer (350 µl) that contained 10 µl β-ME per 1 ml Buffer RLT was added into each tube followed by 30 sec vortexing to facilitate cell breakage and RNA release. RLT lysis buffer is strong enough to inactivate RNase according to the manufacturer's protocol. The tubes were stored in the -80 °C freezer until RNA extraction.

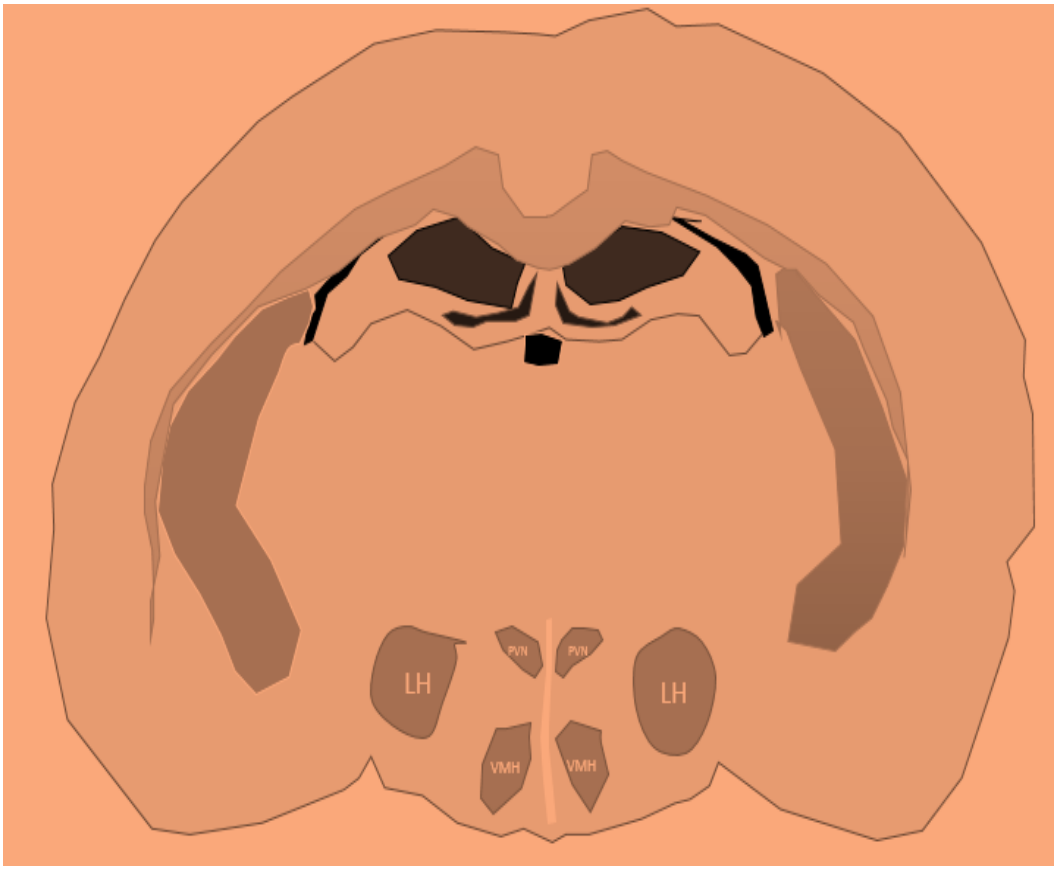


Figure 8 Scheme of locations of PVN, VMH and LH.

The scheme gives the general location and the relative size of the areas of interest in the current study including PVN, VMH and LH.

RNA extraction and cDNA synthesis

The tissue lysates were thawed in a 37℃ water bath until all the salts were dissolved. The lysates of VMH and LH were centrifuged (Eppendorf centrifuge model 5424) at full speed for 3 min to remove the cell debris, as per the manufacturer's instructions (Qiagen RNeasy micro kit). The PVN lysates were not centrifuged. DNase I was used to remove any DNA which may affect the downstream applications. RNA quality was examined using a NanoDrop 2000 spectrophotometer based on A260/A280 values. cDNA synthesis was carried out using iScript™ cDNA Synthesis Kit (Bio-Rad) following the manufacturer's protocol. The cDNA products were stored at -20°C until used.

Quantitative Real Time PCR

The PCR reaction with iQ SYBR Green Supermix was carried out in two replicates using a CFX96 Bio-Rad system. RPLP1 or β-actin were used as reference gene. The program used for all PCR reactions was 95 °C for 3 min and 40 cycles of 95 °C for 15 sec, annealing temperature (Ta) for 30 sec (

Table 4) and 68 °C for 30 sec.

Table 4 Primer sets for Chapter 2

Primer Name	**sequences (5' to 3')**	**Ta (℃)**
RPLP1 sense	GAAGAATCCGAGGATGACA	51
RPLP1 antisense	CAGGTTCAGCTCTTTATTGG	
β actin sense	TGTCACCAACTGGGACGATA	60
β actin antisense	GGGGTGTTGAAGGTCTCAAA	
11β-HSD1 sense	GTGTCTCGCTGCCTTGAAC	55
11β-HSD1 antisense	AGTGGTCTGTGTGATGTGATTG	
OXY sense	ACCCTGAGTCTGCCTTCT	54
OXY antisense	ATGGGGAATGAAGGAAGCG	
AVP sense	ACCTCTGCCTGCTACTTC	53
AVP antisense	ACACTGTCTCAGCTCCAT	
NPY sense	AATGAGAGAAAGCACAGAAA	46
NPY antisense	AAGTCAGGAGAGCAAGTT	
AgRP sense	GAGTTCTCAGGTCTAAGTCT	49
AgRP antisense	GTGGATCTAGCACCTCTG	

Data Analysis

The Ct mean value from ribosomal protein large P1 (RPLP1) [139] or β actin [62] was used as the reference gene as before. All values were expressed as means ± SEM. Outlier data was removed using Dixon's Q test. Student's t tests were applied to $2^{-\Delta Ct}$ values to determine significance between groups using JMP Pro 10.0.2. P values less than 0.05 were considered statistically significant.

Results

Quality of samples

Twenty four RNA samples extracted from the rat brains had good qualities evidenced by 260/A280 >1.8 (see Table 5). The melting curve confirmed the eligibility of the test primers which were designed from Beacon Designer 7 in our lab.

Table 5 260/A280 values of the twenty samples from of PVN, VMH and LH

Labels	PVN	VMH	LH
A1	1.94	2.10	2.06
A2	2.13	2.14	2.07
A3	2.13	2.09	2.08
A4	2.12	2.11	2.06
B1	2.19	2.09	2.04
B2	1.97	2.10	1.81
B3	2.09	2.08	2.06
B4	1.92	2.13	2.08

Expression of PVN markers

The expression of the PVN markers - CRH, OXT, AVP and TRH were significantly higher in the PVN than those in the VMH or the LH ($p<0.0001$) (refer to Figure 9A-C).

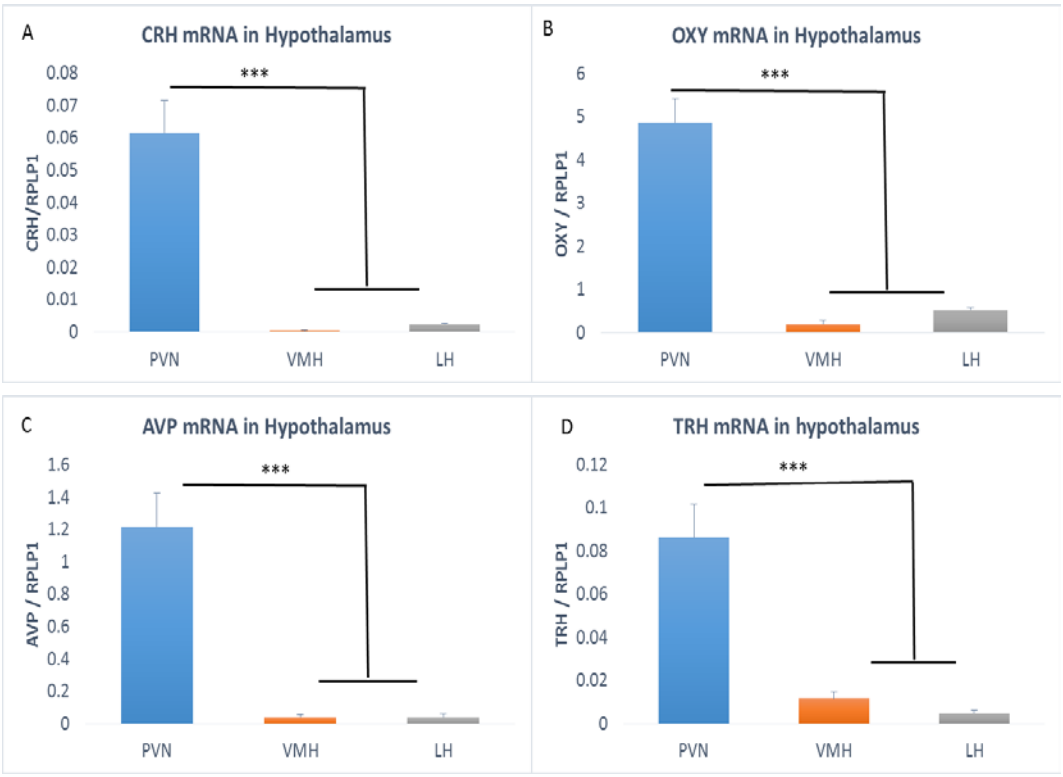


Figure 9 Expression of PVN markers.

(A) Expression of CRH mRNA in the PVN, VMH and LH. Expression of CRH mRNA in the PVN is significantly higher than that in VMH and LH. (B) Expression of OXT mRNA in the PVN, VMH and LH. Expression of OXT mRNA in the PVN is significantly higher than that in VMH and LH. (C) Expression of AVP mRNA in the PVN, VMH and LH. Expression of AVP mRNA in the PVN is significantly higher than that in VMH and LH. (D) Expression of TRH mRNA in the PVN, VMH and LH. Expression of TRH mRNA in the PVN is significantly higher than that in VMH and LH.

***: $P<0.0001$

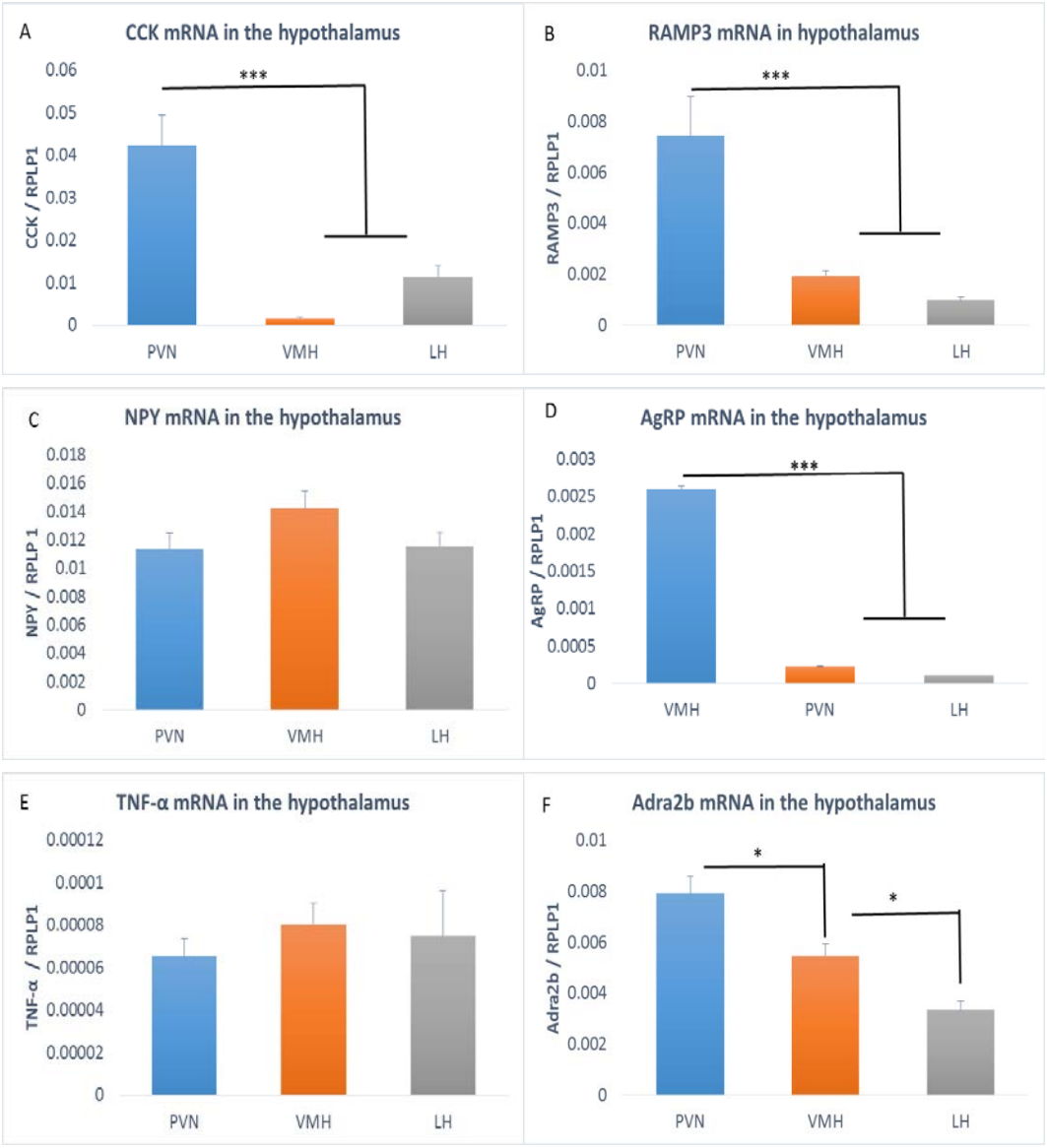
Distribution of other neuropeptides in the hypothalamus

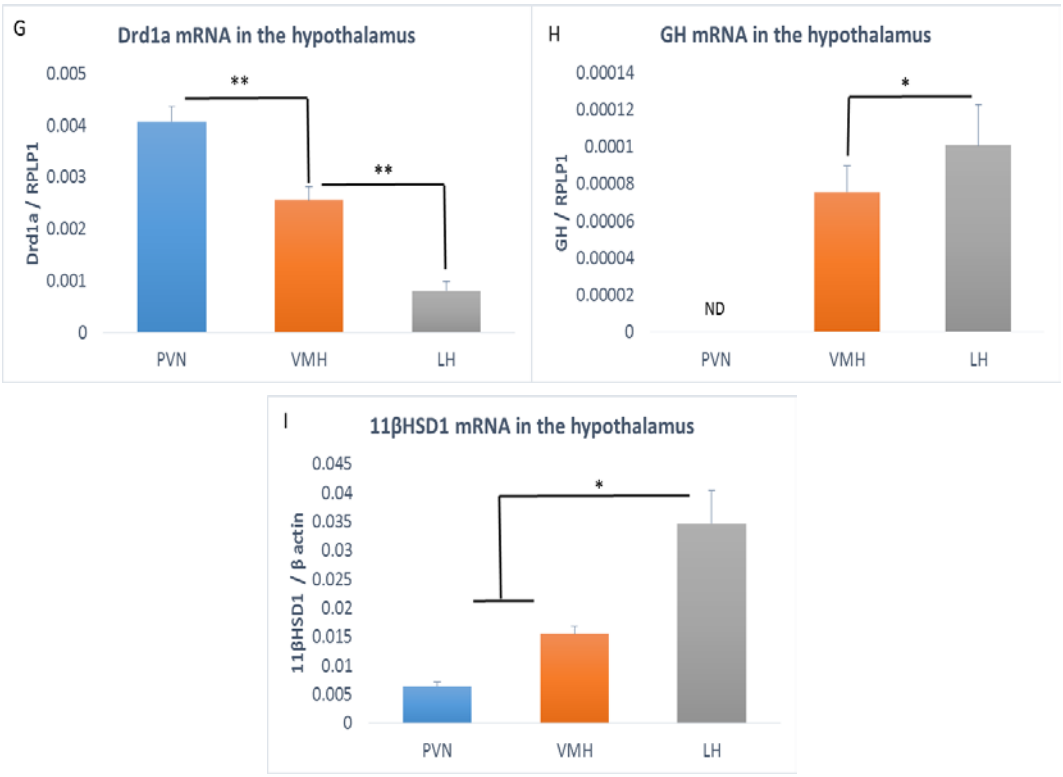
CCK was expressed in the PVN at a higher level than that in the VMH or LH. RAMP3 expression was also much higher compared with that in other two regions tested. NPY and TNF-α were uniformly expressed in the hypothalamus. AgRP was highly expressed in the VMH. Drd1a and Adra2b had the similar distribution pattern, i.e. both of them were mostly expressed in the PVN, and following is VMH and LH. GH was only expressed in the VMH and LH. 11β-HSD1 was abundantly expressed in the hypothalamus. Specially, 11β-HSD1 is mostly expressed in the LH (refer to Figure 10A-I).

Figure 10 Expression of several hypothalamic neuropeptide mRNA in the hypothalamus.

(A) CCK was mainly expressed in the PVN of the hypothalamus. (B) RAMP3 expression was also much higher compared with that in other two regions tested. (C) NPY was uniformly expressed in the hypothalamus. (D) AgRP was highly expressed in the VMH. (E) TNF-α were uniformly expressed in the hypothalamus (F-G) Drd1a and Adra2b had the similar distribution patterns, i.e. both of them were mostly expressed in the PVN followed by VMH and LH. (H) GH was only detected in the VMH and LH. (I) 11β-HSD1 was widely detected in the hypothalamus with its expression in the LH at the highest level.

*: $P<0.01$, **: $P<0.001$, ***: $P<0.0001$


A
CCK mRNA in the hypothalamus
CCK / RPLP1
0.06
0.05
0.04
0.03
0.02
0.01
0
***
PVN
VMH
LH
B
RAMP3 mRNA in hypothalamus
RAMP3 / RPLP1
0.01
0.008
0.006
0.004
0.002
0
***
PVN
VMH
LH
C
NPY mRNA in the hypothalamus
NPY / RPLP 1
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
PVN
VMH
LH
D
AgRP mRNA in the hypothalamus
AgRP / RPLP1
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
***
VMH
PVN
LH
E
TNF-α mRNA in the hypothalamus
TNF-α / RPLP1
0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
PVN
VMH
LH
F
Adra2b mRNA in the hypothalamus
Adra2b / RPLP1
0.01
0.008
0.006
0.004
0.002
0
*
*
PVN
VMH
LH


G
Drd1a mRNA in the hypothalamus
Drd1a / RPLP1
0.005
0.004
0.003
0.002
0.001
0
**
**
PVN
VMH
LH
H
GH mRNA in the hypothalamus
GH / RPLP1
0.00014
0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
*
ND
PVN
VMH
LH
I
11βHSD1 mRNA in the hypothalamus
11βHSD1 / β actin
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
*
PVN
VMH
LH

Discussions

Two methodological issues encouraged us to develop the alternative method described above. First, many labs have applied staining (typically cresyl violet) to identify the PVN [140-142]. However, brain slices after standard staining can lose as much as 10% of its RNA within 30 minutes [138]. Here we have demonstrated that the micro-dissection of brain slices can be performed under a microscope without staining. Additionally, this method allowed us the use of thick slices for dissection/sampling and that way minimized steps that could have affected the structure or intracellular molecules for downstream analysis. Secondly, immunohistochemical methods have been used to identify peptides within a known anatomical region such as the PVN [141, 143]. However, this technique is both costly and labor-intensive. As an alternative, we have sampled regions of the PVN and verified the accuracy by amplifying mRNA that is known to be localized only in the PVN. Our findings confirmed that we have been able to sample the PVN, as CRH, OXT, AVP and TRH messages were not measured to any extent in samples of the VMH or LH.

Another way to further validate the PVN samples is to examine mRNAs that wouldn't be in PVN but would be in surrounding areas. We examined GH that was only detected in the VMH and LH. The data were shown in the next chapter. It

should be noted that a similar validation was not performed on VMH and LH samples. When compared with the PVN, these two regions are much larger and anatomically distinct, making it unnecessary to further examine if they were accurately dissected.

Consistent with previous report, CCK was mainly expressed in the PVN of the hypothalamus. CCK is a hormone peptide both synthesized in the intestine and brain. Our result is consistent with a previous report that hypothalamic CCK is primarily expressed in PVN, typically in the parvocellular sub-nuclei [144].

We found that RAMP3 is also mainly expressed in the PVN. RAMP3 is a member of RAMP family of single-transmembrane-domain proteins. Adrenomedullin receptors and amylin receptors are two well-studied complex forms that require RAMP3. Adrenomedullin is a potent endogenous vasodilatory peptide [145]. Amylin is a hormone that is co-secreted with insulin from pancreatic β cells.

We further showed that NPY and TNF-α were uniformed expressed in the tested regions. AgRP was highly expressed in the VMH. TNF-α is an important chemokine involved in systemic inflammation. NPY and ArRP are two important player in ARC as orexigenic neurohormones. Our result also detected these two hormones in the hypothalamic regions other than ARC. NPY mRNA was hardly

detected in other hypothalamic areas using hybridization, but we found that NPY mRNA was also expressed in the PVN, VMH and LH, in ascending order of expression. AgRP mRNA was also highly expressed in the VMH, consistent with the previously reported data [91].

The distributions of Drd1a and Adra2b showed the similar distribution pattern, i.e. mostly expressed in the PVN followed by the VMH and LH. Drd1a plays an important role in dopamine pathway. Adra2b has a critical role in regulating neurotransmitter release from sympathetic nerves and from adrenergic neurons in the CNS. Our result found that both Drd1a and Adra2b were widely expressed in the hypothalamus especially in the PVN.

GH was only expressed in the VMH and LH. GH is synthesized and secreted from the pituitary gland. A report showed that GH is also biosynthesized in the LH [146]. Our result showed that except PVN, GH can also be synthesized in the VMH region.

Glucocorticoid plays an essential role in the normal physiology, typically through hypothalamic-pituitary-adrenal axis. Adipose and hepatic 11β-HSD1 has been hotly studied for its possible roles in the obesity development, whereas the cerebral 11β-HSD1 is rarely reported. Early report showed that 11β-HSD1 was expressed in the forebrain -hypothalamus, hippocampus, and cortex of Wister rats

[147]. One group recently investigated the distribution of 11β-HSD1 in the human brain, which showed that 11β-HSD1 was also abundantly detected in the human hypothalamus [148]. However, the expression pattern of specific neuropeptide can vary based on different species [149]. Hypothalamus is the food intake control center playing a pivotal role in energy balance. Here we determined where the 11β-HSD1 mRNA was distributed in the hypothalamic regions using quantitatively PCR in the Sprague Dawley rats. Sprague Dawley rats are widely used as a study model like Wistar rats. We found that 11β-HSD1 was most expressed in the LH, but also abundantly expressed in the other two hypothalamic regions – PVN and VMH. This result is consistent with the previous report on Wister rats that 11β-HSD1 was highly expressed in the preoptic area and also posterior arcuate nucleus (ARC) (not included in the current study) [147]. The previous report failed to clearly detect 11β-HSD1 expression in other hypothalamic areas. This is probably because the in situ hybridization is less sensitive than the quantitative PCR technique. In the human study, the 11β-HSD1 was found to be coexpressed with CRH, OXT and AVP in the PVN [148]. Similarly, we found 11β-HSD1 was detected in the PVN, where CRH, OXT and AVP were highly expressed. This indicates hypothalamic 11β-HSD1 in the PVN possibly participates in the hypothalamic-pituitary-adrenal axis and/or other biological metabolism, e.g. food intake control. Our result clearly showed that 11β-HSD1 mRNA was not expressed in different hypothalamic

regions to the same extent in the Sprague Dawley rats. As 11β-HSD1 mRNA is mainly expressed in the LH, the 11β-HSD1 may also play an important role in the ultra-hypothalamic regions. Further research is needed to validate the physiological function of hypothalamic 11β-HSD1 or glucocorticoids.

CHAPTER 3. EFFECTS OF SUGARS ON NEUROPEPTIDES IN DIFFERENT HYPOTHALAMIC REGIONS [150]

Abstract

It's been reported that rats with long term additional sugar solutions access gained weight. Ample evidence has shown that hypothalamic neuropeptides are pivotal in energy homeostasis. In this chapter, we present the results of our studies of the effects of various sugars on the expression of hypothalamic neuropeptides. We provided Sprague Dawley rats 24 h access to 15% solutions of glucose, fructose, sucrose or HFCS and then dissected portions of the paraventricular hypothalamic nuclei (PVN), the ventromedial hypothalamus (VMH) and the lateral hypothalamus (LH). We then evaluated the expression of several neuropeptides in these tissues, all of which were previously shown to be influenced by sugar consumption.

Rats that had access to sugars consumed less chow. However, although rats fed with additional sucrose and HFCS solutions consumed around 10% higher calories compared with other groups on average, this differences failed to reach statistically significance. In other words, all groups maintained a similar amount of energy intake compared with control.

Of the four sugar solutions tested, only fructose decreased expression of CCK significantly, whereas glucose and sucrose significantly increased the expression of

TNF-α only in the PVN, not in the VMH or the LH. Fructose and sucrose decreased GH in the VMH. Glucose increased Drd1a expression in the PVN only.

We also found differences of several neuropeptides between different sugar groups. Fructose decreased CCK expression compared with glucose. Fructose and sucrose decreased GH in both VMH and LH compared with glucose. HFCS decreased Drd1a compared with glucose in both PVN and VMH. Both HFCS and fructose significantly decreased RAMP3 expression compared with glucose in the VMH. By contrast, RAMP3 expression in the LH was significantly reduced in the HFCS group when compared to the glucose group. Both fructose and HFCS reduced TRH expression when compared with sucrose in the PVN. No differences were found for NPY, AgRP, OXT, AVP and Adra2b.

We conclude that 24 h free access to different sugars can influence the expression of several hypothalamic neuropeptides in different ways. Changes in the expression of these neuropeptides do not disrupt energy intake immediately but may contribute to the obesity caused by long term intake of different sugars.

Introduction

It has been known for more than 40 years that long term *ad libitum* access to sugar solutions (glucose, sucrose or fructose) can induce excessive weight gain and obesity [45, 151]. Similarly, long term free access to HFCS -55 solutions can also induce overweight and obesity [48]. Interestingly, rats adjust their intake of sugar solutes from different concentrations [45]. Under normal conditions, energy balance is maintained: the body matches energy expenditure and energy intake [51]. Long term access to sugar solutions disrupts energy balance so as to induce obesity when sugar is freely accessible. The question is: "Which mechanism(s) is(are) involved in promoting sugar induced obesity?"

The system of energy control is composed of both peripheral and central regulators. Not all sugars are equally effective in promoting changes in peripheral metabolic controls. For example, unlike glucose intake, fructose intake can quickly induce hypertriglyceridemia [152], a condition that frequently coexists with obesity, type 2 diabetes and metabolic syndrome [153]. Our lab previously reported that fructose consumption can quickly suppress the expression of 11β -HSD1 in liver and visceral adipose tissues [67] leading to an increase in intracellular levels of glucocorticoids [154]. Increased intracellular glucocorticoids are commonly observed in obesity in human and animal models [155, 156]. Both of these effects

are examples of how a nutrient (in this case fructose) can have a dramatic effect on gene expression and in that way change short term regulation.

Sugars can have differing effects on the brain's food intake control center- the hypothalamus, where appetite, motivation and reward processing functions are regulated [26]. Different hypothalamic structures and regions influence hunger and satiety. For example, Stellar proposed that the VMH and LH acted together to control food intake [101]. The "Dual Center Hypothesis" was one of the most studied theses in 20th century neurophysiology. The PVN was added to this mix later, noting that there were differences in metabolic and behavioral controls of hunger [102]. Many neuropeptides synthesized in these hypothalamic regions play critical roles in energy maintenance [51].

We hypothesize that brief access to sugar solutions can change the expression pattern of specific hypothalamic neuropeptides that control energy balance. Testing this hypothesis is the first step so as to understand whether the change of neuropeptides may contribute to the sugar induced obesity. We recently measured 84 obesity-related genes (using PCR arrays) in the hypothalamus of Sprague Dawley rats fed different sugar solutions. We found that several hypothalamic neuropeptides are affected differently by different sugars [139]. These neuropeptides include CCK, TNF-α, RAMP3, CRH, TRH and GH. Interestingly,

two important orexigenic neuropeptides - NPY and AgRP in the ARC were not changed after sugar treatment. It might be that brief sugar intake somehow bypasses ARC and acts on the other hypothalamic regions. As a result, the purpose of the present study was to examine how these neuropeptides were affected by different sugars in the remaining hypothalamic regions - the PVN, the VMH and the LH.

Methods

Animal treatment

Adult male Sprague-Dawley (CD strain) rats (Charles River Laboratories, Wilmington, MA) were used. The rats weighed approximately 300 g upon arrival to our laboratory. All animals were individually housed. They were maintained on a 12 h light/dark cycle in a temperature controlled animal room (22± 1 °C). During a 1 week acclimation period the rats were given free access to water and chow. The chow is a nutritionally complete low fat diet [Rodent diet 7012] prepared by Harlan Teklad (Bethlehem, PA) containing 3.41 kcal/g of diet, of which 2.14 kcals were derived from carbohydrate per gram of chow. All animals were given free access to water throughout the experiment.

Rats were randomly assigned to one of five weight-matched groups (n= 8/group). One group of rats had *ad libitum* chow and water and served as the control group. Rats assigned to the other groups had *ad libitum* access to the chow and water and to one of four solutions: a 15% weight/volume (w/v) fructose (Tate & Lyle, Decatur IL) solution, a 15% (w/v) glucose (Sigma Aldrich, St Louis MO) solution, a 15% (total solute per volume) high fructose corn syrup (HFCS) (IsoSweet® 5500, 55% fructose–41% glucose, 77% solids, Tate & Lyle, Decatur IL) or a 15% (w/v) sucrose (Domino Foods, Baltimore MD) solution. All sugar

solutions were prepared 24 h in advance and stored at 4 °C until use. The rats were maintained with free access to their respective diets for 24 h before sacrifice. This method was chosen so as to minimize the stress associated with administering fixed volumes of solution intragastrically, as weight gain is thought to be facilitated by increased glucocorticoids. All rats were killed by slow replacement of air in a specialized chamber with pure CO_2 followed by rapid decapitation and exsanguination. This method has been approved for use by the Panel on Euthanasia of the American Veterinary Medical Association as well as the UM IACUC. All procedures described herein are in compliance with the University of Maryland's ACUC guidelines. At the time of sacrifice, the brains were dissected, snap frozen in isopentane/dry ice and then stored at -80 °C until use.

Brain sectioning

Frozen brains were embedded using M1 embedding matrix (Lipshaw, Pittsburgh, PA) on dry ice. An IEC Minot Custom Microtome (Damon/IEC Division) was used for cryosectioning. The cryostat's blade and antiroll plate were pretreated with RNaseZap® to remove any possibility of RNase contamination and then cleaned with a paper towel soaked in DEPC-treated water. The brain was sectioned using the Paxinos and Watson rat brain atlas in stereotaxic coordinates

(second edition) at -10°C. Slices were obtained from Interaural 7.70 mm (Bregma -1.30 mm) to Interaural 4.48 mm (Bregma -4.52 mm) at a thickness of 110 µm each and carefully transferred to pre-cleaned slides (Fisher Scientific, Pittsburgh, PA). The slices were then stored at -80 °C until sampled.

Sampling of hypothalamic regions

RNA is unstable and subject to degradation. When following standard staining procedures, brain slices can lose as much as 10% of their initial RNA in as little as 30 minutes [138]. Water is the key factor that facilitates RNA degradation, however RNA integrity can be preserved up to 90 min following ethanol dehydration [138]. Preliminary tests showed that cresyl violet staining does not reveal internal hypothalamic structures quickly. Rather than attempt to stain the sections in order to facilitate accurate dissection we employed a three-step alcohol dehydration procedure (specifically 95% alcohol immersion for 30 seconds, followed by 100 % alcohol immersion for 1min and then another 100 % alcohol immersion for at least an additional minute).

After dehydration, the slices were immediately dissected under a light microscope. Centered on the third ventricle, several 110 µm slices of each region (PVN, VMH and LH) were dissected using sterile 23 G x 1” hypodermic needles

(B-D PrecisionGlide, Franklin Lakes. NJ). Specifically, 6-7 brain slices were used for PVN dissection. PVN sampling was initiated approximately at -1.30 mm behind Bregma. VMH and LH samples were dissected starting at around -2.12 mm behind Bregma. VMH samples were approximately 1.5 mm thick while LH samples were approximately 3 mm thick. Captured tissues were carefully transferred into 1.5 ml polypropylene eppendorf tubes on ice. 350 µl RLT lysis buffer (containing10 µl β-mercaptoethanol per 1 ml Buffer RLT) was added and samples were then subjected to 30 sec vortexing for cell breakage and RNA release. Samples were stored at -80 °C until RNA extraction.

RNA extraction and cDNA synthesis

Tissue lysates were thawed in a 37 ℃ water bath until all the salts were dissolved. VMH and LH lysates were centrifuged using an Eppendorf centrifuge (model 5424) at full speed for 3 min to remove the cell debris. Samples from all three regions were then processed using a Qiagen RNeasy micro kit. This kit uses DNase I to remove DNA that may affect the downstream applications. RNA quality was examined using a NanoDrop 2000 spectrophotometer (A260/A280 >1.8). The cDNA synthesis was completed using iScript™ cDNA Synthesis Kit (Bio-Rad)

following the manufacturer's protocol. The final cDNA products were stored at -20 °C until use.

Quantitative real time PCR

PCR reactions were performed in two replicates using iQ SYBR Green Supermix and a Bio-Rad CFX96 Bio-Rad system. The program used for all PCR reactions was 95°C for 3 min and 40 cycles of 95°C for 15 sec, annealing temperature (Ta) for 30 sec (see Table 1) and 68°C for 30 sec. A melt curve analysis was then appended. All primers that were designed using Beacon Designer 7 software met the ΔΔCt requirement for the product length less than 200 bp.

Data analysis

The formula below was used to plot the final result from qPCR data:

$$\Delta Ct(test) = Ct(target, test) - Ct(ref, test)$$

$$\Delta Ct(calibrator) = Ct(target, calibrator) - Ct(ref, calibrator)$$

$$2^{-\Delta\Delta Ct} = 2^{\Delta Ct(\ calibrator) - \Delta Ct(test)}$$

The Ct mean value from RPLP1 was used as the reference gene as before [139] and the water treated group mean Ct was used as the calibrator. All values were

expressed as means ± SEM. One way ANOVA with Duncan post hoc testing was applied to food intake and energy intake using IBM SPSS Statistics 21. Data were pre-screened using Dixon's Q test. Student's t test option was applied to the $2^{-\Delta Ct}$ values to determine significance between groups using JMP Pro 10.0.2. Pearson's correlation coefficients were calculated to evaluate the relationship between sugar intake and the expression of the neuropeptides. P values less than 0.05 were considered statistically significant.

Table 6 Primer sets for chapter 3

Primer Name	**sequences (5' to 3')**	**Len (bp)**	**Ta (℃)**
RPLP1 sense	GAAGAATCCGAGGATGACA	81	51
RPLP1 antisense	CAGGTTCAGCTCTTTATTGG		
CCK sense	GCGTTTATTTATTAAGTCC	133	43
CCK antisense	ATAGCATAGCAACATTAG		
Tnf-α sense	CCAATCTGTGTCCTTCTAA	85	47
Tnf-α antisense	TTCTGAGCATCGTAGTTG		
RAMP3 sense	CAAGGTCATCTGGAAGGT	109	50
RAMP3 antisense	GACTCCTAACAACTCCATTC		
GH sense	GTCTGTTTGCCAATGCTGTG	152	55
GH antisense	TGGGATGGTCTCTGAGAAGC		
TRH sense	AAAGACATTGAAGCTGAAGAGAGG	75	55
TRH antisense	GGGGTGCTGTCGTTTGTG		
CRH sense	TGGAGATTATCGGGAAAT	158	47
CRH antisense	TACATCTTCTATGCTTCAAG		

Results

Food and energy intake

When sugars were presented, the chow intake in all sugar fed groups decreased significantly compared with the control group. However, the average total caloric intakes of each group over the 24 h experimental period were maintained, and did not differ from one another. The percentage of total calories derived from sugar intake ranged from 44 to 53%. Rats fed fructose consumed significantly less sugar than rats fed sucrose. No other statistically significant differences between treatment groups were found. Refer to Table 6 for details.

Table 7 Calorie intake of rats fed with different sugars

Group	Water	Glucose	Sucrose	HFCS	Fructose
Chow Intake (g)	28.8 ± 3.3^{a}	14.1 ± 1.6^{b}	15.2 ± 2.3^{b}	17.5 ± 2.7^{b}	17.6 ± 3.7^{b}
Sugar Intake (g)	NA	11.5 ± 0.6^{ab}	13.2 ± 1.0^{a}	11.1 ± 0.9^{ab}	9.7 ± 1.4^{b}
Chow Calorie (Kcal)	93.1 ± 10.8^{a}	46.2 ± 5.2^{b}	49.56 ± 7.4^{b}	57.18 ± 8.7^{b}	57.67 ± 12.7^{b}
Sugar Calorie (Kcal)	NA	46.2 ± 2.2^{ab}	52.71 ± 3.9^{a}	44.6 ± 3.4^{ab}	38.8 ± 5.7^{b}
Total Calorie (Kcal)	93.1 ± 10.8^{a}	92.3 ± 4.8^{a}	102.3 ± 7.8^{a}	101.8 ± 7.0^{a}	96.4 ± 15.2^{a}
% calories intake as sugar	NA	51.1±4.3	52.8±4.9	45.9±5.6	44.3±5.2

Note: Values are means ± SEM. Different letter indicated significant difference at $p<0.05$

Neuropeptides regulated by sugars

CCK

Fructose downregulated CCK expression in the PVN ($p<0.05$). (Refer to Figure 11A). CCK expression was not changed in the VMH or in the LH (refer to Figure 11B and C). No other differences were statistically significant.

TNF-α

Glucose and sucrose intake significantly increased the expression of TNF-α in the PVN. HFCS and fructose groups failed to change TNF-α in the PVN (Refer to Figure 11D). No other differences between groups were found in the VMH or LH (Refer to Figure 11E and F).

GH

We failed to detect any GH in the PVN. In the VMH, sucrose and fructose decreased GH expression. Compared with glucose solution, the other three sugars decreased GH expression (Refer to Figure 11G). In the LH, only sucrose and fructose decreased GH expression compared with glucose solution (Refer to Figure 11H). Compared with glucose, both sucrose and fructose decreased its expression in both VMH and LH (Refer to Figure 11G and H).

Drd1a

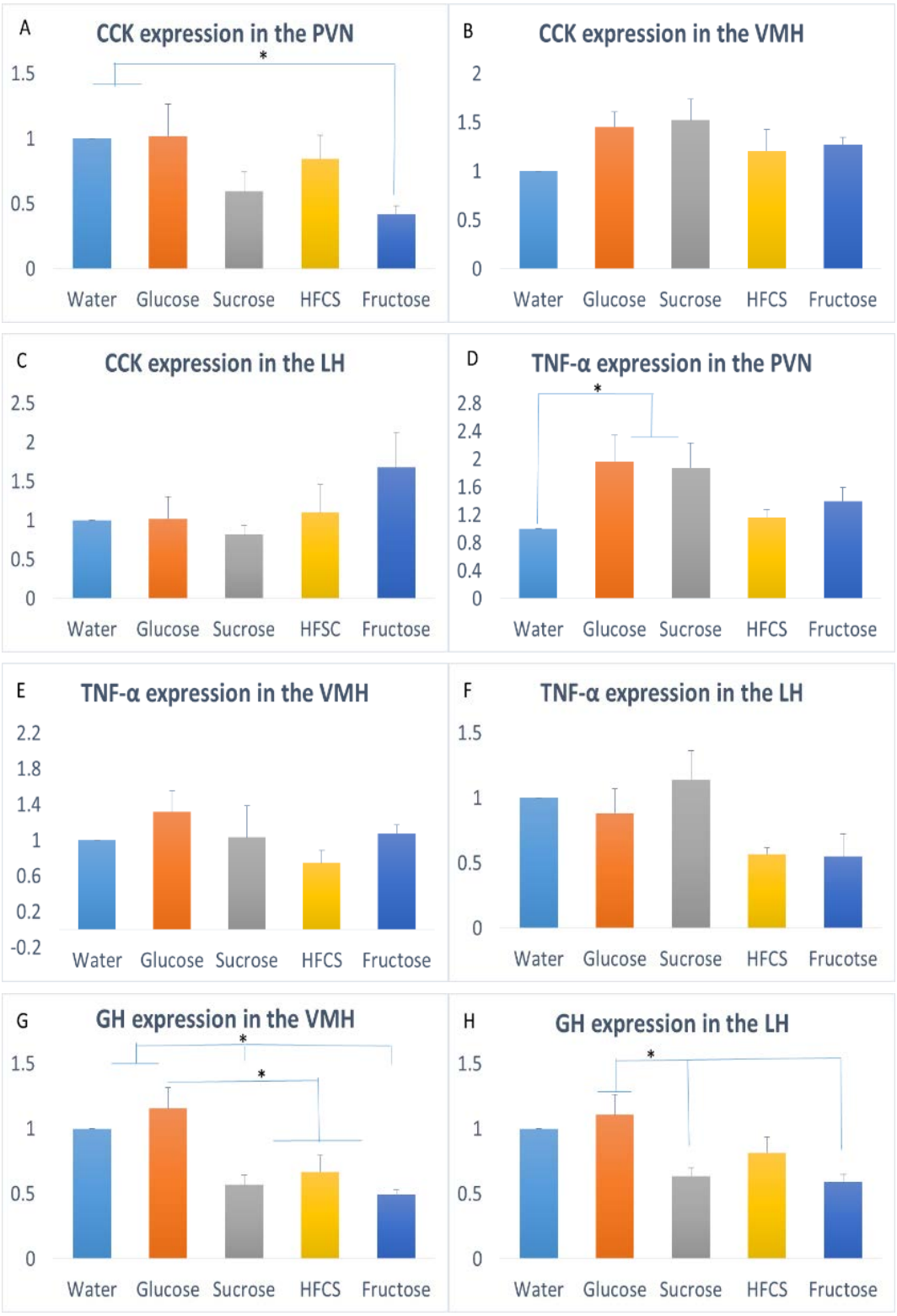
We found that glucose increased expression of hypothalamic Drd1a in the PVN only. Compared with glucose, HFCS decreased its expression in both PVN and VMH.

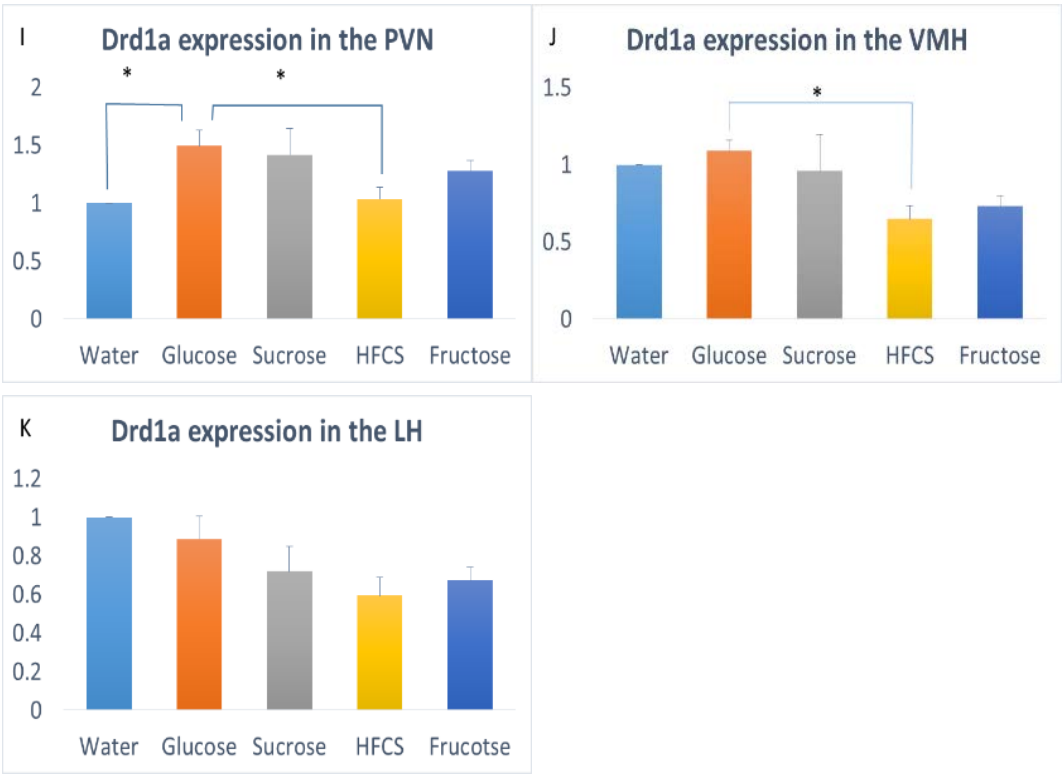
Figure 11 Different sugars affect the expression of CCK, TNF-α, GH and Drd1a in the PVN, VMH and LH.

(A-C) Expression of CCK mRNA in different groups was expressed in the PVN, VMH and LH. Of the four sugar solutions tested, only fructose decreased expression of CCK significantly only in the PVN. (D-F) Expression of TNF-α mRNA in different sugar groups was expressed in the PVN, VMH and LH. Glucose and sucrose significantly increased the expression of TNF-α only in the PVN, not in the VMH or the LH (G-H) Expression of GH mRNA in different sugar groups was observed in the VMH and LH. Fructose and sucrose decreased GH in the VMH. Compared with glucose, sucrose, HFCS and fructose decreased GH significantly in the VMH and sucrose and fructose decreased GH significantly in the LH. (I-K) Expression of Drd1a mRNA in different sugar groups was observed in the PVN, VMH and LH. Glucose increased Drd1a expression in the PVN only. Compared with the glucose, HFCS decreased Drd1a expression in the PVN and VMH.

All values on Y axis were expressed as $2^{-\Delta Ct}$

* $p<0.05$


A
CCK expression in the PVN
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
B
CCK expression in the VMH
2
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
C
CCK expression in the LH
2.5
2
1.5
1
0.5
0
Water
Glucose
Sucrose
HFSC
Fructose
D
TNF-α expression in the PVN
2.8
2.4
2
1.6
1.2
0.8
0.4
0
Water
Glucose
Sucrose
HFCS
Fructose
E
TNF-α expression in the VMH
2.2
1.8
1.4
1
0.6
0.2
-0.2
Water
Glucose
Sucrose
HFCS
Fructose
F
TNF-α expression in the LH
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Frucotse
G
GH expression in the VMH
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
H
GH expression in the LH
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose


I
Drd1a expression in the PVN
2
1.5
1
0.5
0
*
*
Water
Glucose
Sucrose
HFCS
Fructose
J
Drd1a expression in the VMH
1.5
1
0.5
0
*
Water
Glucose
Sucrose
HFCS
Fructose
K
Drd1a expression in the LH
1.2
1
0.8
0.6
0.4
0.2
0
Water
Glucose
Sucrose
HFCS
Frucotse

RAMP3, TRH and 11βHSD1

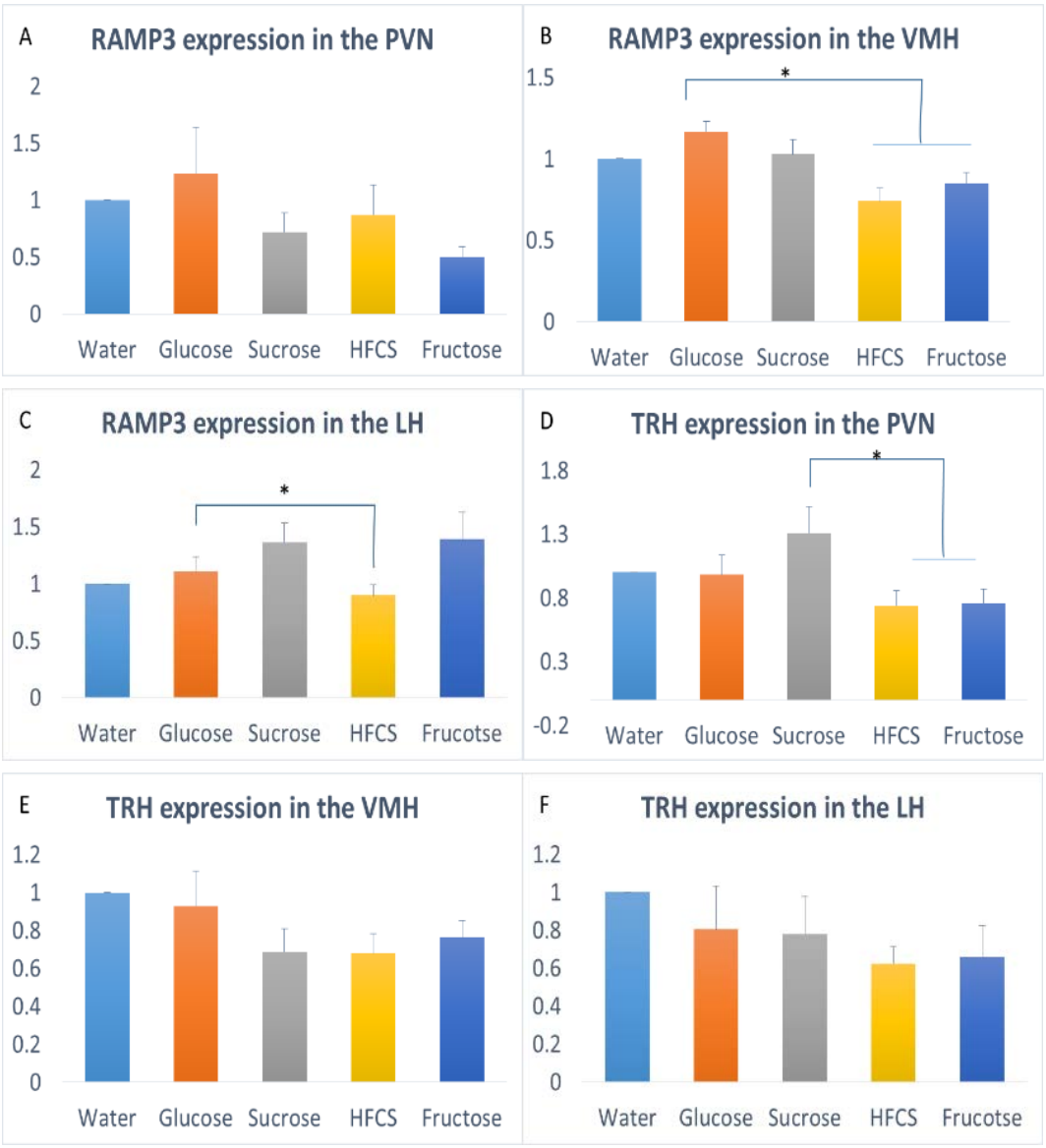
When compared to controls, none of the sugars tested had a significant effect on RAMP3, TRH or CRH messages (Refer to Figure 12). However, some differences in RAMP3 and TRH between sugar groups were found. Both HFCS and fructose significantly decreased RAMP3 expression compared with glucose in the VMH (Refer to Figure 12B). By contrast, in the LH RAMP3 expression was significantly reduced in the HFCS group when compared to the glucose group (Refer to Figure 12C). Both fructose and HFCS reduced TRH expression when compared with sucrose in the PVN (Refer to Figure 12D). No other differences were found in the VMH or LH (Refer to Figure 12E and F).

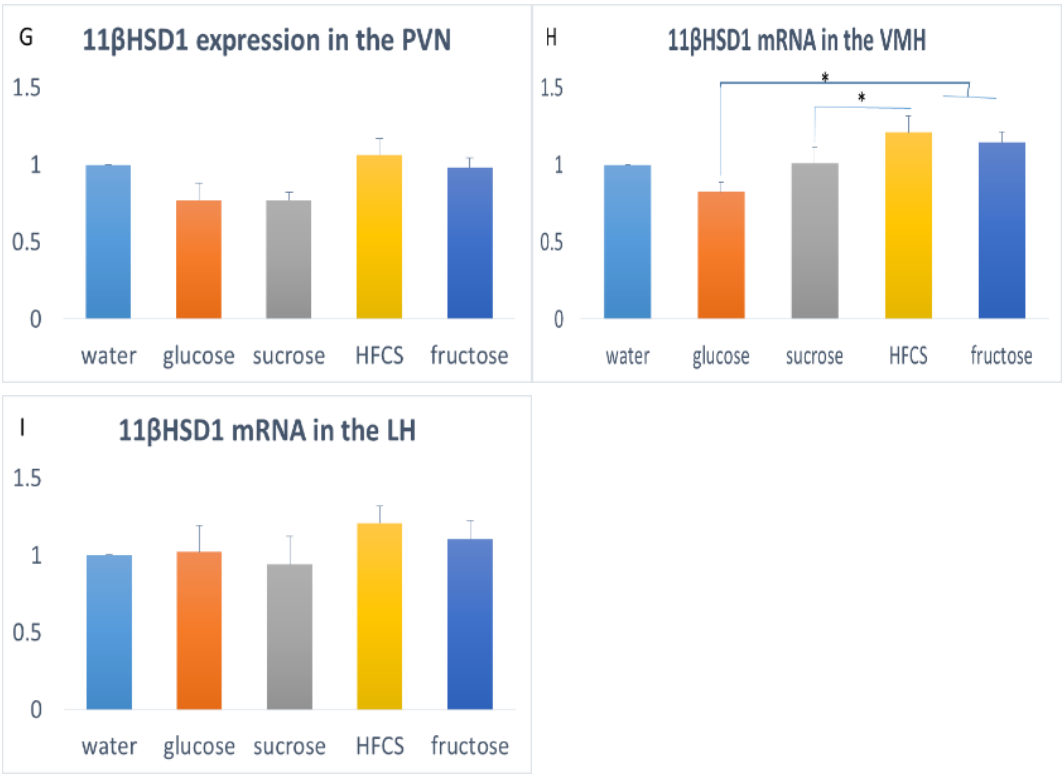
Figure 12 Different sugars affect the expression of RAMP3, TRH and 11βHSD1 in the PVN, VMH and LH.

(A-C) Expression of RAMP3 mRNA in different groups was expressed in PVN, VMH and LH. Both HFCS and fructose significantly decreased RAMP3 expression compared with glucose in the VMH. By contrast, RAMP3 expression in the LH was significantly reduced in the HFCS group when compared to the glucose group. (D-F) Expression of TRH mRNA in different sugar groups was observed in PVN, VMH and LH. Both fructose and HFCS reduced TRH expression when compared with sucrose in the PVN. (G-I) Expression of 11βHSD1 mRNA in different sugar groups was observed in PVN, VMH and LH. Sugars did not significantly change 11β-HSD1 mRNA expression in VMH compared with water group. HFCS significantly increased 11β-HSD1 mRNA expression in the VMH compared with glucose or sucrose.

All values on Y axis were expressed as $2^{-\Delta Ct}$

* $p<0.05$


A
RAMP3 expression in the PVN
2
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
B
RAMP3 expression in the VMH
*
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
C
RAMP3 expression in the LH
*
2
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Frucotse
D
TRH expression in the PVN
*
1.8
1.3
0.8
0.3
-0.2
Water
Glucose
Sucrose
HFCS
Fructose
E
TRH expression in the VMH
1.2
1
0.8
0.6
0.4
0.2
0
Water
Glucose
Sucrose
HFCS
Fructose
F
TRH expression in the LH
1.2
1
0.8
0.6
0.4
0.2
0
Water
Glucose
Sucrose
HFCS
Frucotse


G
11βHSD1 expression in the PVN
1.5
1
0.5
0
water
glucose
sucrose
HFCS
fructose
H
11βHSD1 mRNA in the VMH
*
*
1.5
1
0.5
0
water
glucose
sucrose
HFCS
fructose
I
11βHSD1 mRNA in the LH
1.5
1
0.5
0
water
glucose
sucrose
HFCS
fructose

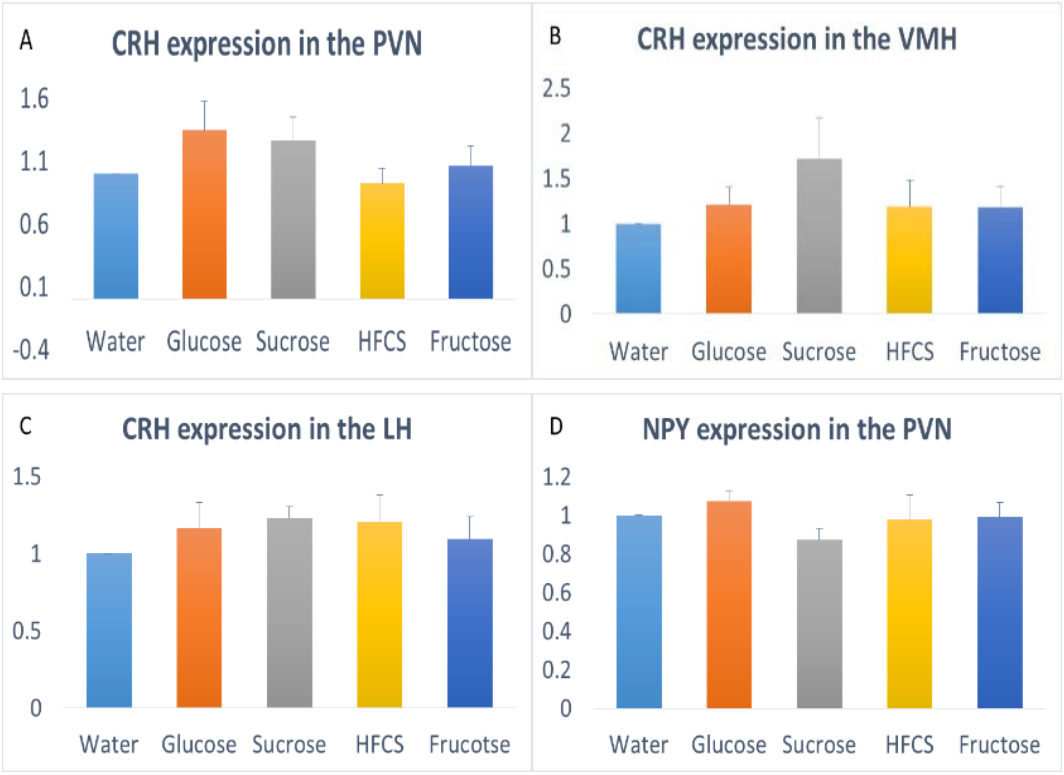
Several neuropeptides that were not affected by sugar intake

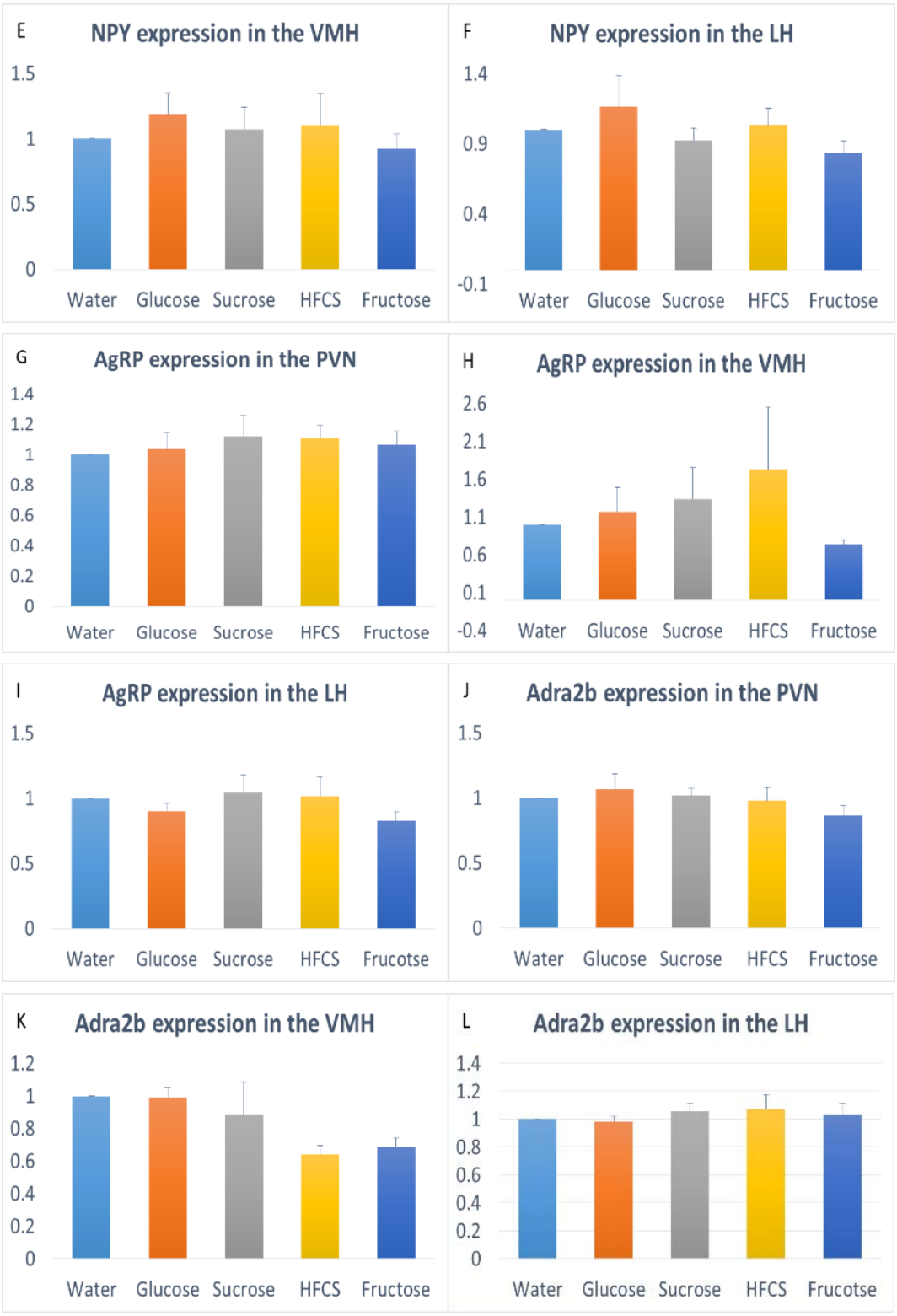
The other neuropeptides tested that were not significantly affected by sugar intake include CRH, NPY, AgRP, Adra2b, OXT and AVP. (See Figure 13)

Figure 13 Hypothalamic neuropeptides that were not affected by 24 hour exposure to sugar solutions.

(A-R) expression of CRH, NPY, AgRP, Adra2b, OXT and AVP in different sugar groups in PVN, VMH and LH. All sugars failed significantly change the mRNA expression of these neuropeptides in PVN, VMH or LH.

All values on Y axis were expressed as $2^{-\Delta Ct}$




E
NPY expression in the VMH
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
F
NPY expression in the LH
1.4
0.9
0.4
-0.1
Water
Glucose
Sucrose
HFCS
Fructose
G
AgRP expression in the PVN
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Water
Glucose
Sucrose
HFCS
Fructose
H
AgRP expression in the VMH
2.6
2.1
1.6
1.1
0.6
0.1
-0.4
Water
Glucose
Sucrose
HFCS
Fructose
I
AgRP expression in the LH
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Frucotse
J
Adra2b expression in the PVN
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
K
Adra2b expression in the VMH
1.2
1
0.8
0.6
0.4
0.2
0
Water
Glucose
Sucrose
HFCS
Fructose
L
Adra2b expression in the LH
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Water
Glucose
Sucrose
HFCS
Frucotse


M
OXY expression in the PVN
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
N
OXY expression in the VMH
2
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
O
OXY expression in the LH
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Frucotse
P
AVP expression in the PVN
1.5
1
0.5
0
Water
Glucose
Sucrose
HFCS
Fructose
Q
AVP expression in the VMH
1.2
1
0.8
0.6
0.4
0.2
0
Water
Glucose
Sucrose
HFCS
Fructose
R
AVP expression in the LH
1.2
1
0.8
0.6
0.4
0.2
0
Water
Glucose
Sucrose
HFCS
Frucotse

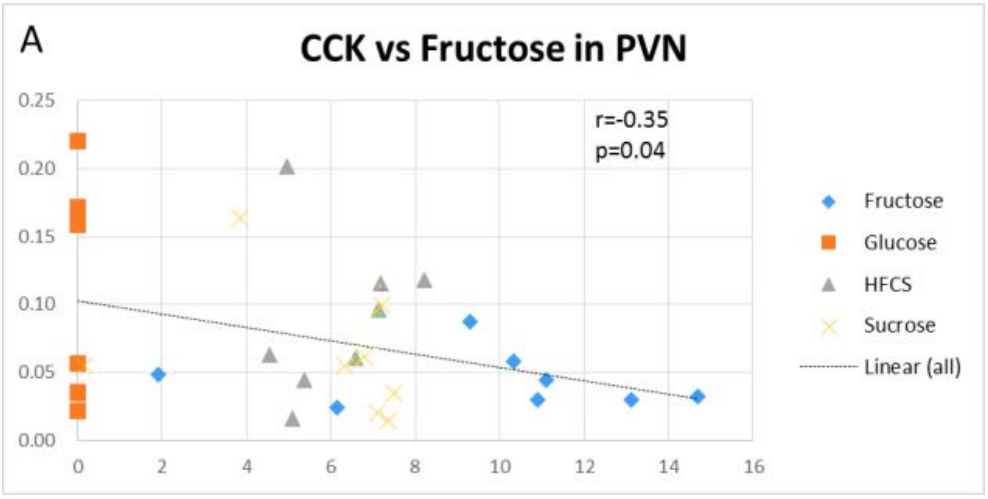
Correlation between fructose/glucose intake and neuropeptide expression

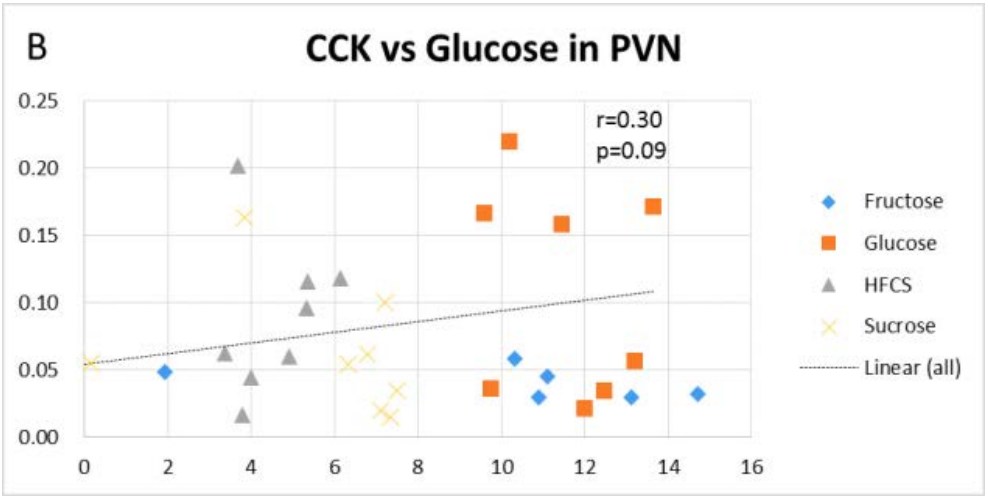
Pearson correlation analyses revealed that CCK expression was regulated by fructose but not by glucose. A statistically significant inverse correlation between CCK expression in the PVN and fructose intake ($r= -0.36$, $p<0.05$) was found (refer to Figure 14A-B). No correlations between fructose or glucose intake and TNF-α were statistically significant (refer to Figure 14C-D). GH expression is inversely correlated with fructose intake ($r=-0.44$, $p=0.01$) and positively correlated with glucose intake ($r=0.59$, $p=0.00$) (refer to Figure 14E-F). The correlations between fructose or glucose intakes with other neuropeptides in any of the three hypothalamic regions sampled failed to achieve statistical significance.

Figure 14 Effect of fructose or glucose on the expression of several neuropeptides.

Expression of CCK in the PVN is negatively correlated with fructose intake. (B) CCK expression in the PVN is not significantly correlated with glucose intake. (C) Expression of TNF-α in the PVN is not correlated with fructose intake. (D) TNF-α expression in the PVN is not significantly correlated with glucose intake. (E) Expression of GH in the VMH is negatively correlated with fructose intake. (F) GH expression in the VMH is significantly correlated with glucose intake.

Values on Y axis were expressed as $2^{-\Delta Ct}$, and values on X axis were express as gram of fructose.


A
CCK vs Fructose in PVN
r=-0.35
p=0.04
0.25
0.20
0.15
0.10
0.05
0.00
0
2
4
6
8
10
12
14
16
Fructose
Glucose
HFCS
Sucrose
Linear (all)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0.20
0.15
0.10
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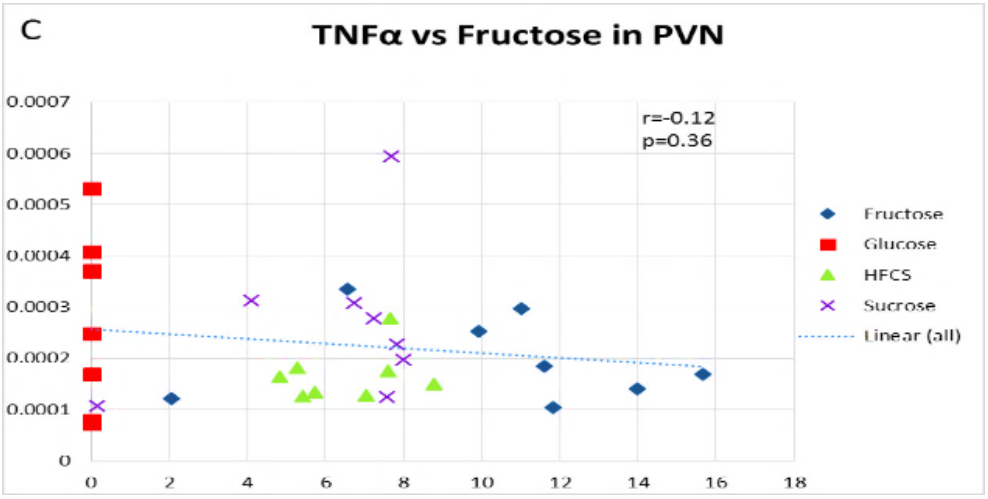
0.00
0
2
4
6
8
10
12
14
16
Fructose
Glucose
HFCS
Sucrose
Linear (all)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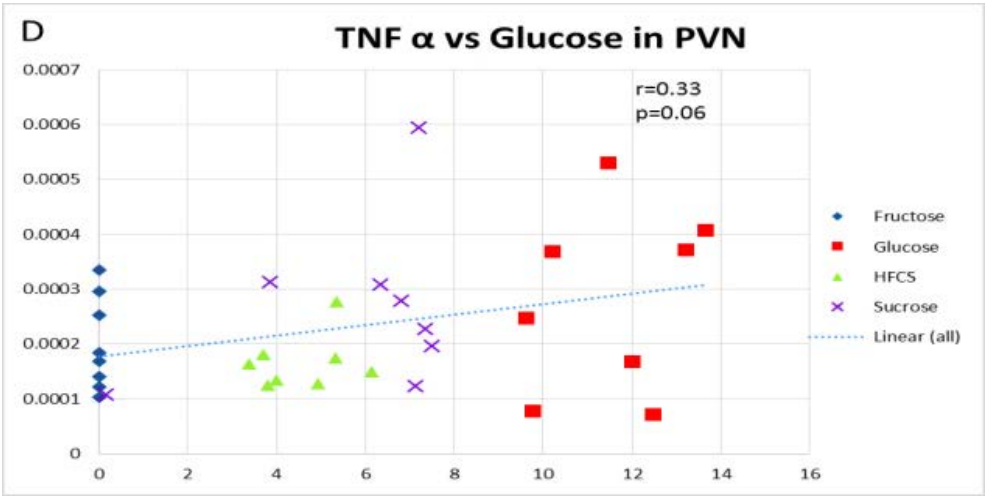
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
0
2
4
6
8
10
12
14
16
18
Fructose
Glucose
HFCS
Sucrose
Linear (all)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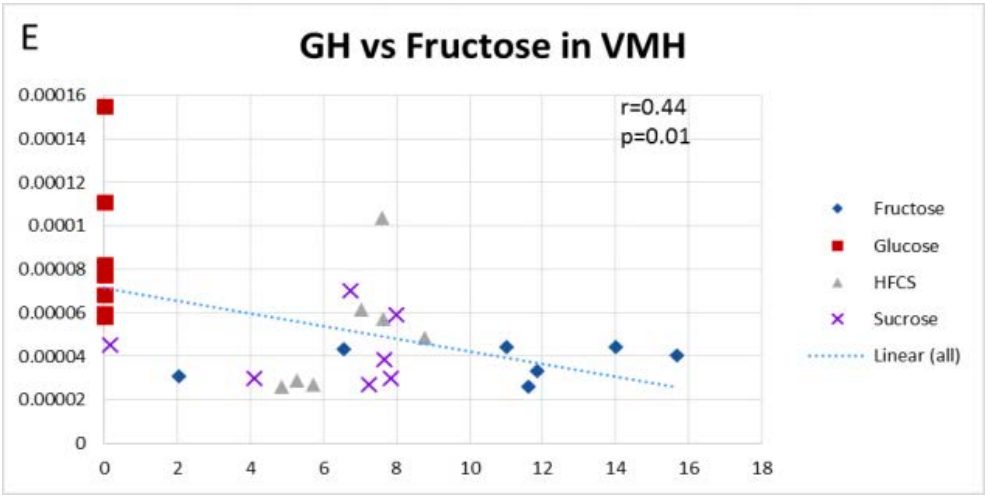
p=0.06
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
0
2
4
6
8
10
12
14
16
Fructose
Glucose
HFCS
Sucrose
Linear (all)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p=0.01
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0.00012
0.0001
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0.00004
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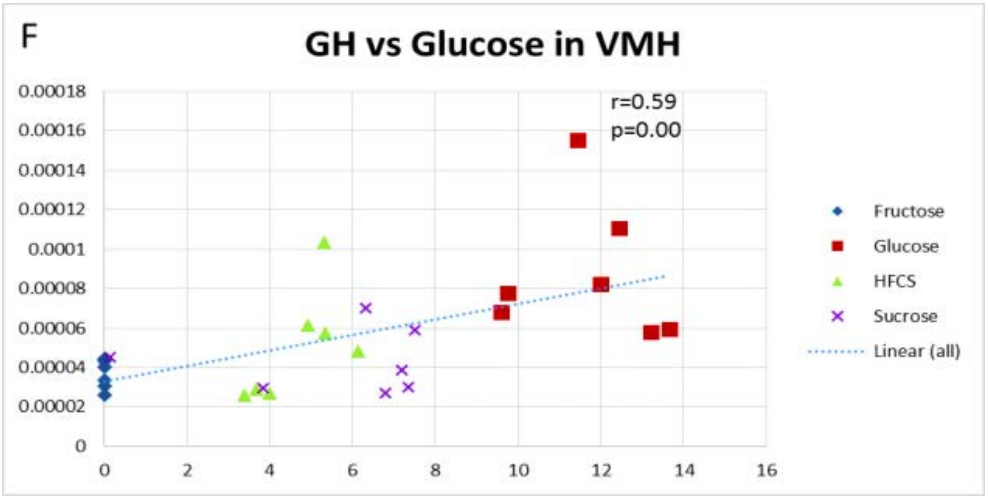
0
0
2
4
6
8
10
12
14
16
18
Fructose
Glucose
HFCS
Sucrose
Linear (all)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GH vs Glucose in VMH
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p=0.00
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0.00006
0.00004
0.00002
0
0
2
4
6
8
10
12
14
16
Fructose
Glucose
HFCS
Sucrose
Linear (all)

Discussion

Sugar induced obesity is well documented, whereas the mechanisms behind it are still not clear. Differences in sugar metabolism in the liver are not sufficient to explain how long term *ad libitum* sugar access can cause weight gain and/or even obesity. We hypothesize that sugars can affect the expression pattern of hypothalamic neuropeptides that may bias energy balance in the long term. To test the initial response of the rats, we gave the rats a brief exposure of different sugar solutions. Our results showed that under *ad libitum* conditions, fructose intakes were comparable (or even a little lower(see also [106])) to intakes of other sugars. This *ad libitum* method more closely mimics conditions that in humans lead to excess intake and obesity. It should be noted that some popular sweetened drinks may have over 12% sugar content[38] and some juices even have higher sugar content[39].

Free access to glucose, fructose, sucrose or HFCS can lead to excessive weight gain and/or obesity in the Sprague Dawley rat [45, 48, 151]. We found evidence that brief exposure to different sugars can change the expression of CCK and TNF-α in the PVN as well as GH in the VMH. The responses to sugars are not the result from excess energy intake, as total caloric intake did not differ among all five groups (refer to Table 2).

CCK is well known as an effective hunger suppressant [157]. Brain CCK receptor deficiency results in hyperphagia and decreased responsiveness to high fat diet in rats [158-160]. Dorsal medial hypothalamic CCK inhibits food intake for at least 22 h [161, 162]. CCK microinfused in the PVN inhibits gastric emptying and stimulates colonic transit in a dose dependent way [163]. It has been reported that CCK synthesis in the hypothalamus is disrupted during diabetes development [144]. CCK release from PVN in response to a gavaged meal is also compromised in the obese (fa/fa) Zucker rats [164]. These observations support the view that hypothalamic CCK plays an important role in energy control. We recently reported that 24 hour access to glucose solution upregulates CCK expression whereas access to fructose results in significant downregulation of CCK [139]. In the current study, we found that fructose affects the hypothalamus by down-regulating CCK mainly in the PVN, not in the VMH or the LH. It has been previously reported that hypothalamic CCK is primarily expressed in PVN, typically in the parvocellular sub-nuclei [144]. This may suggest that effect of sugars on CCK is confined to the PVN region. CCK expression is not significantly different in response to sucrose or HFCS.

TNF-α is an important chemokine involved in systemic inflammation. High doses of exogenous TNF-α can reduce food intake with weight loss being

proportional to the decrease of both food and water intake [165]. It needs to be noted that the effect of TNF-α on food intake is mild compared with the effects of either insulin or leptin[166]. On the contrary, hypothalamic pro-inflammatory signaling can lead to impaired insulin sensitivity [167], increasing the potential for excess weight gain [168]. In the current study we found glucose and sucrose increased TNF-α. This effect is similar to that caused by high fat diet[169]. By contrast HFCS and fructose had no significant effect on TNF-α expression. The slight increase of TNF-α caused by glucose or sucrose was probably insufficient to have an effect on energy balance in the short term. Although fructose was reported to increase TNF-α in the whole hypothalamus[139], that observation is probably a reflection of the fact that the entire hypothalamus was sampled.

GH is known to be a peptide that contributes to the energy expenditure and lipid oxidation[170]. GH is critical for fat mobilization during fasting or starvation state when insulin is suppressed[171, 172]. Adults with GH deficiency usually have increased body fat mass and decreased extracellular fluid volume[173]. We recently reported that GH expression was upregulated by glucose intake, in the current study we further found fructose decreased GH expression in specific hypothalamic areas[139]. Although the effect of glucose intake on the GH expression failed to reach statistical significance, the positive correlation between

GH expression and glucose intake is robust ($P<0.05$). Because fructose can induce hypertriglyceridemia, our observation is also consistent with the fact that plasma triglycerides inhibit GH release[174].

Drd1a is a receptor for dopamine. Dopamine signalling is proposed as part of the mechanism of the overeating[175]. Activation of Drd1a expressing neurons in the medial prefrontal cortex (mPFC) can increase feeding[176]. Activation of amygdala D2 receptor can reduce food intake and operant behavior for sucrose in Male Sprague Dawley rats, whereas Drd1a stimulation or blockade cannot[177]. High level of D1 receptor expression in the VMH may contribute to the specific feeding pattern in obese rats[178]. Interestingly, we found that glucose can increase the expression of Drd1a in the PVN. HFCS had opposite effect on Drd1a expression in PVN and VMH.

RAMP3 can interact with several protein-coupled receptors. Adrenomedullin receptors and amylin receptors are two well-studied complex forms that require RAMP3. Adrenomedullin is a potent endogenous vasodilatory peptide. Amylin is secreted in the gut in response to nutrient intake and suppresses glucagon secretion. It also slows gastric emptying and delays glucose entering into the circulation. Its agonist can be used for type 2 diabetic patients to control postprandial glucose levels[179]. Increased amylin is associated with reduced body weight gain and

adiposity [180]. Unlike CCK, glucose and fructose failed to affect the expression of RAMP3 in the PVN. Interestingly, although RAMP3 is mainly expressed in PVN, HFCS suppressed RAMP3 in both the VMH and the LH when compared to the glucose group, but not with the water-fed control group.

Adipose tissue 11β-HSD1 has been studied for its possible roles in obesity development [78-80], whereas the brain 11β-HSD1 is rarely reported. We found 11β-HSD1 was detected in the PVN, where CRH, OXT and AVP were highly expressed. Our observations make it plausible that hypothalamic 11β-HSD1 in the PVN participates in the hypothalamic-pituitary-adrenal axis and/or other biological metabolism, e.g. food intake control. Central glucocorticoids play an important role in energy homeostasis [181, 182]. Intraventricular glucocorticoid injection increases the body weight gain in adrenalectomized rats [182] and 11β-HSD1 deficiency increases glycolysis and energy substrate (lactate) in the brain [183], indicating that hypothalamic 11β-HSD1 may play a critical role in the control of energy homeostasis. We have previously shown that sugars can affect the expression of several neuropeptides in the hypothalamus. Here we further showed that sugars also increased the hypothalamic expression of 11β-HSD1 in both PVN and VMH. Interestingly, when we combined the PVN, VMH and LH data together, we failed to detect any significant differences. Therefore, we believe the effect of

sugars on the hypothalamic glucocorticoids is probably mainly in the PVN and VMH areas.

Finally, TRH is mainly located in the PVN and its secretion is connected with pituitary hormone release. TRH has an anorexigenic effect both by central or peripheral administration [111]. Differently from the results from the whole hypothalamus reported earlier [139], fructose failed to significantly decrease TRH in the PVN, the VMH or the LH. It has been pointed out elsewhere [184] that TRH expression is highly specific to the PVN. We failed to replicate the previously reported finding (that fructose promoted decreases in TRH message and that HFCS intake downregulated CRH message). It is likely that this difference in observation is attributable to the differences in dissection/sampling. Earlier work from our laboratory used tissue from a much larger region of the hypothalamus, whereas the sampling performed in this paper was confined to discreet hypothalamic nuclei or regions.

When we examined food intake and energy intake, we found that standard chow consumption was reduced but total energy intake remained unchanged. Although the reduction of CCK and GH or increase of TNF-α failed to affect energy intake immediately, the expression change of these neuropeptides could possibly contribute to the obesity induced by long term use of sugars.

We have compared the effects of HFCS to the effects of other sugars including glucose, sucrose and fructose. Sucrose and HFCS have differing effects on several neuropeptides including TNF-α, GH, RAMP3 and TRH. Fructose intake, when unaccompanied by comparable glucose intake, can lead to malabsorption[41]. As a result HFCS (55% fructose, 41% glucose) has the potential to result in greater malabsorption than does sucrose. Different sugars may also have different effects on the gut microbiota. All these factors may lead to their distinct role in energy metabolism.

Conclusion

We have presented evidence here that makes it clear that different sugars can and do have different effects on different targets, even within the hypothalamus. Our study clearly indicated that brief sugar intake can change hypothalamic neuropeptides. We found a close link between the expression of the neuropeptide CCK and GH and fructose intake. No differences in CCK or GH between HFCS and sucrose groups were observed. The mRNA measurements in the current study do not predict protein levels but reflects on the expression changes of the neuropeptides which are critical for control of energy homeostasis. Further study is needed to confirm

whether the change of these neuropeptides is sufficient for inducing obesity induced by the long term use of sugars.

CHAPTER 4. SUGARS AND ADIPOSITY HORMONES

Abstract

Several neuroendocrine systems are important for energy balance. We have shown evidence that different sugars can affect expression of different neuropeptides. In this chapter, we will focus on the effects of sugars on the important circulating regulator adiposity hormones insulin and leptin. We recently reported that fructose can quickly increase circulating triglycerides but insulin was not affected. We then measured the other adiposity hormone leptin in the sera of Sprague Dawley rats fed with different 15% sugar solutions for 24 hour. Surprisingly, sugars slightly increased leptin level on average but failed to reach significant level. However, literature research showed that long term use of sugars can lead to the disorder of insulin and leptin signaling pathways. Therefore, we believe that sugar is not the direct factor that affects these adiposity hormones. However, sugar induced obesity with accumulated body fat mass will finally generate more leptin accompanied with increased insulin secretion.

Introduction

Fructose that accounts for much of the increase in sugar intake in the American diet has a potential role in contributing to the current obesity problem [2]. Unlike glucose intake, fructose intake can quickly induce hypertriglyceridemia[152], a

condition that frequently coexists with obesity, type 2 diabetes and metabolic syndrome [153]. Our lab previously reported that fructose consumption can quickly suppress the expression of 11β -HSD1 in liver and visceral adipose tissues [62] leading to an increase in intracellular levels of glucocorticoids [154]. Increased intracellular glucocorticoids are commonly observed in obesity in human and animal models [155, 156].

To evaluate the effects of sugars on obesity development, we have also measured two hormones known to participate in the control of intake: insulin and leptin. To date, only insulin and leptin meet all the criteria of adiposity hormones: 1) they circulate at levels proportional to body fat content, which further determines the amount they enter the CNS. 2) their receptors are expressed by brain neurons involved in energy intake, specifically high level of both hormones in the brain reduces food intake, whereas deficiency of either hormone has the opposite effect [185, 186].

Insulin is an important adiposity hormone which is secreted from β cells of the pancreas. Deficiency of insulin can lead to an increase in NPY expression and intracerebroventricular insulin infusion can reduce diabetic hyperphagia [187]. Knockout of neuron specific insulin receptors in mice results in the diet-sensitive

obesity with increases in body fat composition [188]. However, we previously showed that sugars failed to affect insulin after 24 hour exposure.

Similarly, leptin knockout mice become obese. Leptin can inhibit NPY gene expression [189] and activate POMC/CART neurons located in ARC. Loss of NPY can even attenuates the obesity syndrome of ob/ob mice [97]. Although leptin activates POMC via PI3K signaling pathway, PI3K inhibition only cannot induce obesity in a long term [190]. Therefore, POMC is probably not the only target of leptin to control food intake. Exogenous leptin infusion can prevent the diabetic hyperphagia in a rat model of insulin deficient diabetes [191]. How sugars can affect another adiposity - leptin in short term is unknown. In the current project, we measured leptin levels in rats fed with different sugars and then we will deeply discuss how sugars affect the adiposity hormones as well as other peripheral regulators.

Materials and Methods

Animal treatment

Adult male Sprague-Dawley (CD strain) rats (Charles River Laboratories, Wilmington, MA) weighted approximately 300 g were used. Upon arrival, all animals were individually housed and maintained on a 12 h light/dark cycle at room temperature of 22 °C ± 1 °C. During 1 week acclimation period the rats were given

free access to the water and chow. The chow is a nutritionally complete low fat diet [Rodent diet 7012] prepared by Harlan Teklad (Bethlehem, PA) containing 3.41 kcal/g of diet of which 2.14 kcal were derived from carbohydrate per gram of chow. All animals were given free access to water throughout the experiment.

Rats were randomly assigned to one of five weight-matched groups (n= 8/group). One group of rats had *ad libitum* chow and water and served as the control group. Rats assigned to the other groups had *ad libitum* access to the chow and water and to one of the four solutions: the 15%weight/volume (w/v) fructose (Tate & Lyle, Decatur IL) solution, the 15% (w/v) glucose (Sigma Aldrich, St Louis MO) solution, the 15% (w/v) high fructose corn syrup (HFCS) (IsoSweet® 5500, 55 % fructose–41 % glucose, 77 % solids, Tate & Lyle, Decatur IL) or the 15%(w/v) sucrose (Domino Foods, Baltimore MD) solution. All sugar solutions were prepared 24 h in advance and stored at 4 °C until use. The rats were maintained on their respective diets for 24 h before sacrifice. All rats were killed by slow replacement of air in a specialized chamber with pure CO2 followed by rapid decapitation and exsanguination. This method has been approved for use by the Panel on Euthanasia of the American Veterinary Medical Association as well as the UM IACUC. All procedures described herein are in compliance with the University of Maryland's ACUC guidelines.

Plasma Leptin Measures

Leptin concentrations were determined using the EZRL-83K, Rat Leptin ELISA kit (EMD Millipore) from sera of Sprague Dawley rats fed with different sugar solutions.

Statistics

One way ANOVA with Duncan post hoc testing was applied to the $2^{-\Delta Ct}$ values using IBM SPSS Statistics 21. P values of less than 0.05 were considered statistically significant.

Results

Leptin level was not changed by 24 h treatment of sugar solutions (see Figure 15).

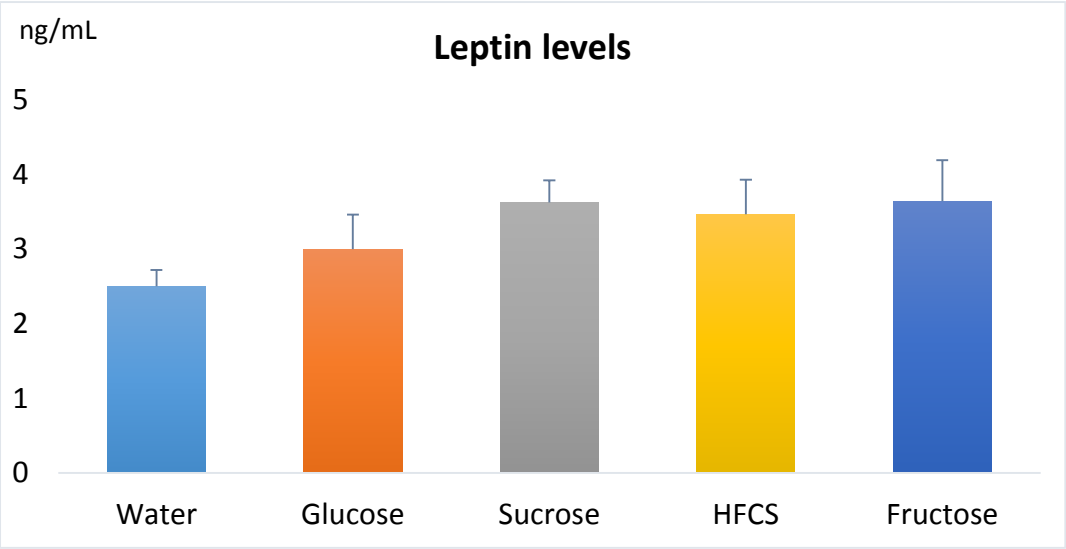

Figure 15 Plasma leptin level of rats fed with different sugars.

Leptin were determined using EZRL-83K, Rat Leptin ELISA kit (EMD Millipore) from sera of Sprague Dawley rats. These rats were fed with different sugar solutions including water, 15% glucose, 15% sucrose, 15% HFCS and 15% fructose. On average, all sugars slightly increased leptin level, but this change failed to reach significant level ($P<0.05$)

Discussions

Insulin

Glucose sweetened drinks are more effective than fructose sweetened drinks in their ability to increase insulin in humans within one hour [26]. This effect disappears quickly, as evidenced that 24 hour sugar exposure failed to change insulin level in in Sprague Dawley rats [63, 70]. Rats initiate more meals/day (approximately 12 meals/day) than do humans[192], so glucose-induced increases in insulin should be higher than that caused by fructose intake. Although insulin can inhibit food intake, it is also required for adiposity. Hyperphagia with low levels of insulin fails to promote obesity. High insulin levels in the long term can drive obesity in a brain insulin independent pathway[193]. As a result, long term frequent use of glucose may also induce obesity.

One important obesity associated disorder is type 2 diabetes featured with insulin resistance. Fructose enriched diets or solutions can induce hyperinsulinemia after two weeks treatment [106, 194, 195]. This is an early signal of insulin resistance. Indeed, even moderate amounts of fructose or sucrose can alter hepatic insulin sensitivity and lipid metabolism compared with glucose [196]. Human studies confirmed that short term high fructose intake can lead to dyslipidemia and

hepatic and/or muscle/adipose tissue insulin resistance [197, 198]. Long term use of fructose, e.g. in the form of sucrose form can cause insulin insensibility [199].

Fructose and glucose share several of the same metabolic pathways. The main reason that fructose can cause insulin resistance is probably due to the unique metabolism of fructose in the liver. Fructose is mainly metabolized in the liver and quickly stimulates an increase in fasting and postprandial triglycerides. This is not only because of hepatic de novo lipogenesis but also due to the increased VLDL-triglyceride secretion as well as a decreased VLDL-triglyceride clearance. A case report showed that hypertriglyceridemia may not be an obligatory cause for insulin resistance or vice versa [200].

In addition to insulin resistance, fructose can also induce glucose intolerance. Glucose intolerance is a pre-diabetic state of hyperglycemia. It is often associated with insulin resistance. A high-fructose diet can cause hyperinsulinemia, while a high-fat diet can result in impaired pancreatic function of insulin secretion. Both of them can induce glucose intolerance [201]. This is partly due to impaired suppression of hepatic glucose output as well as fructose induced gluconeogenesis via hypothalamic AMPK activation [60].

Leptin

Neither glucose nor fructose sweetened solutions affect circulating leptin in humans within one hour [26]. We also failed to detect significant change of plasma leptin in 24 hour sugar-treated rats. However, glucose enriched diet (60% by calorie), but not fructose enriched diet, increases circulating leptin of 5 hour fasted Sprague Dawley rats after two weeks treatment [194]. Similarly, glucose, sucrose and fructose solutions increased fasting leptin after two weeks [106]. Sugar solutions may cause leptin resistance that can further accelerates diet induced obesity [202]. Obesity with leptin resistance has been repeatedly reported. In addition to the defect of leptin receptors [203], other factors that can induce leptin resistance include: brown adipose tissue removal [204], unknown factors from obesity independent of melanocortin-4 (MC4) receptors [205], melanocortin receptor blockade associated with mutation of Ay gene [206]. Dysfunction of leptin transport may lead to leptin resistance and obesity evidenced by the obese humans with low leptin levels in cerebrospinal fluid [207]. The expression of the suppressors-of-cytokine-signaling (SOCS) 3 is another mechanism that can contribute to leptin resistance [208]. The mechanism of how sugars can induce leptin resistance is not quite clear yet, but understanding this is undoubtedly crucial to unravel the enigma of sugar induced obesity.

CHAPTER 5. SUMMARY AND PROSPECT

Time course of the sugar study

Obesity is a complicated disease that can be induced in many ways. Diet induced obesity is one of the most widely used models, as this closely mimics the obesity and/or associated metabolic diseases emerging in the world. High fat, high sugar or both can generate diet-induced obesity. With the increasing interest in the sugar hypothesis, typically HFCS, in the current obesity epidemic in US, our lab designed several experiments to investigate the effect of brief sugar intake on the energy control of Sprague Dawley rats. Below is a list of previous findings from our lab about sugar induced obesity.

11β-HSD-1, that interconverts active and inactive glucocorticoid, is frequently confirmed to be closely related with obesity, typically central obesity. For example, 11β-HSD-1 in liver and adipose is deregulated in both human and animal models of obesity. Between the years 2006 to 2009, our lab found that ten weeks' exposure to sucrose solution increased both the body fat, fasting insulin and the 11β-HSD-1 mRNA in adipose tissues. Fructose solutions can effectively increase 11βHSD1 message in mesenteric adipose and liver just within 24 hours. This result clearly explained why fructose is more effective to increase visceral fats than glucose. However, the hepatic 11βHSD1 mRNA and protein expression were suppressed

after one week treatment. Additionally, our lab observed that fructose can also quickly increase plasma triglycerides which is a well-known risk factor for obesity and metabolic diseases. The sugars including sucrose, fructose and glucose have their distinct metabolic and endocrine responses, of which fructose can uniquely induce glucocorticoid dysregulation in liver and adipose within 24 hour.

Because of the high efficiency of fructose induced hypertriglyceridemia as well as increased 11βHSD1 expression, our lab continued using this model to further study the effects of initial sugar intake on the brain, typically, the hypothalamus. The hypothalamus is considered as the control center for food/caloric intake with complicated neuronal signaling network. Between the years 2010 to 2012, our lab briefly treated Sprague Dawley rats with different sugar solutions for 24 hours. In addition to glucose, sucrose and fructose, HFCS was also included since it was increasingly used in the food market today. As expected, our lab repeated the previous finding that fructose containing sugars can quickly cause hypertriglyceridemia. However, circulating insulin as well as glucose were not significantly changed. This is not surprising, since it normally takes months for free access to sugar solutions to cause significant body weight gain in rodent animals. The sugar increased insulin is probably the consequence of increased body fat caused by long term sugar use. PCR array was then applied to examine 84 obesity

related genes in the hypothalamus. Those genes that were found be significantly changed by sugars were re-examined by individual PCR assays. Several hypothalamic neuropeptides that were found to be changed by sugar intake included CCK, TNF-α, RMAP3, CRH, TRH and GH. Interestingly, the well-studied neuropeptides in the ARC like NPY were not affected.

However, the hypothalamus is composed of different functional regions, each of which have their district functions in food/caloric intake. For example, ablation of PVN or VMH can cause hyperphagia and obesity whereas ablation of LH will do the opposite. This stimulated the idea of the current project to further examine these neuropeptides in specific hypothalamic regions.

Summary of the current study

There are many neuropeptides that are associated with energy balance. Thanks to the preliminary PCR array experiment, we only focused on those neuropeptides that were indicated to be affected by sugar access. These neuropeptides usually have their distinct distributions. For example, CCK, RAMP3, TRH, CRH are mainly expressed in the PVN whereas GH are mainly synthesized in the VMH and LH. However, TNF-α is widely and nearly uniformly expressed in the whole

hypothalamus. We early expected that the effects of sugars on these neuropeptides should be mainly restricted to the regions that mainly biosynthesized them.

In the current dissertation, we showed evidence that brief sugar intake affected expression of several hypothalamic neuropeptides. Specifically, sugars containing fructose can repress the expression of anorexic neuropeptide CCK in the PVN, and GH in the VMH which may facilitate fat burning. Sucrose and glucose can both increase the TNF-α expression in the PVN, a chemokine involved in inflammation and may impair insulin signaling to cause obesity. Additionally, glucose solutions also quickly increased Drd1a expression, which might amply dopamine activity and increase the risk of overeating and excess energy intake. Although we failed to detect significant change of the other neuropeptides, we believe our result is convincing since we measured these neuropeptides within specific hypothalamic regions that were accurately dissected compared with the whole hypothalamus.

This experimental design is an important and also necessary step for us to understand how sugars affect the energy control in the long term. It's too early to give a conclusive assertion about sugar induced obesity. Based on what have so far, we attempt to propose that sugars can cause energy imbalance via both peripheral and hypothalamic signals including increased circulating triglycerides, increased intracellular glucocorticoids as well as regulation of specific brain neuropeptides

[Figure 16]. The final consequence is the increased body fat as well as adiposity hormones. All of these can contribute to obesity, type II diabetes and metabolic syndrome.

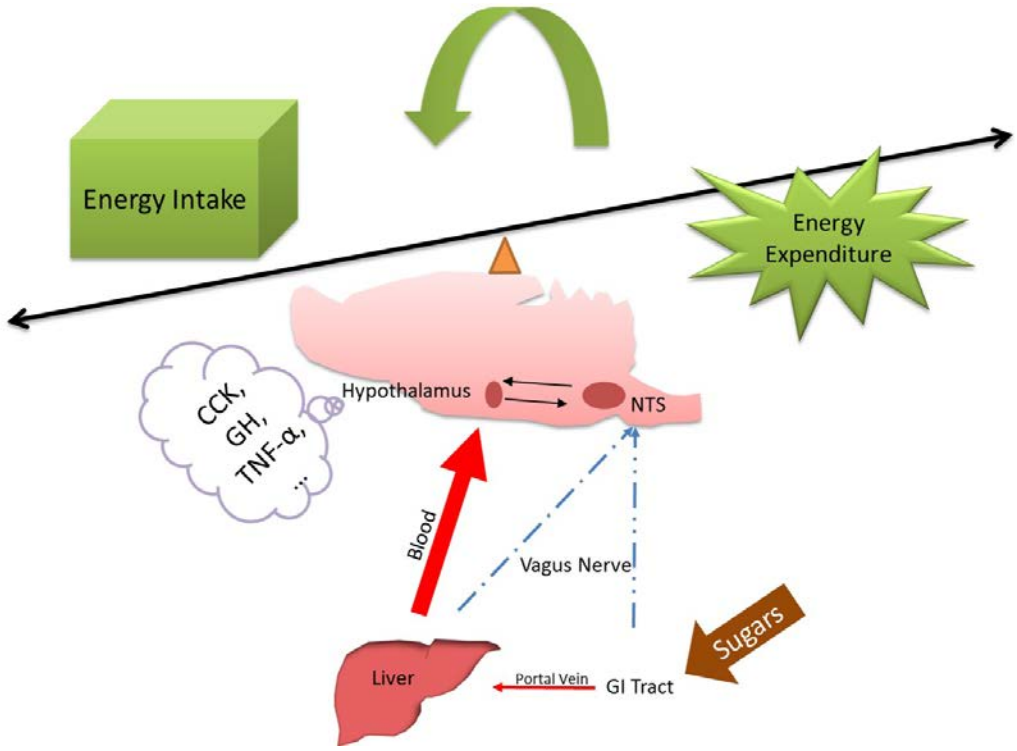

Figure 16 Sugar induced energy imbalance.

The dietary sugars can reach the neural circuitry in specific brain areas including hypothalamus and NTS via both the circulation system as well as the vagus nerve system. As a consequence, the disrupted energy homeostasis leads to body weight gain and/or obesity.

Prospects

Sucrose and HFCS are two of the most widely used sweeteners in the US market. As both of them have the ability of inducing obesity, the argument focusing on the HFCS or fructose itself is a little bit “off-target”. Although fat also can also induce obesity, it is excluded as the main cause of the current obesity epidemic because years of promotion of low fat or fat free products has failed to slow down the obesity increasing rate. HFCS use has dropped in recent years. This decrease hasn’t significantly affected the increasing obesity rate either. It’s not known whether there is a lagged effect caused by long-term use of sugars. Perhaps there is a threshold for sugar use to cause obesity? The promotion of low sugar or sugar free products in the future is a good strategy to examine whether sugars or other factors involved contribute to the current obesity epidemic. It really makes things more complicated when some people argue about whether animal models are capable of mimicking human behaviors. We have used rodent models to do multiple studies and found a close similarity in both physiology and response to many diet stimuli.

We admit that there are limitations for the current study, for example, whether change of hypothalamic CCK expression is sufficient to explain part of fructose induced obesity needs further investigation. To continue this project future work

can be done based on the current results. Below is a brief proposal for the downstream study.

Title: Investigation of the role of hypothalamic CCK in the fructose induced obesity.

Hypothesis: hypothalamic CCK plays a central role in fructose induced obesity.

Rationale:

1. CCK is an anorexic neuropeptide. For example, inactivation of the brain CCK receptors results in hyperphagia whereas increased hypothalamic CCK inhibits food intake, increase gastric emptying and stimulate bowel movement. On the other hand, the hypothalamic CCK pathway is disrupted in both diabetic and obese rats.
2. Fructose can effectively reduce hypothalamic CCK expression. This is both found in the PVN as well as the whole hypothalamus.

Objective 1: to understand the relationship between the hypothalamic CCK and the obesity

Experiment 1: we can measure CCK levels in PVN in both fed and fasted state of rats as well as in lean vs obese rats. Manipulation of CCK level in the hypothalamus can cause changes of feeding behaviors. Measuring CCK levels in PVN in both fed

and fasted state can further find out whether CCK expression in the PVN is a regulator during the fasted/fed states. Moreover, if CCK is closely related with obesity, then the CCK expression will be probably different between obese and lean rats.

Objective 2: to confirm how effective of hypothalamic CCK can promote obesity and/or its associative metabolic change.

Experiment 2: we can modulate CCK level in rats by downregulating CCK in the PVN using siRNA/knockout techniques and measure several metabolic parameters in rats. Previous research did show that CCK is an anorexic neuropeptide, but how effective CCK can cause obesity is still not quite understood. The siRNA or conditional knockout technique is undoubtedly is a powerful tool to evaluate the effect of CCK repression on the obesity development. After knockdown or knockout, several metabolic parameters can be recorded. These include body weight, lipid profile, and adiposity hormone as well as insulin sensitivity and so on.

Objective 3: To find out the relationship between long term access to fructose solution and CCK expression.

Experiment 3: as is known, free access to fructose solutions can cause significant body weight gain after several months' treatment. The current study only lasted for 24 hour which cannot extrapolate to long term effect of fructose on CCK expression.

To evaluate whether CCK expression is closed related with fructose induced obesity. CCK level in the PVN should be monitored during the whole process to fructose treatment until body weight changes significantly.

Objective 4: to investigate whether CCK manipulation can stop or reverse fructose induced obesity.

Experiment 4: if CCK is the main contributor to the fructose induced obesity. CCK overexpression should significantly ameliorate fructose induced obesity. During this experiment we can use hypothalamic CCK overexpressed rats as experimental group to examine whether fructose induced obesity as well as metabolic syndrome can be modified in the CCK genetic modified rats. Another way to do this is to inject CCK to the PVN of obese rats fed with fructose on a regular manner. This is to further examine whether CCK can reverse fructose induced obesity.

The above is just a brief example for the possible future work following the current project. Further work can also be done for protein measurement, varying fructose/glucose ratio, effect of sugars on the other areas of the brain, vagotomy studies and so on. We believe the sugar induced obesity is complicated and may involve many pathways. More work should be done to address these questions, which are important for us to understand the mechanism of sugar induced obesity.

GLOSSARY

1. Obesity: a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health, leading to reduced life expectancy and/or increased health problems. (Wikipedia)
2. Energy homeostasis: The biological process by which the body maintains body fat stores by balancing energy intake with energy expenditure over time. [52]
3. Insulin resistance: a physiological condition in which cells fail to respond to the normal actions of the hormone insulin. (Wikipedia)
4. Leptin resistance: the failure of endogenous or exogenous leptin to promote anticipated salutary metabolic outcomes in states of over-nutrition or obesity, although the hormone's inability to promote desired responses in specific situations results from multiple molecular, neural, behavioral, and environmental mechanisms. [209] A state in which the body is no longer responsive to the anorexic effect of exogenous leptin. [52]
5. Fructose hypothesis: the fructose component common to all major caloric sweeteners (sucrose, high-fructose corn syrup, honey, and fruit juice concentrates) plays a unique and causative role in the increasing rates of

cardiovascular disease, hypertension, diabetes, cancer, and nonalcoholic fatty liver disease. [210]

6. Adiposity hormones: Hormones that circulate in direct proportion to body fat and convey the state of total energy stores to the CNS. [52]
7. Satiety: The state of feeling full to the point of satisfaction after the consumption of food. [52]
8. Neuropeptide: A small protein-like molecule that is used by neurons to communicate with each other, often in a paracrine manner. [52]
9. Neurotransmitters: Chemical messengers that are released by the end of a nerve fibre, causing an impulse to be passed from once cell to another. [52]

BIBLIOGRAPHY

1. Bray, G.A., S.J. Nielsen, and B.M. Popkin, *Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity.* The American journal of clinical nutrition, 2004. **79**(4): p. 537-543.

2. Johnson, R.J., et al., *Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease.* The American journal of clinical nutrition, 2007. **86**(4): p. 899-906.

3. Malik, V. and F. Hu, *Sweeteners and Risk of Obesity and Type 2 Diabetes: The Role of Sugar-Sweetened Beverages.* Current Diabetes Reports, 2012. **12**(2): p. 195-203.

4. Wang, Y.C., et al., *Health and economic burden of the projected obesity trends in the USA and the UK.* The Lancet, 2011. **378**(9793): p. 815-825.

5. Stanhope, K.L., et al., *Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans.* The Journal of clinical investigation, 2009. **119**(5): p. 1322.

6. Hinnouho, G.-M., et al., *Metabolically Healthy Obesity and Risk of Mortality Does the definition of metabolic health matter?* Diabetes Care, 2013. **36**(8): p. 2294-2300.

7. Adolph, E.F., *Urges to eat and drink in rats.* American Journal of Physiology -- Legacy Content, 1947. **151**(1): p. 110-125.

8. Kral, T.V., L.S. Roe, and B.J. Rolls, *Combined effects of energy density and portion size on energy intake in women.* The American Journal of Clinical Nutrition, 2004. **79**(6): p. 962-968.

9. Rolls, B. and E. Bell, *Intake of fat and carbohydrate: role of energy density.* European journal of clinical nutrition, 1999. **53**: p. S166-73.

10. Harris, R., *Factors influencing energy intake of rats fed either a high-fat or a fat mimetic diet.* International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity, 1994. **18**(9): p. 632-640.

11. Nordmann, A.J., et al., *Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials.* Archives of Internal Medicine, 2006. **166**(3): p. 285-293.

12. Gower, B.A. and A.M. Goss, *A Lower-Carbohydrate, Higher-Fat Diet Reduces Abdominal and Intermuscular Fat and Increases Insulin Sensitivity in Adults at Risk of Type 2 Diabetes.* The Journal of Nutrition, 2015. **145**(1): p. 177S-183S.

13. Chiavaroli, L., et al., *Fructose in obesity and cognitive decline: is it the fructose or the excess energy?* Nutrition journal, 2014. **13**(1): p. 27.

14. Forshee, R.A., et al., *A critical examination of the evidence relating high fructose corn syrup and weight gain.* Critical reviews in food science and nutrition, 2007. **47**(6): p. 561-582.

15. Nakagawa, T., et al., *Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome.* Nat Clin Pract Neph, 2005. **1**(2): p. 80-86.

16. Bray, G. and B. Popkin, *Calorie‐sweetened beverages and fructose: what have we learned 10 years later*. Pediatric obesity, 2013. **8**(4): p. 242-248.

17. Stanhope, K.L., *Role of fructose-containing sugars in the epidemics of obesity and metabolic syndrome.* Annual review of medicine, 2012. **63**: p. 329-343.

18. Vos, M.B., et al., *Dietary Fructose Consumption Among US Children and Adults: The Third National Health and Nutrition Examination Survey.* The Medscape Journal of Medicine, 2008. **10**(7): p. 160-160.

19. Mellor, K.M., et al., *Myocardial autophagy activation and suppressed survival signaling is associated with insulin resistance in fructose-fed mice.* Journal of Molecular and Cellular Cardiology, 2011. **50**(6): p. 1035-1043.

20. Gao, H., et al., *Treatment with ginger ameliorates fructose-induced Fatty liver and hypertriglyceridemia in rats: modulation of the hepatic carbohydrate response element-binding protein-mediated pathway.* Evidence-Based Complementary and Alternative Medicine, 2012. **2012**.

21. Sánchez-Lozada, L.G., et al., *Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats.* American Journal of Physiology-Renal Physiology, 2007. **292**(1): p. F423-F429.

22. Iannelli, A., et al., *Inflammation, insulin resistance, lipid disturbances, anthropometrics, and metabolic syndrome in morbidly obese patients: a case control study comparing laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy.* Surgery, 2011. **149**(3): p. 364-370.

23. Nakagawa, T., et al., *A causal role for uric acid in fructose-induced metabolic syndrome.* American Journal of Physiology-Renal Physiology, 2006. **290**(3): p. F625-F631.

24. Blevins, J.E., et al., *Chronic oxytocin administration inhibits food intake, increases energy expenditure, and produces weight loss in fructose-fed obese rhesus monkeys.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2014: p. ajpregu. 00441.2014.

25. Xu, X., et al., *Increased CYP2J3 Expression Reduces Insulin Resistance in Fructose-Treated Rats and db/db Mice.* Diabetes, 2010. **59**(4): p. 997-1005.

26. Page, K.A., et al., *Effects of Fructose vs Glucose on Regional Cerebral Blood Flow in Brain Regions Involved With Appetite and Reward PathwaysFructose Consumption and Weight Gain.* JAMA, 2013. **309**(1): p. 63-70.

27. Erlanson-Albertsson, C. and A. Lindqvist, *Fructose affects enzymes involved in the synthesis and degradation of hypothalamic endocannabinoids.* Regulatory peptides, 2010. **161**(1): p. 87-91.

28. Gersch, M.S., et al., *Fructose, but not dextrose, accelerates the progression of chronic kidney disease.* American Journal of Physiology-Renal Physiology, 2007. **293**(4): p. F1256-F1261.

29. Niewoehner, C.B., et al., *Metabolic effects of oral fructose in the liver of fasted rats.* Am. J. Physiol, 1984. **247**: p. E505-E512.

30. He, K., et al., *Changes in intake of fruits and vegetables in relation to risk of obesity and weight gain among middle-aged women.* Int J Obes Relat Metab Disord, 2004. **28**(12): p. 1569-1574.

31. Epstein, L.H., et al., *Increasing Fruit and Vegetable Intake and Decreasing Fat and Sugar Intake in Families at Risk for Childhood Obesity.* Obesity Research, 2001. **9**(3): p. 171-178.

32. DiNicolantonio, J.J. and S.C. Lucan, *The wrong white crystals: not salt but sugar as aetiological in hypertension and cardiometabolic disease.* Open Heart, 2014. **1**(1): p. e000167.

33. van Buul, V.J., L. Tappy, and F.J. Brouns, *Misconceptions about fructose-containing sugars and their role in the obesity epidemic.* Nutrition research reviews, 2014: p. 1-12.

34. Varady, K.A., et al., *Effects of weight loss via high fat vs. low fat alternate day fasting diets on free fatty acid profiles.* Scientific reports, 2015. **5**.

35. Gaby, A.R., *Adverse effects of dietary fructose.* Alternative medicine review, 2005. **10**(4): p. 294-306.

36. Niewoehner, C.B., *Metabolic effects of dietary versus parenteral fructose.* Journal of the American College of Nutrition, 1986. **5**(5): p. 443-450.

37. Lê, K.-A., et al., *A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans.* The American journal of clinical nutrition, 2006. **84**(6): p. 1374-1379.

38. Ventura, E.E., J.N. Davis, and M.I. Goran, *Sugar content of popular sweetened beverages based on objective laboratory analysis: focus on fructose content.* Obesity, 2011. **19**(4): p. 868-874.

39. Walker, R.W., K.A. Dumke, and M.I. Goran, *Fructose content in popular beverages made with and without high-fructose corn syrup.* Nutrition, 2014. **30**: p. 928–935.

40. Ackroff, K. and A. Sclafani, *Rats' preferences for high fructose corn syrup vs. sucrose and sugar mixtures.* Physiology & Behavior, 2011. **102**(5): p. 548-552.

41. Rumessen, J.J., *Fructose and related food carbohydrates: sources, intake, absorption, and clinical implications.* Scandinavian journal of gastroenterology, 1992. **27**(10): p. 819-828.

42. Le, M.T., et al., *Effects of high-fructose corn syrup and sucrose on the pharmacokinetics of fructose and acute metabolic and hemodynamic responses in healthy subjects.* Metabolism, 2012. **61**(5): p. 641-651.

43. Sánchez-Lozada, L.G., et al., *Comparison of free fructose and glucose to sucrose in the ability to cause fatty liver.* European journal of nutrition, 2010. **49**(1): p. 1-9.

44. Suez, J., et al., *Artificial sweeteners induce glucose intolerance by altering the gut microbiota.* Nature, 2014. **514**(7521): p. 181-186.

45. Castonguay, T.W., E. Hirsch, and G. Collier, *Palatability of sugar solutions and dietary selection?* Physiology & Behavior, 1981. **27**(1): p. 7-12.

46. Thorburn, A.W., et al., *Fructose-induced in vivo insulin resistance and elevated plasma triglyceride levels in rats.* The American Journal of Clinical Nutrition, 1989. **49**(6): p. 1155-1163.

47. Soto, M., et al., *Intermittent access to liquid sucrose differentially modulates energy intake and related central pathways in control or high-fat fed mice.* Physiology & Behavior, 2014.

48. Bocarsly, M.E., et al., *High-fructose corn syrup causes characteristics of obesity in rats: Increased body weight, body fat and triglyceride levels.* Pharmacology Biochemistry and Behavior, 2010. **97**(1): p. 101-106.

49. Ishimoto, T., et al., *Opposing effects of fructokinase C and A isoforms on fructose-induced metabolic syndrome in mice.* Proceedings of the National Academy of Sciences, 2012. **109**(11): p. 4320-4325.

50. Kawasaki, T., et al., *Long-term sucrose-drinking causes increased body weight and glucose intolerance in normal male rats.* British journal of nutrition, 2005. **93**(05): p. 613-618.

51. Schwartz, M.W., et al., *Central nervous system control of food intake.* Nature, 2000. **404**(6778): p. 661-671.

52. Morton, G.J., T.H. Meek, and M.W. Schwartz, *Neurobiology of food intake in health and disease.* Nature Reviews Neuroscience, 2014. **15**(6): p. 367-378.

53. Colley, D.L., *Effects of sugar solutions on hypothalamic appetite regulation*, 2013.

54. Shiota, M., et al., *Small amounts of fructose markedly augment net hepatic glucose uptake in the conscious dog.* Diabetes, 1998. **47**(6): p. 867-873.

55. SCHAFTINGEN, E. and A. VANDERCAMMEN, *Stimulation of glucose phosphorylation by fructose in isolated rat hepatocytes.* European Journal of Biochemistry, 1989. **179**(1): p. 173-177.

56. BERRY, M., *Long-term maintenance of low concentrations of fructose for the study of hepatic glucose phosphorylation.* Biochem. J, 1999. **337**: p. 497-501.

57. Petersen, K.F., et al., *Stimulating effects of low-dose fructose on insulin-stimulated hepatic glycogen synthesis in humans.* Diabetes, 2001. **50**(6): p. 1263-1268.

58. Niewoehner, C.B., et al., *Metabolic effects of oral fructose in the liver of fasted rats.* American Journal of Physiology-Endocrinology And Metabolism, 1984. **247**(4): p. E505-E512.

59. Niewoehner, C.B., B.Q. Nuttall, and F.Q. Nuttall, *Effects of graded intravenous doses of fructose on glycogen synthase in the liver of fasted rats.* Metabolism, 1987. **36**(4): p. 338-344.

60. Kinote, A., et al., *Fructose-induced hypothalamic AMPK activation stimulates hepatic PEPCK and gluconeogenesis due to increased corticosterone levels.* Endocrinology, 2012. **153**(8): p. 3633-3645.

61. Kelley, G.L., G. Allan, and S. Azhar, *High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation.* Endocrinology, 2004. **145**(2): p. 548-555.

62. London, E. and T.W. Castonguay, *High Fructose Diets Increase 11β‐Hydroxysteroid Dehydrogenase Type 1 in Liver and Visceral Adipose in Rats Within 24‐h Exposure.* Obesity, 2011. **19**(5): p. 925-932.

63. Colley, D.L. and T.W. Castonguay, *Effects of sugar solutions on hypothalamic appetite regulation.* Physiology & Behavior, 2014.

64. Tappy, L., et al., *Fructose and metabolic diseases: New findings, new questions.* Nutrition (Burbank, Los Angeles County, Calif.), 2010. **26**(11): p. 1044-1049.

65. Teff, K.L., et al., *Dietary Fructose Reduces Circulating Insulin and Leptin, Attenuates Postprandial Suppression of Ghrelin, and Increases Triglycerides in Women.* Journal of Clinical Endocrinology & Metabolism, 2004. **89**(6): p. 2963-2972.

66. Swarbrick, M.M., et al., *Consumption of fructose-sweetened beverages for 10 weeks increases postprandial triacylglycerol and apolipoprotein-B concentrations*

in overweight and obese women. British Journal of Nutrition, 2008. **100**(05): p. 947-952.

67. London, E. and T.W. Castonguay, *High Fructose Diets Increase 11β-Hydroxysteroid Dehydrogenase Type 1 in Liver and Visceral Adipose in Rats Within 24-h Exposure.* Obesity, 2011. **19**(5): p. 925-932.

68. Schultz, A., et al., *Hepatic Adverse Effects of Fructose Consumption Independent of Overweight/Obesity.* International Journal of Molecular Sciences, 2013. **14**(11): p. 21873-21886.

69. Zavaroni, I., Y.-D.I. Chen, and G.M. Reaven, *Studies of the mechanism of fructose-induced hypertriglyceridemia in the rat.* Metabolism, 1982. **31**(11): p. 1077-1083.

70. Campbell, E. and T.W. Castonguay, *Fructose Intake and Circulating Triglycerides: An Examination of the Role of APOC 3.* J Diabetes Obes 2014. **1**(1): p. 1-8.

71. Stanhope, K.L., et al., *Consumption of fructose and high fructose corn syrup increase postprandial triglycerides, LDL-cholesterol, and apolipoprotein-B in young men and women.* The Journal of Clinical Endocrinology & Metabolism, 2011. **96**(10): p. E1596-E1605.

72. Castonguay, T.W., M. Dallman, and J.S. Stern, *Corticosterone prevents body weight loss and diminished fat appetite following adrenalectomy.* Nutrition & Behavior, 1984.

73. Bista, B. and N. Beck, *Cushing Syndrome.* The Indian Journal of Pediatrics, 2013: p. 1-7.

74. Chapman, K., M. Holmes, and J. Seckl, *11β-Hydroxysteroid Dehydrogenases: Intracellular Gate-Keepers of Tissue Glucocorticoid Action.* Physiological Reviews, 2013. **93**(3): p. 1139-1206.

75. Jamieson, P.M., et al., *11 beta-hydroxysteroid dehydrogenase is an exclusive 11 beta-reductase in primary cultures of rat hepatocytes: effect of physicochemical and hormonal manipulations.* Endocrinology, 1995. **136**(11): p. 4754-4761.

76. Bujalska, I.J., et al., *A switch in dehydrogenase to reductase activity of 11β-hydroxysteroid dehydrogenase type 1 upon differentiation of human omental adipose stromal cells.* The Journal of Clinical Endocrinology & Metabolism, 2002. **87**(3): p. 1205-1210.

77. Stewart, P.M. and J.W. Tomlinson, *Cortisol, 11β-hydroxysteroid dehydrogenase type 1 and central obesity.* Trends in Endocrinology & Metabolism, 2002. **13**(3): p. 94-96.

78. Morton, N.M., et al., *Novel Adipose Tissue–Mediated Resistance to Diet-Induced Visceral Obesity in 11β-Hydroxysteroid Dehydrogenase Type 1–Deficient Mice.* Diabetes, 2004. **53**(4): p. 931-938.

79. Masuzaki, H., et al., *Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice.* The Journal of clinical investigation, 2003. **112**(1): p. 83-90.

80. Morton, N.M., et al., *Improved Lipid and Lipoprotein Profile, Hepatic Insulin Sensitivity, and Glucose Tolerance in 11β-Hydroxysteroid Dehydrogenase Type 1 Null Mice.* Journal of Biological Chemistry, 2001. **276**(44): p. 41293-41300.

81. Livingstone, D.E.W. and B.R. Walker, *Is 11β-Hydroxysteroid Dehydrogenase Type 1 a Therapeutic Target? Effects of Carbenoxolone in Lean and Obese Zucker Rats.* Journal of Pharmacology and Experimental Therapeutics, 2003. **305**(1): p. 167-172.

82. Holmes, M.C., et al., *11β-hydroxysteroid dehydrogenase type 1 expression is increased in the aged mouse hippocampus and parietal cortex and causes memory impairments.* The Journal of Neuroscience, 2010. **30**(20): p. 6916-6920.

83. Bursać, B.N., et al., *Fructose consumption enhances glucocorticoid action in rat visceral adipose tissue.* The Journal of nutritional biochemistry, 2013. **24**(6): p. 1166-1172.

84. Zhao, C., A.E. Tschiffely, and T.W. Castonguay, *Effect of sugars on mRNA expression of 11β-HSD1 in the hypothalamus of rats after 24 hour exposure (accepted).* Journal of Agriculture and Life Sciences, 2015.

85. Grill, H.J. and M.R. Hayes, *Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance.* Cell metabolism, 2012. **16**(3): p. 296-309.

86. Briggs, D.I. and Z.B. Andrews, *Metabolic Status Regulates Ghrelin Function on Energy Homeostasis.* Neuroendocrinology, 2011. **93**(1): p. 48-57.

87. Balthasar, N., et al., *Leptin Receptor Signaling in POMC Neurons Is Required for Normal Body Weight Homeostasis.* Neuron, 2004. **42**(6): p. 983-991.

88. Shi, H., et al., *The roles of leptin receptors on POMC neurons in the regulation of sex-specific energy homeostasis.* Physiology & Behavior, 2010. **100**(2): p. 165-172.

89. van de Wall, E., et al., *Collective and individual functions of leptin receptor modulated neurons controlling metabolism and ingestion.* Endocrinology, 2008. **149**(4): p. 1773-1785.

90. Ring, L.E. and L.M. Zeltser, *Disruption of hypothalamic leptin signaling in mice leads to early-onset obesity, but physiological adaptations in mature animals stabilize adiposity levels.* The Journal of Clinical Investigation, 2010. **120**(8): p. 2931-2941.

91. Hahn, T.M., et al., *Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons.* Nature neuroscience, 1998. **1**(4): p. 271-272.

92. Elias, C.F., et al., *Leptin Activates Hypothalamic CART Neurons Projecting to the Spinal Cord.* Neuron, 1998. **21**(6): p. 1375-1385.

93. Baskin, D.G., J.F. Breininger, and M.W. Schwartz, *Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus.* Diabetes, 1999. **48**(4): p. 828-833.

94. MARKS, J.L., et al., *Localization of insulin receptor mRNA in rat brain by in situ hybridization.* Endocrinology, 1990. **127**(6): p. 3234-3236.

95. Satoh, N., et al., *The arcuate nucleus as a primary site of satiety effect of leptin in rats.* Neuroscience Letters, 1997. **224**(3): p. 149-152.

96. Luckett, B.S., et al., *Arcuate nucleus injection of an anti-insulin affibody prevents the sympathetic response to insulin.* American Journal of Physiology-Heart and Circulatory Physiology, 2013. **304**(11): p. H1538-H1546.

97. Erickson, J.C., G. Hollopeter, and R.D. Palmiter, *Attenuation of the Obesity Syndrome of ob/ob Mice by the Loss of Neuropeptide Y.* Science, 1996. **274**(5293): p. 1704-1707.

98. Erickson, J.C., K.E. Clegg, and R.D. Palmiter, *Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y.* Nature, 1996. **381**(6581): p. 415-418.

99. Cone, R.D., et al., *The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation.* Recent progress in hormone research, 1996. **51**: p. 287-317; discussion 318.

100. Purnell, J.Q., et al., *Brain functional magnetic resonance imaging response to glucose and fructose infusions in humans.* Diabetes, Obesity and Metabolism, 2011. **13**(3): p. 229-234.

101. Stellar, E., *The physiology of motivation.* Psychological review, 1954. **61**(1): p. 5.

102. Weingarten, H.P., P. Chang, and T. McDonald, *Comparison of the metabolic and behavioral disturbances following paraventricular-and ventromedial-hypothalamic lesions.* Brain research bulletin, 1985. **14**(6): p. 551-559.

103. Page, K.A., et al., *EFfects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways.* JAMA, 2013. **309**(1): p. 63-70.

104. Kruse, M.S., et al., *Alterations of LXRα and LXRβ expression in the hypothalamus of glucose-intolerant rats.* Journal of Endocrinology, 2012. **215**(1): p. 51-58.

105. Burmeister, M.A., et al., *Central glucagon-like peptide 1 receptor-induced anorexia requires glucose metabolism-mediated suppression of AMPK and is impaired by central fructose.* American Journal of Physiology - Endocrinology and Metabolism, 2013. **304**(7): p. E677-E685.

106. Lindqvist, A., A. Baelemans, and C. Erlanson-Albertsson, *Effects of sucrose, glucose and fructose on peripheral and central appetite signals.* Regulatory peptides, 2008. **150**(1): p. 26-32.

107. Cha, S.H., et al., *Differential effects of central fructose and glucose on hypothalamic malonyl–CoA and food intake.* Proceedings of the National Academy of Sciences, 2008. **105**(44): p. 16871-16875.

108. Hu, Z., et al., *Hypothalamic malonyl-CoA as a mediator of feeding behavior*. Proceedings of the National Academy of Sciences, 2003. **100**(22): p. 12624-12629.

109. Cha, S.H. and M.D. Lane, *Central lactate metabolism suppresses food intake via the hypothalamic AMP kinase/malonyl-CoA signaling pathway.* Biochemical and Biophysical Research Communications, 2009. **386**(1): p. 212-216.

110. de Greef, W.J., et al., *Regulation of hypothalamic TRH production and release in the rat.* Acta medica Austriaca, 1992. **19 Suppl 1**: p. 77-79.

111. Schuhler, S., et al., *Thyrotrophin-Releasing Hormone Decreases Feeding and Increases Body Temperature, Activity and Oxygen Consumption in Siberian Hamsters.* Journal of neuroendocrinology, 2007. **19**(4): p. 239-249.

112. Jeanneteau, F.D., et al., *BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus.* Proceedings of the National Academy of Sciences, 2012. **109**(4): p. 1305-1310.

113. Xi, D., et al., *Ablation of Sim1 neurons causes obesity through hyperphagia and reduced energy expenditure.* PloS one, 2012. **7**(4): p. e36453.

114. Ludwig, M. and G. Leng, *Dendritic peptide release and peptide-dependent behaviours.* Nature Reviews Neuroscience, 2006. **7**(2): p. 126-136.

115. Trajkovic, M., et al., *Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8.* Journal of Clinical Investigation, 2007. **117**(3): p. 627-635.

116. LECHAN, R.M. and I.M. JACKSON, *Immunohistochemical Localization of Thyrotropin-Releasing Hormone in the Rat Hypothalamus and Pituitary*.* Endocrinology, 1982. **111**(1): p. 55-65.

117. Sawchenko, P., L. Swanson, and W. Vale, *Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat.* Proceedings of the National Academy of Sciences, 1984. **81**(6): p. 1883-1887.

118. Smith, S.M. and W.W. Vale, *The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress.* Dialogues in clinical neuroscience, 2006. **8**(4): p. 383.

119. Dallman, M.F., et al., *Minireview: glucocorticoids—food intake, abdominal obesity, and wealthy nations in 2004.* Endocrinology, 2004. **145**(6): p. 2633-2638.

120. Maes, M., et al., *Interleukin-1 beta: a putative mediator of HPA axis hyperactivity in major depression?* The American journal of psychiatry, 1993. **150**(8): p. 1189-1193.

121. Padgett, D.A. and R. Glaser, *How stress influences the immune response.* Trends in immunology, 2003. **24**(8): p. 444-448.

122. Reiche, E.M.V., S.O.V. Nunes, and H.K. Morimoto, *Stress, depression, the immune system, and cancer.* The lancet oncology, 2004. **5**(10): p. 617-625.

123. Levy, F., et al., *Intracerebral oxytocin is important for the onset of maternal behavior in inexperienced ewes delivered under peridural anesthesia.* Behavioral neuroscience, 1992. **106**(2): p. 427.

124. Brown, C., et al., *Physiological regulation of magnocellular neurosecretory cell activity: integration of intrinsic, local and afferent mechanisms.* Journal of neuroendocrinology, 2013. **25**(8): p. 678-710.

125. Arletti, R., A. Benelli, and A. Bertolini, *Oxytocin inhibits food and fluid intake in rats.* Physiology & behavior, 1990. **48**(6): p. 825-830.

126. Ott, V., et al., *Oxytocin reduces reward-driven food intake in humans.* Diabetes, 2013. **62**(10): p. 3418-3425.

127. Meyer, A., W. Langhans, and E. Scharrer, *Vasopressin reduces food intake in goats.* Experimental Physiology, 1989. **74**(4): p. 465-473.

128. Konturek, P., et al., *Neuro-hormonal control of food intake: basic mechanisms and clinical implications.* Journal of physiology and pharmacology: an official journal of the Polish Physiological Society, 2005. **56**: p. 5-25.

129. Benicky, J., et al., *Angiotensin II AT1 receptor blockade ameliorates brain inflammation.* Neuropsychopharmacology, 2011. **36**(4): p. 857-870.

130. Wang, Q., et al., *Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat.* Diabetes, 1997. **46**(3): p. 335-341.

131. Sahu, A., et al., *Neuropeptide Y release from the paraventricular nucleus increases in association with hyperphagia in streptozotocin-induced diabetic rats.* Endocrinology, 1992. **131**(6): p. 2979-2985.

132. Liposits, Z., C. Phelix, and W. Paull, *Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the*

hypothalamic paraventricular nucleus of the rat. Histochemistry, 1987. **86**(6): p. 541-549.

133. Palkovits, M., et al., *Norepinephrine and dopamine content of hypothalamic nuclei of the rat.* Brain research, 1974. **77**(1): p. 137-149.

134. Cook, C.B., et al., *Expression of thyroid hormone receptor beta 2 in rat hypothalamus.* Endocrinology, 1992. **130**(2): p. 1077-1079.

135. Meyer, A., W. Langhans, and E. Scharrer, *Vasopressin reduces food intake in goats.* Quarterly Journal of Experimental Physiology, 1989. **74**(4): p. 465-473.

136. Langhans, W., E. Delprete, and E. Scharrer, *Mechanisms of vasopressin's anorectic effect.* Physiology & Behavior, 1991. **49**(1): p. 169-176.

137. Stomby, A., et al., *Tissue-specific dysregulation of cortisol regeneration by 11βHSD1 in obesity: has it promised too much?* Diabetologia, 2014. **57**(6): p. 1100-1110.

138. Clément-Ziza, M., et al., *Stabilization of RNA during laser capture microdissection by performing experiments under argon atmosphere or using ethanol as a solvent in staining solutions.* Rna, 2008. **14**(12): p. 2698-2704.

139. Colley, D.L. and T.W. Castonguay, *Effects of sugar solutions on hypothalamic appetite regulation (in press).* Physiology & Behavior, 2014.

140. Balsalobre, A., et al., *Resetting of Circadian Time in Peripheral Tissues by Glucocorticoid Signaling.* Science, 2000. **289**(5488): p. 2344-2347.

141. Fuller, P.J., J.A. Clements, and J.W. Funder, *Localization of Arginine Vasopressin-Neurophysin II Messenger Ribonucleic Acid in the Hypothalamus of Control and Brattleboro Rats by Hybridization Histochemistry with a Synthetic Pentadecamer Oligonucleotide Probe.* Endocrinology, 1985. **116**(6): p. 2366-2368.

142. Weiser, M.J., C. Osterlund, and R.L. Spencer, *Inhibitory effects of corticosterone in the hypothalamic paraventricular nucleus (PVN) on stress-induced ACTH secretion and gene expression in the PVN and anterior pituitary.* J Neuroendocrinol., 2011. **23**(12): p. 1231–1240.

143. Cho, E.S., et al., *Organotypic slice culture of the hypothalamic paraventricular nucleus of rat.* Journal of veterinary science, 2007. **8**(1): p. 15-20.

144. Abramov, A., et al., *Changes in the cholecystokinin-synthesizing system of the hypothalamus in experimental diabetes mellitus in rats.* Neuroscience and behavioral physiology, 1999. **29**(6): p. 621-624.

145. Cockcroft, J.R., et al., *Haemodynamic effects of adrenomedullin in human resistance and capacitance vessels.* British journal of clinical pharmacology, 1997. **44**(1): p. 57-60.

146. Yoshizato, H., et al., *The growth hormone (GH) gene is expressed in the lateral hypothalamus: enhancement by GH-releasing hormone and repression by restraint stress.* Endocrinology, 1998. **139**(5): p. 2545-2551.

147. Moisan, M.-P., J.R. SECKL, and C.R. EDWARDS, *1lβ-Hydroxysteroid Dehydrogenase Bioactivity and Messenger RNA Expression in Rat Forebrain: Localization in Hypothalamus, Hippocampus, and Cortex*.* Endocrinology, 1990. **127**(3): p. 1450-1455.

148. Bisschop, P.H., et al., *Expression of 11β-Hydroxysteroid Dehydrogenase Type 1 in the Human Hypothalamus.* Journal of neuroendocrinology, 2013. **25**(5): p. 425-432.

149. Schlussman, S., et al., *Regional mRNA expression of the endogenous opioid and dopaminergic systems in brains of C57BL/6J and 129P3/J mice: strain and heroin effects.* Pharmacology Biochemistry and Behavior, 2011. **100**(1): p. 8-16.

150. Zhao, C., et al., *Overnight Access to Sugar Solutions Affects mRNA Expression of Several Neuropeptides in Different Hypothalamic Regions in Rats.* Journal of Food and Nutrition Research, 2015. **3**(1): p. 69-76.

151. Kanarek, R.B. and N. Orthen-Gambill, *Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats.* The Journal of nutrition, 1982. **112**(8): p. 1546-1554.

152. Campbell, E.S. and T.W. Castonguay, *Fructose intake and circulating triglycerides: an examination of the roles of APOC 3 and FOXO1.* The FASEB Journal, 2013. **27**: p. 1074.8.

153. Yuan, G., K.Z. Al-Shali, and R.A. Hegele, *Hypertriglyceridemia: its etiology, effects and treatment.* Canadian Medical Association Journal, 2007. **176**(8): p. 1113-1120.

154. Kotelevtsev, Y., et al., *11β-Hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress.* Proceedings of the National Academy of Sciences, 1997. **94**(26): p. 14924-14929.

155. Masuzaki, H. and J. Flier, *Tissue-specific glucocorticoid reactivating enzyme, 11 beta-hydroxysteroid dehydrogenase type 1 (11 beta-HSD1)--a promising drug target for the treatment of metabolic syndrome.* Current drug targets. Immune, endocrine and metabolic disorders, 2003. **3**(4): p. 255-262.

156. Masuzaki, H., et al., *A transgenic model of visceral obesity and the metabolic syndrome.* Science, 2001. **294**(5549): p. 2166-2170.

157. Kraly, F.S., et al., *Effect of cholecystokinin on meal size and intermeal interval in the sham-feeding rat.* Journal of comparative and physiological psychology, 1978. **92**(4): p. 697.

158. Schwartz, G.J., et al., *Decreased responsiveness to dietary fat in Otsuka Long-Evans Tokushima fatty rats lacking CCK-A receptors.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1999. **277**(4): p. R1144-R1151.

159. Chen, H., S. Kent, and M.J. Morris, *Is the CCK2 receptor essential for normal regulation of body weight and adiposity?* European Journal of Neuroscience, 2006. **24**(5): p. 1427-1433.

160. Weiland, T.J., N.J. Voudouris, and S. Kent, *The role of CCK_2 receptors in energy homeostasis: insights from the CCK_2 receptor-deficient mouse.* Physiology & Behavior, 2004. **82**(2): p. 471-476.

161. Chen, J., et al., *Characterization of the feeding inhibition and neural activation produced by dorsomedial hypothalamic cholecystokinin administration.* Neuroscience, 2008. **152**(1): p. 178-188.

162. Zhu, G., et al., *Roles of dorsomedial hypothalamic cholecystokinin signaling in the controls of meal patterns and glucose homeostasis.* Physiology & Behavior, 2012. **105**(2): p. 234-241.

163. Tebbe, J., et al., *Cholecystokinin (CCK) microinfused into the paraventricular nucleus of the hypothalamus (PVN) inhibits gastric emptying and stimulates colonic motor activity in the conscious rat.* Gastroenterology, 1998. **114**: p. A1184-A1185.

164. De Fanti, B.A., et al., *Lean (Fa/Fa) but not obese (fa/fa) Zucker rats release cholecystokinin at PVN after a gavaged meal.* American Journal of Physiology-Endocrinology and Metabolism, 1998. **275**(1): p. E1-E5.

165. Mahony, S. and M. Tisdale, *Induction of weight loss and metabolic alterations by human recombinant tumour necrosis factor.* British journal of cancer, 1988. **58**(3): p. 345.

166. Romanatto, T., et al., *TNF-α acts in the hypothalamus inhibiting food intake and increasing the respiratory quotient—effects on leptin and insulin signaling pathways.* Peptides, 2007. **28**(5): p. 1050-1058.

167. De Souza, C.T., et al., *Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus.* Endocrinology, 2005. **146**(10): p. 4192-4199.

168. Swaroop, J.J., D. Rajarajeswari, and J. Naidu, *Association of TNF-α with insulin resistance in type 2 diabetes mellitus.* The Indian journal of medical research, 2012. **135**(1): p. 127.

169. Wang, X., et al., *Increased hypothalamic inflammation associated with the susceptibility to obesity in rats exposed to high-fat diet.* Experimental diabetes research, 2012. **2012**.

170. Jørgensen, J.O.L., et al., *Fuel metabolism, energy expenditure, and thyroid function in growth hormone-treated obese women: A double-blind placebo-controlled study.* Metabolism, 1994. **43**(7): p. 872-877.

171. Sakharova, A.A., et al., *Role of growth hormone in regulating lipolysis, proteolysis, and hepatic glucose production during fasting.* The Journal of Clinical Endocrinology & Metabolism, 2008. **93**(7): p. 2755-2759.

172. Gahete, M.D., et al., *The rise in growth hormone during starvation does not serve to maintain glucose levels or lean mass but is required for appropriate adipose tissue response in female mice.* Endocrinology, 2012. **154**(1): p. 263-269.

173. Rosen, T., et al., *Increased body fat mass and decreased extracellular fluid volume in adults with growth hormone deficiency.* Clinical Endocrinology, 1993. **38**(1): p. 63-71.

174. Coxam, V., M.-J. Davicco, and J.-P. Barlet, *Effect of triglycerides on growth hormone (GH)-releasing factor-mediated GH secretion in newborn calves.* Domestic animal endocrinology, 1989. **6**(4): p. 389-393.

175. Volkow, N.D., G.-J. Wang, and R.D. Baler, *Reward, dopamine and the control of food intake: implications for obesity.* Trends in cognitive sciences, 2011. **15**(1): p. 37-46.

176. Land, B.B., et al., *Medial prefrontal D1 dopamine neurons control food intake.* Nat Neurosci, 2014. **17**(2): p. 248-253.

177. Anderberg, R.H., et al., *Dopamine signaling in the amygdala, increased by food ingestion and GLP-1, regulates feeding behavior.* Physiology & behavior, 2014.

178. Fetissov, S.O., et al., *Expression of dopaminergic receptors in the hypothalamus of lean and obese Zucker rats and food intake.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2002. **283**(4): p. R905-R910.

179. Mayhew, M.S., *Amylin and Incretin Enhancers for Diabetes Type 2.* The Journal for Nurse Practitioners, 2010. **6**(7): p. 551-552.

180. Lutz, T.A., *Control of energy homeostasis by amylin.* Cellular and molecular life sciences, 2012. **69**(12): p. 1947-1965.

181. Fietta, P., P. Fietta, and G. Delsante, *Central nervous system effects of natural and synthetic glucocorticoids.* Psychiatry and clinical neurosciences, 2009. **63**(5): p. 613-622.

182. Green, P.K., C.W. Wilkinson, and S.C. Woods, *Intraventricular corticosterone increases the rate of body weight gain in underweight adrenalectomized rats.* Endocrinology, 1992. **130**(1): p. 269-275.

183. Verma, M., et al., *Decreased brain 11β-HSD1 expression following inflammation; a role in regulating brain energy homeostasis?* Endocrine Abstracts, 2014. **34**: p. 243.

184. Fekete, C., et al., *α-Melanocyte-stimulating hormone is contained in nerve terminals innervating thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus and prevents fasting-induced suppression of prothyrotropin-releasing hormone gene expression.* The Journal of Neuroscience, 2000. **20**(4): p. 1550-1558.

185. Schwartz, M.W., *Central nervous system of food intake.* Nature, 2000. **404**: p. 661-671.

186. Benoit, S.C., et al., *Insulin and leptin as adiposity signals.* Recent progress in hormone research, 2004. **59**: p. 267-286.

187. Sipols, A.J., D.G. Baskin, and M.W. Schwartz, *Effect of Intracerebroventricular Insulin Infusion on Diabetic Hyperphagia and Hypothalamic Neuropeptide Gene Expression.* Diabetes, 1995. **44**(2): p. 147-151.

188. Brüning, J.C., et al., *Role of brain insulin receptor in control of body weight and reproduction.* Science, 2000. **289**(5487): p. 2122-2125.

189. Schwartz, M.W., et al., *Specificity of Leptin Action on Elevated Blood Glucose Levels and Hypothalamic Neuropeptide Y Gene Expression in ob/ob Mice.* Diabetes, 1996. **45**(4): p. 531-535.

190. HILL, et al., *Acute effects of leptin require P13K signaling in hypothalamic proopiomelanocortin neurons in mice*. Vol. 118. 2008, Ann Arbor, MI, ETATS-UNIS: American Society for Clinical Investigation. 10.

191. Sindelar, D.K., et al., *Low plasma leptin levels contribute to diabetic hyperphagia in rats.* Diabetes, 1999. **48**(6): p. 1275-1280.

192. West, D.B., D. Fey, and S.C. Woods, *Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats.* Am J Physiol, 1984. **246**(5 Pt 2): p. R776-R787.

193. Mehran, A.E., et al., *Hyperinsulinemia drives diet-induced obesity independently of brain insulin production.* Cell metabolism, 2012. **16**(6): p. 723-737.

194. Suga, A., et al., *Effects of fructose and glucose on plasma leptin, insulin, and insulin resistance in lean and VMH-lesioned obese rats*. Vol. 278. 2000. E677-E683.

195. Hwang, I.-S., et al., *Fructose-induced insulin resistance and hypertension in rats.* Hypertension, 1987. **10**(5): p. 512-516.

196. Aeberli, I., et al., *Moderate Amounts of Fructose Consumption Impair Insulin Sensitivity in Healthy Young Men A randomized controlled trial.* Diabetes Care, 2013. **36**(1): p. 150-156.

197. Faeh, D., et al., *Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men.* Diabetes, 2005. **54**(7): p. 1907-1913.

198. Lê, K.-A., et al., *Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes.* The American journal of clinical nutrition, 2009. **89**(6): p. 1760-1765.

199. Hallfrisch, J., et al., *Insulin and glucose responses in rats fed sucrose or starch.* The American journal of clinical nutrition, 1979. **32**(4): p. 787-793.

200. Holzl, B., et al., *Hypertriglyceridemia and insulin resistance.* Journal of internal medicine, 1998. **243**(1): p. 79-82.

201. Huang, B.W., et al., *The effect of high-fat and high-fructose diets on glucose tolerance and plasma lipid and leptin levels in rats.* Diabetes, Obesity and Metabolism, 2004. **6**(2): p. 120-126.

202. Shapiro, A., et al., *Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2008. **295**(5): p. R1370-R1375.

203. Campfield, L.A., et al., *Recombinant Mouse OB Protein: Evidence for a Peripheral Signal Linking Adiposity and Central Neural Networks.* Science, 1995. **269**(5223): p. 546-549.

204. Mantzoros, C.S., et al., *Severe Leptin Resistance in Brown Fat-Deficient Uncoupling Protein Promoter-Driven Diphtheria Toxin A Mice Despite Suppression of Hypothalamic Neuropeptide Y and Circulating Corticosterone Concentrations.* Diabetes, 1998. **47**(2): p. 230-238.

205. Marsh, D.J., et al., *Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides.* Nature genetics, 1999. **21**(1): p. 119-122.

206. Wilson, B.D., et al., *Physiological and Anatomical Circuitry between Agouti-Related Protein and Leptin Signaling.* Endocrinology, 1999. **140**(5): p. 2387-2397.

207. Caro, J.F., et al., *Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance.* The Lancet, 1996. **348**(9021): p. 159-161.

208. Bjørbæk, C., et al., *Identification of SOCS-3 as a Potential Mediator of Central Leptin Resistance.* Molecular cell, 1998. **1**(4): p. 619-625.

209. Myers Jr, M.G., et al., *Defining Clinical Leptin Resistance-Challenges and Opportunities.* Cell metabolism, 2012. **15**(2): p. 150.

210. White, J.S., *Challenging the Fructose Hypothesis: New Perspectives on Fructose Consumption and Metabolism.* Advances in Nutrition: An International Review Journal, 2013. **4**(2): p. 246-256.

CURRICULUM VITAE

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EDUCATION:	BS in Food Science and Engineering: Harbin Institute of Technology, 2009
	Master student in Food Science: Harbin Institute of Technology, 2010
	Doctorate student in Veterinary Medicine Science: Mississippi State University, 2012
	Ph.D in Nutrition and Food Science: University of Maryland, 2015
EXPERIENCE:	Teaching assistant, Department of Nutrition and Food Science, University of Maryland, August 2012 - January

2013, August 2013 - January 2014, August 2014 - June 2015.

Research assistant, Department of Basic Science, College of Veterinary Medicine, Mississippi State University, August 2010- January 2012.

Teaching assistant, English summer camp, Harbin New Oriental School, June 2008 – July 2008.

RESEARCH ACTIVITIES

a. Books

i. Chapters in Books:

1. 2015 Zhao, C. and T.W. Castonguay. Selenium Binding Protein 1: A Moonlighting Protein. In: Foods in Dietary Supplements in the Prevention and Treatment of Disease in Older Adults. R. Watson, (ed.), Elsevier Press, New York.

b. Peer-reviewed Journal Articles:

1. 2015 Zhao, C. and T. W. Castonguay. Effects of Free Access to Sugar Sweetened Drinks on Energy Control. (Submitted)

2. 2015 Schlappal, A.E., C. Zhao, E.S. Campbell, and T.W. Castonguay. Fructose increases liver 11 beta-hydroxysteroid dehydrogenase-1 mRNA within 24h. (Submitted).

3. 2015 Zhang Y., X. Qi, J. Zheng, Y. Luo, C. Zhao, J. Hao, X. Li, K. Huang and W. Xu. Lipid rafts disruption increases ochratoxin A cytotoxicity to hepatocytes. (Submitted)

4. 2015 Zhao, C., Tschiffely, A.E., and T.W. Castonguay. Effect of sugars on mRNA expression of 11β-HSD1 in the hypothalamus of rats after 24 hour exposure. Journal of Agriculture and Life Sciences. (Accepted)

5. 2015 Zhao, C., E. S. Campbell, A.E. Tschiffely and T.W. Castonguay. Overnight access to sugar solutions affects mRNA expression of several neuropeptides in different hypothalamic regions in rats. Journal of Food and Nutrition Research. 3(1): 69-76.

6. 2015 Guo, M., S. Ding, C. Zhao, X. Gu, X. He, K. Huang, Y. Luo, Z. Liang, H. Tian, W. Xu. Red Ginseng and Semen Coicis can improve the structure of gut microbiota and relieve the symptoms of ulcerative colitis. Journal of Ethnopharmacology. 162: 7-13.

7. 2015 Liang, R., X. Shen, B. Zhang, Y. Li, W. Xu, C. Zhao, Y. Luo, K. Huang. Apoptosis Signal-regulating Kinase 1 promotes Ochratoxin A-induced renal cytotoxicity. Scientific Reports. 5.

8. 2014 Guo, M., S. Ding, C. Zhao, X. Gu, X. He, K. Huang, Y. Luo, Z. Liang, H. Tian, W. Xu. Red Ginseng and Semen Coicis can improve the structure of gut microbiota and relieve the symptoms of ulcerative colitis. Journal of Ethnopharmacology. 162: 7–13.

9. 2014 Xia, K, X. He, Q. Dai, WH. Cheng, X. Qi, M. Guo, Y. Luo, K. Huang, C. Zhao and W. Xu. Discovery of systematic responses and potential biomarkers induced by ochratoxin A using metabolomics. Food Additives & Contaminants: Part A. 25:1-10.

10. 2014 Shen, X., B. Zhang, R Liang, WH. Cheng, W. Xu, Y. Luo, C. Zhao, K. Huang. Central role of Nix in the autophagic response to ochratoxin A. Food and Chemical Toxicology. 69: 202–209.

11. 2014 Zhang, B, X. Shen, R. Liang, Y. Li, K. Huang, C. Zhao, Y. Luo and W Xu. Protective role of the mitochondrial Lon protease 1 in ochratoxin A-induced cytotoxicity in HEK293 cells. Journal of Proteomics 101: 154–168.

12. 2010 He, S., Y. Ma, J. Wang, Q. Li, S. Tang, C. Zhao, H. Li and J. Maubois. Characterization of fat globules and milk fat globule membrane proteins in milk of different yak breeds. Dairy Sci. Technol. 90: 601–609.

13. 2010 Chen, L., Y. Ma, L. Chen, C. Zhao, J. Maubois, T. Jiang, H. Li and S. He. Antioxidant activity of two yeasts and their attenuation effect on 4-

Nitroquinoline 1-oxide induced in vitro lipid peroxidation. International Journal of Food Science and Technology 45: 555–561.

14. 2010 Zhao, C., Y. Li, D. He and W. Lu. The Research on the Constituents, Functions and Application of Maize Pollens. (In Chinese) Science and Technology of Food Industry 9: 414-416.

15. 2009 Zhao, C., D. Cheng, C. Luo, Z. Lu and D. Xu. Study and application of sugarbeet pigment. (In Chinese) Chinese Beet & Sugar 1: 29-31.

16. 2008 Yang, X., H. Zhang, C. Zhao. Y. Zhang, A. Dong and Y. Ma. Analysis of Volatile Constituents in Pinecone of Pinus sylvesris L.var.mongolica Litvin by Gas Chromatography/Mass Spectrometry. (In Chinese) Chinese Traditional Patent Medicine11: 1704-1707.

c. Articles in Preparation:

1. Zhao, C., R. T.Y, Wu, H. Zeng and WH Cheng. Loss of selenium binding protein 1 promotes resistance to clastogens in HeLa cells.

2. Geng, X., C. Zhao, Q. Li, Corn Bran Soluble Dietary Fibers Prevent Immunosuppression in Streptozotocin-induced Diabetic Mice.

ABSTRACTS/PAPERS PRESENTED AT PROFESSIONAL MEETINGS

1. 2015 Zhao, C., E. Campbell, A. Schlappal and T.W. Castonguay. Sugar and the regulation of hypothalamic anorectic peptides. Poster presented at 2015 NFSC Research Day, Beltsville, MD. USA. (Abstract submitted)

2. 2014 Zhao, C., E. Campbell, A. Schlappal and T.W. Castonguay. Sugar and the regulation of hypothalamic anorectic peptides. Poster presented at 2014 Neuroscience Conference, Washington, DC. USA.

3. 2014 Schlappal, A., C. Zhao and T.W. Castonguay. Sugar decreases 11 beta-hydroxysteroid dehydrogenase mRNA within 24h. Poster presented at 2014 Neuroscience Conference, Washington, DC. USA.

4. 2014 Changhui Zhao, Eric Campbell, Anna Schlappal and Thomas W. Castonguay. Effects of sugars on several hypothalamic neuropeptides. Poster presented at 2014 Mid-Atlantic Diabetes Research Symposium, Bethesda, MD. USA.

5. 2014 Zhao, C., E. Campbell, A. Schlappal and T.W. Castonguay. Opposing roles of glucose and fructose in two hypothalamic anorexigenic peptides. Poster presented at 2014 NFSC Research Day, Beltsville, MD. USA.

6. 2013 Zhao, C., WH. Cheng and H. Zeng. A new role of selenium binding protein 1 in the response to oxidative stress. Poster presented at 2013 NFSC Research Day, Beltsville, MD. USA.

7. 2011 Zhao, C. D. Russell and X. Wan. Competition between Co-infected Influenza A Viruses. Poster presented at American Society for Microbiology (ASM) conference, New Orleans, LA. USA.

GRANTS

a. Contracts and grants

2007-2010 Investigation on the anti-radioactive function of Maize Pollens. National College Students Creative Research Project Funding, Harbin Institute of Technology, Harbin, China; ¥20,000 (around $3,200) Role: co-PI.

SERVICE

a. Reviewing activities:

i. Served as a reviewer for the following peer-reviewed scientific journals:

Current diabetes reviews, Journal of Integrative Agriculture