ABSTRACT

Title of Document: EFFECTS OF SUGAR SOLUTIONS ON HYPOTHALAMIC APPETITE REGULATION

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There are multiple hypotheses for the causes of the obesity epidemic. One such hypothesis is that dietary intake patterns have significantly shifted to include unprecedented amounts of refined sugar. We set out to determine some the unique metabolic changes that occur with initial exposure to dilute glucose, sucrose, high fructose corn syrup, or fructose solutions. Rats were given access to food, water and a sugar solution for 24 h, after which blood and tissues were collected. Fructose access (as opposed to other sugars investigated) resulted in a doubling of circulating triglycerides. Glucose consumption resulted in upregulation of 7 satiety related hypothalamic peptides whereas changes in gene expression were mixed for remaining sugars. Also, following multiple verification assays, 6 satiety related peptides were verified as being affected by sugar intake. These data provide evidence that not all sugars are equally effective in affecting the control of intake.

EFFECTS OF SUGAR SOLUTIONS ON HYPOTHALAMIC APPETITE REGULATION

By

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Thank you to the love of my life Bob for all the compassion, support and understanding. Without you, I wouldn't have made it. You are my greatest cheerleader. I love you.

To Dr. C, thank you for being the world's greatest advisor! You have taught me so much I am so thankful for the impact you have made on my life. Thank you for EVERYTHING! "Stay the course."

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List of Abbreviations

ACTH.....Adrenocorticotropic hormone

ADIPOQ .....Adiponectin

ADRA2b ..... Adrenergic, alpha-2B-, receptor

ADX..... Adrenalectomized

AGRP..... Agouti-related peptide

ANOVA .....Analysis of variance

AP ..... Area postrema

APOA4.....Apolipoprotein A-IV

ARC .....Arcuate nucleus

BP.....Base pair

CCK ..... Cholecystokinin

Cdna ..... Complementary DNA

CHO .....Carbohydrate

CLPS ..... Colipase, pancreatic

CLR..... Calcitonin receptor-like receptor

CNS.....Central nervous system

CRH/CRF.....Corticotropin-releasing hormone/factor

CSF .....Cerebral spinal fluid

Ct.....Threshold Cycle

DRD1a .....Dopamine receptor D1A

GAPDH.....Glyceraldehyde 3-phosphate dehydrogenase

GH..... Growth hormone

GHS-R..... Growth hormone secretagogue receptor

GPCR ..... G protein-coupled receptor

HFCS.....High fructose corn syrup

IAPP ..... Islet amyloid polypeptide, Amylin

Il1a ..... Interleukin 1 alpha

Il6 .....Interleukin 6

INS2 ..... Insulin 2

IV .....Intravenous

KO.....Knock out

LDHA .....Lactate dehydrogenase A

MCH .....Melanin-concentrating hormone

mRNA.....Messenger ribonucleic acid

MSH..... Melanocyte-stimulating hormone

NAC .....Nucleus accumbens

NMU .....Neuromedin U

NPY.....Neuropeptide Y

NTRK1..... Neurotrophic tyrosine kinase, receptor, 1

NTS ..... Nucleus tractus solitarius

PCR ..... Polymerase chain reaction

POMC ..... Pro-opiomelanocortin

PPG ..... Preproglucagon

PVN.....Periventricular nucleus

RAMP2 ..... Receptor activity modifying protein 2

RAMP3 ..... Receptor activity modifying protein 3

RPLP1 .....Ribosomal protein, large, P1

RNA .....Ribonucleic acid

RT-PCR..... Real time polymerase chain reaction

Ta .....Annealing temperature

TaOpt .....Optimum annealing temperature

TCA..... Tricarboxylic acid

TG .....Triglyceride

TNF ..... Tumor necrosis factor

TRH/TRF .....Thyrotropin- releasing hormone/factor

UCN ..... Urocortin

Chapter 1: Literature Review

Introduction

Despite years of research, and millions of dollars spent every year in prevention and treatment, obesity has become the nation's most preventable health problem (1, 2). The current obesity epidemic is not attributable to any one factor, and is not restricted by race, nationality, age or gender (3). Despite its prevalence, there is no known cure for this disease. Several hypotheses about how obesity develops have been proposed that help guide research efforts.

One such hypothesis is that dietary patterns of Americans have significantly shifted over the past fifty years by including unprecedented amounts of refined sugar (4). Others have added that not all sugars are the same, and that fructose in particular accounts for much of the increase in sugar intake in the American diet (5, 6). From 1970 to 1990, consumption of high fructose corn syrup (HFCS) increased more than 1000% and currently accounts for 40% of all added caloric sweeteners (7, 8). Evidence that fructose is capable of promoting excessive weight gain has been reported in animal models and in humans. Figure 1 depicts the trends of overweight and obesity with the increase in fructose consumption in the US over the years. Rats maintained on a diet rich in HFCS for 6 or 7 months show abnormal weight gain, increased circulating triglycerides (TG) and augmented fat deposition (9). This hypothesis is not without its critics. Several groups have reported that when tested side-by-side, fructose is no more or less effective in promoting excess weight gain (10, 11).

It has long been established that the hypothalamus, particularly the arcuate nucleus, is the center of appetite regulation in the brain (12). Many researchers have

examined the role of peripheral hunger and satiety messages. However in the past 15 years exploration of the connection of the CNS to energy homeostasis has accelerated (13). This increase is due to increases in scientific technology including genetic assays as well as improvements in the study of neuroscience. In the past, ablation studies were the fundamental method for understanding how changes in the brain affect energy balance. In fact, these ablation studies are how it was originally established the hypothalamus is so important for appetite regulation (14).

For the purpose of this review, literature pertaining to appetite regulatory genes of the hypothalamus, particularly those impacted by sugar consumption, will be considered. Of course there are probably hundreds of genes in the hypothalamus that relate to energy balance in one form or another. Therefore, genes included in this review were selected based upon recommendations from the manufacturer of an obesity related PCR array that has been used in our laboratory (SABioscience, Frederick, MD). The PCR system used for this analysis was a rat obesity RT² Profiler™ PCR Array (PARN-017A). A PCR array is a multi-well plate that has been pre-loaded with specified primer pairs, in this case primers for genes relating to obesity. RNA and PCR reagents are added to the plate and using a thermal cycler, quantitative changes in gene expression are measured. The benefit of this system is it allows for analysis of multiple genes in a single experiment in a very short time. For the purpose of this study, gene expression in hypothalamic tissue of rats given 24 hour access to either glucose, sucrose, HFCS, or fructose was examined. From these data 'significant' changes in gene expression were identified. From that list the most

'significant' and interesting changes in gene expression were selected for inclusion in this review.

Amylin

Amylin, also known as Islet Amyloid Polypeptide (IAPP), is a 37 amino acid peptide hormone. Amylin reduces eating via meal size effect by promoting meal ending satiation. In the periphery it has been observed that amylin reduces gastric acid secretion, slows gastric emptying, and reduces pancreatic glucagon secretion (15). Pancreatic β-cells are the major source of circulating amylin and it is generally believed that the role of amylin in appetite regulation is as a result of secretion from β-cells (16).

Amylin acts centrally via action in the area postrema (AP), which is rich in amylin receptors. The presence of amylin synthesized centrally is still highly debated. According to Lutz (2011), there is currently no evidence for central amylin synthesis (17). One possible reason that amylin synthesis has not been identified centrally may be in part due to the fact that most studies thus far have focused on male rats. Dobolyi and colleagues have reported that IAPP was upregulated in the preoptic area of the hypothalamus in the early postpartum period. Utilizing micro array technology, RT-PCR and *in situ* hybridization for verification, amylin expression was found to be significantly increased in dams and mothers whose pups were removed immediately after delivery. These findings suggest that in female rats, central amylin synthesis may be important for maternal regulatory mechanisms (18).

Agouti Related Peptide

Agouti-related protein (AGRP) is a 132 amino acid obesogenic peptide produced in the ventromedial portion of the arcuate nucleus in the hypothalamus. AGRP and neuropeptide Y (NPY) are co-expressed and both are produced in AGRP/NPY neurons. AGRP is one of the most potent and long-lasting of appetite stimulators.

Release of AGRP and NPY from AGRP/NPY neurons is inhibited by insulin. By inactivating the insulin receptor on AGRP neurons, Konner and colleagues found that insulin failed to regulate hepatic glucose production (19). This result demonstrates the importance of AGRP in insulin-induced suppression of hepatic glucose production. These findings suggest that AGRP may be a new target for type 2 diabetes therapies.

Given that AGRP and NPY are involved in insulin regulation, it is not surprising that they are activated by sugar intake. Sucrose intake causes direct changes in NPY and AGRP expression in the ARC. Following a sucrose preload, AGRP and NPY mRNA expression was suppressed for the first 10 min following preload. During this interval there was also in increase in plasma and CSF glucose. In the subsequent interval (30-60 min) AGRP and NPY mRNA expression then increased (20).

Sucrose preloads increased intake in the subsequent meal as compared to a starch preload. Leibowitz and colleagues have suggested that sucrose causes increased intake and hunger following intake due to increased expression of orexogenic peptides NPY and AGRP (21). Gaysinskaya also suggests that NPY and

AGRP's orexegenic mechanisms are directed toward increased consumption of CHO where as other orexogenic peptides may act to increase intake of fats (20).

Cholecystokinin

Cholecystokinin (CCK) is an anorexogenic peptide that is expressed in both the small intestine and the brain. CCK was discovered in 1928 in a study examining the ability of intestinal extracts to stimulate gallbladder contraction in dogs. However, CCK was not purified and sequenced until 1966 by Jorpes and Mutt (22). CCK comes in many forms, denoted by size. For example, CCK-33, which was the first to be discovered, is a 33-amino acid peptide. The most common CCK form is CCK-8, which is an 8 amino acid peptide. All forms of CCK are derived from the same *CCK* gene by post translational or extracellular processing. Expression of CCK results in short duration reduction of meal size (23).

Despite much of the literature surrounding CCK being focused on its expression in the gut, CCK is one of the must abundantly expressed peptides in the brain (24). In the brain, CCK is expressed in the cerebellum, cortex, midbrain, hypothalamus and hippocampus (25).

Woods et al have theorized that CCK has two roles in satiation. First, CCK signals a reduction in meal size and second, CCK acts as a mediator of the satiation process (13). CCK is a mediator of the satiation process via many mechanisms including increasing hypothalamic permeability to leptin (26). Leptin is a signal for overall body adiposity status and CCK is a gatekeeper for this message entering the hypothalamic area (26).

It has been suggested that obesity, as a result of leptin resistance, may be due to impaired transport of leptin from the blood into the CNS (26). Rats that lack the CCK1 receptor are resistant to peripheral leptin but not leptin directly infused into the brain. To explore the connection between CCK and leptin, Cano et al administered leptin in the periphery to animals that had been given a CCK-1 receptor antagonist. They found that although leptin gradually decreased in the plasma, it simultaneous increased in concentration in the CSF (26). This finding suggests that leptin builds up in the periphery when CCK is blocked. Thus CCK increases leptin permeability into the CNS.

Insulin, in its role as another adiposity signal, is also linked to CCK expression. Lo (2011) using CCK-KO mice, found that when maintained on a low-fat diet animals had a reduced acute insulin response to glucose (27). However, when maintained on a high fat diet, CCK-KO mice developed glucose intolerance. The importance of this finding is it shows that CCK is required for regulating both insulin secretion and glucose tolerance in mice on a high fat diet.

Hisadome and colleagues found that an important role of CCK is to regulate neuronal circuitry (28). The aim of their study was to investigate the circuitry which links CCK to nucleus tractus solitarius- preproglucagon (NTS-PPG) neurons. PPG neurons express Glucagon-like peptide-1 which acts to modulate gastric emptying, glucose homeostasis, and appetite control. The researchers found that CCK does modulate the activity of PPG neurons however does so indirectly by acting through adrenergic neurons.

Corticotrophin Releasing Hormone

Corticotropin-releasing hormone (CRH), also known as Corticotropin-releasing factor (CRF) is a 41-amino acid anorectic peptide. CRF was first identified by Vale in 1981 (29). CRH is secreted by neurosecretory cells of the parvocellular region of the periventricular nucleus (PVN) of the hypothalamus and carried via the portal system to the anterior lobe of the pituitary where it promotes ACTH production (30). There are few studies relating CRH to diet and circulating nutrients. CRH has been found to have little response to increases in fat intake (21). Widmaier found that when glucose was removed from an extracted section of rat hypothalamus, there was in increase in CRF production that could be reversed by adding back glucose (31). This increase in CRF as a response to a lack of glucose may be to stimulate glucocorticoid release. Jang and colleagues found that in rats lacking glucocorticoids (via adrenalectomy), leptin administration rapidly increased CRH secretion (and decreased NPY secretion) (32).

Given the connection between CRF and glucose, it is no surprise that there is also a connection between CRH and insulin. When leptin is centrally injected, CRH mRNA expression in the PVN is increased. However, the opposite effect is observed when glucocorticoids are administered (32). This finding suggests that interactions between leptin and glucocorticoids regulate CRH.

The effect of sucrose on CRF expression has also been evaluated. Daily limited access to a sucrose sweetened drink resulted in decreased CRH mRNA expression (33). When adrenalectomized (ADX) rats were allowed to consume sucrose sweetened drinks, they experience normal weight gain and food intake and

have normal CRF levels throughout the brain (34). This finding suggests that CRF may be produced due to a drop in glucose in order to maintain levels of glucocorticoids.

Dopamine

Dopamine is catecholamine (biogenic amine) synthesized from tyrosine. Both are derived. All three catecholamines in this pathway (norepinephrine, epinephrine, and dopamine) function as neurotransmitters. Dopamine is released from the nucleus accumbens and the dorsal striatum in response to intake of palatable foods such as sugar (35). The hypothalamus has also been cited as a site of dopamine action in response to sucrose intake (36-39). Though dopamine has many actions in the brain, actions relating to intake will be the focus of this review.

Dopamine is probably not directly involved in huger and satiety mechanisms but rather influences food intake via reward mechanisms. When palatable foods are consumed, reward pathways, including the nigrostriatal dopaminergic pathway, are activated (40). Foods that are high in sugar and fat are known to activate the reward system and promote eating and prompt conditioning (41). Dopamine antagonists will attenuate this response and supress intake of sweets in rats (40, 42-44).

Overeating obesogenic diets blunts the dopamine signalling pathway. Increased consumption of sugars compensates for this blunted reward pathway (43). Pritchett and colleagues induced obesity in rats with a high fat/high CHO diet. Rats were given dopamine receptor antagonists IV. They found that lean animals

consumed less sugar solution then obese and thus obese animals had reduced dopamine receptor signalling (43).

Genetic differences in the dopamine gene have been shown to result in differences in acquisition of sugar preference. Dym et al. found that genetic differences between standard breeds of mice result in differences in the expression of the learned sucrose preference phenotype (45). Dym has attributed this to be a result of polymorphisms of taste receptors. Other researchers have found that newly discovered taste receptors in the gut may trigger dopamine responses upon exposure to sucrose (46). Similar reports of the impact of sugar on dopamine release have been reported by both Hoebel and Smith. Hoebel postulated that there may a connection with excessive sucrose intake and changes in the dopaminergic pathway in the hypothalamus (36). Smith and colleagues also reported similar conclusions- hypothalamic dopamine plays an integral role in the control of sucrose intake (37-39).

Growth Hormone

Growth hormone (GH) is a 191 amino acid long hormone. GH is released by the lateral wings of the anterior pituitary gland and regulated by neurosecretory nuclei of the hypothalamus. The *GH1* gene encodes the protein somatotropin. GH is thought to be an anorexogenic peptide that not only contributes to the regulation of energy expenditure but also growth. However, there is limited literature surrounding the effects of Growth Hormone (GH) on the regulation of energy balance and intake. Much of the literature surrounding GH is centered around the effects of ghrelin on GH modulation. Ghrelin is a potent orexogenic hormone that functions, in part, to

stimulate the release of GH via binding to growth hormone secretagogue receptor (GHS-R) (47, 48).

Several reports of the effects of GH on fasted or food limited animals have been published. During fasting and anorexia, insulin is suppressed and GH is elevated. The reason behind this elevation is thought to be due to GH antagonizing the actions of insulin so as to promote lipolysis and lipid oxidation (49). Gahete and colleagues examined this connection using female adult onset, isolated GH-deficient mice. These animals showed increased fat depot weight as compared to controls. This suggests that the importance of GH during fasting is more for the support of fat mobilization for gluconeogenesis and muscle function rather than for the maintenance of glucose levels (50).

Strauch and colleagues examined the effects of fructose on plasma GH levels in humans (51). They reported that in humans fructose, when administered IV at a dosage between 0.3 and 0.5 g/kg, is a potent stimulator of GH secretion. These results were in contrast to previous reports surrounding the effects of glucose on GH which suggested that glucose suppressed GH secretion.

Neuropeptide Y

Neuropeptide Y (NPY) is a 36 amino acid, obesogenic peptide that also acts as a neurotransmitter in the arcuate nucleus of hypothalamus. NPY is co-localized on neurons with AGRP (Agouti-related peptide). NPY has a dramatic effect on stimulating feeding (52). NPY/AGRP neurons inhibited (depolarized) by increased

levels of both insulin and leptin (53-55). When insulin and leptin levels in the plasma are low, NPY/AGRP expression is increased (32, 56).

There has also been extensive work done to evaluate the effects of carbohydrates on NPY expression. There is a strong NPY expression peak at the start of the natural feeding period that is thought to support the utilization of carbohydrate (21). In 1988, Rowland reported that animals given injections of NPY into the PVN there is a robust increase in feeding. This affect is then attenuated when an IV dose of glucose is given. The attenuation however does not occur when a dose of fructose is administered (57). This finding demonstrates that these two monosaccharide's have different effects on the regulation of this potent stimulator of intake. It is also significant because it shows that fructose does not have the same ability to inhibit NPY-induced feeding as does glucose.

Receptor Activity Modifying Protein 3

Receptor activity modifying protein 3 (RAMP3) is a G protein-coupled receptor (GPCR) accessory protein in the family of RAMP. RAMP proteins were originally identified in association with calcitonin receptor-like receptors (CLR). One function of RAMP3 is to interact with the calcitonin receptor to produce amylin receptor complexes. Once formed, these complexes allow binding of amylin to the receptor. Another function of RAMP3 is to complex with calcitonin receptor-like receptor (CLR) to form adrenomedullin receptors (15, 58). Because the RAMP family plays such an important role in the transport and modulation of GPCRs, there have been extensive pharmacological studies aimed at examining RAMPs as potential sites

for modulating GPCRs for the sake of disease treatment. Though much is known about the function of this protein, little has been reported on the specific impact of RAMP3 in the control of intake and energy balance.

To examine the life-long impact of RAMP3 deletions and the overlapping effects of RAMP proteins, Dackor and colleagues developed mouse strains with targeted RAMP deletions. Although mice with RAMP2 deletions had severely impaired fertility, RAMP3 deletions did not show any phenotypic differences from control until later in life. In late life, animals lacking the *RAMP3* gene had difficulty maintaining body weight however the researchers note that the mechanism behind this is not yet understood (59).

Tumor Necrosis Factor

Tumor Necrosis Factor-α (TNF-α) is a 212 amino acid inflammatory cytokine found in the hypothalamus. TNF plays in active role in inflammation and obesity and is involved in lipid metabolism regulation, adipocyte differentiation, and insulin sensitivity (60). TNF has been shown to interact with well-known satiety signals such as leptin. Huypens has proposed a model for TNF that suggests the gene has a role in the regulation of the inverse relationship between ADIPOQ and leptin (61).

In a study by Wang and colleagues rats were fed a high fat diet to induce obesity then were switched to a chow diet. Gene expression experiments revealed that many inflammatory cytokines, including TNF, were elevated following induction of obesity. After the switch to a regular chow diet however, the upregulation of these TNF did not return to normal. This suggests that it may not be possible to reduce the

inflammatory response that results from obesity (62). This is an important finding that may impact the way obesity therapies are designed.

Thyrotropin Releasing Hormone

Thyrotropin- releasing hormone (TRH), also called Thyrotropin- releasing factor (TRF), is a 242 amino acid hormone produced in the PVN of the hypothalamus that functions to stimulate the release of thyroid stimulating hormone and prolactin from the anterior pituitary. TRH is an anorectic hormone.

The mechanism of TRH action on appetite regulation is not yet fully understood (63). Zhang and van den Pol found that in addition to reducing intake, body weight and sleep, central injections of TRH also inhibited melanin-concentrating hormone (MCH) neurons. MCH neurons increase food intake (64). This finding may suggest that the reduction of food intake following TRH administration may be through TRH acting on MCH neurons (63).

Leptin has been found to have direct and indirect (through activating POMC neurons that in turn release αMSH to stimulate TRH) effects on stimulation of TRH synthesis (65). NPY also acts on TRH as an inhibitor through activation of NPY receptors on the TRH neurons (66).

Chapter 2: Effects of Sugar Solutions on Hypothalamic Appetite Regulation

Introduction

Despite years of research, and millions of dollars spent every year in prevention and treatment, obesity has become the nation's most preventable health problem (1, 2). The current obesity epidemic is not attributable to any one factor, and is not restricted by race, nationality, age or gender (3). Despite its prevalence, there is no known cure for this disease. Several hypotheses about how obesity develops have been proposed that help guide research efforts.

One such hypothesis is that dietary patterns of Americans have significantly shifted over the past fifty years by including unprecedented amounts of refined sugar (4). Others have added that not all sugars are the same, and that fructose in particular accounts for much of the increase in sugar intake in the American diet (5, 6). From 1970 to 1990, consumption of high fructose corn syrup (HFCS) increased more than 1000% and currently accounts for 40% of all added caloric sweeteners (7, 8). Evidence that fructose is capable of promoting excessive weight gain has been reported in animal models and in humans. Figure 1 depicts the trends of overweight and obesity with the increase in fructose consumption in the US over the years. Rats maintained on a diet rich in HFCS for 6 or 7 months show abnormal weight gain, increased circulating TG and augmented fat deposition (9). This hypothesis is not without its critics. Several groups have reported that when tested side-by-side, fructose is no more or less effective in promoting excess weight gain (10, 11).

However, the rise in circulating TG can be explained by the metabolism of fructose. Fructose is metabolized differently than glucose, the more common monosaccharide. These metabolic pathways are outlined in Figure 2. To summarize,

when glucose enters the cell there are multiple control points that regulate the conversion of glucose to fat (triglycerides). However these control points do not exist in the metabolism of fructose and thus fructose is readily metabolized into triglycerides.

In the laboratory, sugar-induced obesity can be studied by giving rats access to any of a wide variety of sugar solutions, from which they will consume approximately 60% of their total daily caloric intake from the solution, despite differences in concentration and sweetness (12-14). Long term access to sucrose, glucose, and fructose promote increased weight gain and a shift in body composition favoring increased fat deposition (9). Despite the extensive behavioral examinations of the rat's avidity for sugar solutions, relatively little work has been focused on the impact of sugar intake on the hepatic and central mechanisms controlling intake. Exceptions to this characterization are the work of Erlanson-Albertsson and colleagues, who have demonstrated that fructose can upregulate fatty acid amide hydrolase, an enzyme involved in the degradation of hypothalamic endocannabinoids, as well as other enzymes involved in the synthesis of endocannabinoids (67). Hoebel and colleagues have reported on the pronounced effects of sugar solutions on the release of dopamine in the nucleus accumbens (NAc), the brain's so-called "reward center" (68, 69). In addition to dopamine release, this group has demonstrated that sugar consumption can alter receptor gene expression in reward areas of the brain.

We have more recently added to this literature by reporting that fructose consumption can promote increased oxoreductase activity of 11 beta hydroxysteroid

dehydrogenase -1, an enzyme that regulates intracellular glucocorticoids in adipose tissue (70, 71).

The purpose of the present experiment was to directly compare the efficacy of fructose with high fructose corn syrup. To do this, we used a PCR array system which evaluates 86 different obesity related genes on hypothalamic tissue. Once genes with significant changes were identified, we verified the array findings using traditional RT-PCR using primers that were designed in house (pooled verification). To further verify these findings gene expression in individual animals was evaluated with these primers as well (individual verification). It was our intention to establish a sound collection of evidence from these three approaches to determine the effects of sugars on hypothalamic, appetite-regulatory, gene expression.

Materials and Methods

Animals

Adult male Sprague-Dawley (CD strain) rats (Charles River Laboratories, Wilmington, MA) with a mean weight of approximately 300 grams were used. Upon arrival, all animals were individually housed and maintained on a 12h light/dark cycle with a room temperature of 22° C ± 1° C. During the 1 wk acclimation period the rats were given free access to the control diet and water. Animals were weighed and 24h food intake and sugar solution intake (when appropriate) were measured daily at 0900h throughout the experiment.

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 IACUC guidelines.

Animal Diets

The control diet used in these experiments is based upon one previously used in an experiment that manipulated quality and quantity of fat in diet-induced obesity studies with rats (72). The diet is a nutritionally complete low fat diet [Rodent diet 7012] prepared by Harlan Teklad (Bethlehem, PA) and provides 3.41 metabolizable kcal/g of diet. Per gram of chow, 2.14 kcal were derived from carbohydrate, 0.79 kcal was derived from protein, and 0.51 kcal was derived from fat. All animals were given free access to water throughout the experiment.

Study Design

Rats (N=44) were randomly assigned to one of five weight-matched groups (n=8, control n=12) after an initial 1 wk acclimation period during which they had ad lib access to food and water. The rats assigned to the first group had ad libitum control diet (Harlan rodent diet 7012) only, and served as the control group. Rats assigned to the second group had ad libitum access to the control diet and free access to a 16% weight/volume fructose (Tate & Lyle, Decatur IL) solution. Rats assigned to the third group had ad libitum access to the control diet plus free access to a 16% glucose weight/volume (Sigma Aldrich, St Louis MO) solution. Rats assigned to the fourth group had ad libitum access to the control diet and free access to 16% high fructose corn syrup (HFCS) weight/volume (Tate & Lyle, Decatur IL), Finally, rats assigned to the fifth group had ad libitum access to the control diet and free access to a 16% weight/volume sucrose (Domino Foods, Baltimore MD) solution. All sugar solutions were prepared 24h in advance and stored at 4°C. The rats were maintained on their respective diets for 24h and then sacrificed. At the time of sacrifice, livers (lobus lateralis sinister) were dissected and flash frozen for storage at -80°C. Brains were removed and flash frozen at -80°C for subsequent dissection. Frozen brains were sectioned using an IEC Minot Custom Microtome (Damon/IEC Division) and 8-9, 80-100 micron-thick consecutive tissue slices were sectioned from the hypothalamic region starting at the interaural line +6.44 mm. Figure 3 shows the hypothalamic region of interest.

Plasma Measures

Plasma insulin concentrations were measured by ELISA (LINCOplex; LINCO Research). Plasma glucose concentrations were measured enzymatically (Smith-Kline Beecham Laboratories). All reactions were run in duplicate.

The Dimension clinical chemistry system Flex reagent cartridge (Siemens Healthcare Diagnostics, Newark, DE) was used with a Dade Behring Dimension Xpand automated system to quantify plasma triglyceride levels. The assay is based on an enzymatic procedure using a combination of lipoprotein lipase, glycerol kinase, glycerol-3-phosphate-oxidase, and peroxidase. Changes in absorbance (510, 700 nm) resulting from the formation of quinoneimine from hydrogen peroxide reflect the total amount of glycerol and its precursors. All reactions were run in duplicate.

RNA Extraction

Total RNA was extracted from each hypothalamic sample according to RNeasy Mini and RNeasy Lipid (QIAGEN) kit directions. The samples were purified with DNA-*free* (Ambion) and analyzed spectrophotometrically (Nanodrop) to determine concentration and check for quality. cDNA template was created for each sample from 500ng of purified RNA using SuperScript III Reverse Transcriptase (Invitrogen).

cDNA Synthesis

cDNA was made using the SABiosciences RT² First Strand Kit. A genomic DNA elimination mixture was first created using 15 ug RNA samples from each

group (equal quantity from each animal) that was then mixed with 2.0 ul of 5X qDNA elimination buffer and RNase-free H_20 to a final volume of 10 ul. Contents were mixed gently, incubated at 42˚C for 5 minutes and chilled on ice. An RT cocktail was then prepared, containing 5X RT Buffer 3, Primer & External Control Mix, RT Enzyme Mix 3, and RNase free H_20. 10 ul of the RT cocktail was added to each genomic DNA elimination mixture, mixed well, and incubated at 42˚C for exactly 15 min and then immediately stopped by heating at 95˚C for 5 min. 91 ul of ddH_20 was added to each 20 uL of cDNA synthesis reaction and the solution was mixed well and stored at -20˚C. 34 uL of each sample from each experimental group was taken and pooled to create the master stock of cDNA used for the arrays and pooled verification assay. Hypothalamic cDNA templates from each experimental group were pooled by diet.

Measurement of Gene Expression

Changes in gene expression were measured in three ways, RT^2 PCR array, pooled verification, and an individual verification. Hypothalamic cDNA from each group was pooled and served as the genetic material for both the array and the pooled verification assay. After completing the array, 'significant' results were identified and those that were 'significant' and also of interest to the study of sugars effects on hypothalamic appetite regulation were selected for follow up verification assays. Primers were designed for each of these genes and standard RT-PCR experiments were run using pooled hypothalamic cDNA (pooled verification). If quality results were obtained from the pooled verification, the individual verification assay was run.

In the individual verification assay, hypothalamic cDNA from individual rats (n=4 per group) were analyzed using aforementioned primers and standard RT-PCR, results were then averaged for each group. Selection of the four animals included in the individual verification was based on those with the highest quality cDNA as analyzed spectrophotometrically (Nanodrop).

RT² Profiler™ PCR Array

Ten Rat Obesity RT² Profiler™ PCR Arrays (PARN-017A) were purchased from SABiosciences (Frederick, MD). Each array profiled the expression of 84 genes related to obesity, including genes that code for orexigenic peptides, hormones, and receptors, anorectic peptides, hormones, and receptors, and central and peripheral signaling molecules related to energy expenditure. Arrays also include 5 housekeepers and 7 controls. See appendix A for the complete list of genes included in the array.

Diluted First Strand cDNA was added to the 2X SABiosciences RT^2 qPCR Master Mix and aliquoted into each of 96-wells of the obesity arrays. Arrays were run according to the SABiosciences protocol: 95˚C for 10 min, and 40 cycles at 95˚C, 15 s; and 60˚C, 1 min. Results were analyzed using the SABioscience's online RT² Profiler™ PCR Array Data Analysis software. Fold change was calculated as 2^(-ddCt) using SABioscience's online PCR array analysis software. Fold change of 0.5 or less or of 1.5 or greater were considered 'significant.'

Custom Primer Design and Optimization

Using the array data as a guide, primers were designed for the majority of the genes that were significantly upregulated or downregulated in the array to be used in conventional RT PCR reactions. These included: ADIPOQ, ADRA2b, AGRP, APOA4, CCK, CLPS, CRH, DRD1a, GH1, IAPP, NPY, RAMP3, TNF, TRH, and UCN. Additionally, β actin, Ribosomal protein, large, P1 (RPLP1), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and Lactate dehydrogenase A (LDHA) were evaluated as housekeepers. Upon initial evaluation, LDHA was eliminated as an option for housekeeper because the average Ct was much higher than with the other housekeeper options. With the exception of TNF, primers were designed using Beacon Designer 7 software default parameters (PREMIER Biosoft, Palo Alto, CA). TNF was sourced from Yang et al, 2011(73).

Primers were evaluated using the CFX96 (BioRad) protocol, using a gradient that was centered around predicted optimum annealing temperature (TaOpt). The protocol for primer verification PCR is as follows: 95°C, 3 min, and 40 cycles at 95°C for 10 sec, at a gradient of temperatures for 30 sec with subsequent melt curve of 65°- 95°C at increments of 0.5°C for 5 sec. Empirically optimal Ta was determined for each primer set from these results. Housekeepers were selected based on the least variation in cycles between groups. See Table 1 for primer sequences and optimal annealing temperatures.

Pooled Verification Assay

Following determination of TaOpt from the primer verification assay, successful primers were evaluated on pooled group cDNA (glucose, sucrose, HFCS, fructose, and control). RPLP1 served as the housekeeper for each gene of interest. All reactions were carried out in duplicate.

Fold change was calculated as 2^(-ddCt). Fold change of 0.5 or less or of 1.5 or greater were considered 'significant.'

Individual Verification Assay

cDNA was synthesized from individual animals with highest quality RNA, four animals per group, as previously described. Primers that were successful on the group level were evaluated on 20 individual rat cDNA (4 animals from each group). RPLP1 served as the housekeeper for each gene of interest. All reactions were carried out in duplicate.

Fold change was calculated as 2^(-ddCt). Fold change of 0.5 or less or of 1.5 or greater were considered 'significant.'

Findings

Body Weight

Twenty-four hour access to any of the four sugar solutions used failed to promote significant differences in body weight ($p<0.05$). Refer to body weight data presented in Table 2.

Intake

Chow

All four treatment groups consumed significantly less chow ($p<0.001$) than did controls during the 24h access period. HFCS fed animals consumed significantly less chow than either fructose or glucose fed groups ($p<0.05$) Refer to intake data presented in Table 3.

Sugar

HFCS fed animals consumed significantly greater amounts of sugar compared to the group fed glucose ($p<0.05$). No other differences among groups were found. Refer to intake data presented in Table 3.

Total Intake

Control animals consumed significantly fewer total calories than the groups given access to sugar solutions ($p<0.05$). No other differences were observed. Refer to intake data presented in Table 3.

Plasma Analyses

Fructose consumption caused significant upregulation in circulating triglycerides ($p<0.05$). No differences among the five groups in plasma insulin or plasma glucose were found. Refer to plasma data presented in Table 4.

Housekeeper Selection

It has long been recognized that the selection of a housekeeper gene should be determined by that gene's resistance to treatment effects. We examined several standard housekeepers and found that all but one was significantly affected by sugar solution consumption. RPLP1 was the only primer analyzed whose cycle threshold did not vary across treatments (Duncan's new multiple range test $p<0.05$). Refer to housekeeper selection data presented in Table 5.

For these reasons, β-Actin, and GAPDH were excluded as options for housekeeper, leaving RPLP1 to be used as reference gene for all subsequent analyses.

Gene Expression

Inclusion and Exclusion Criteria

In order to expedite the interpretation of the many possible outcomes of the array analysis, we sorted the data into two categories: 'significant' and 'not significant' Fold change of 0.5 or less or of 1.5 or greater were considered 'significant.' Fold change was calculated as 2^(-ddCt) using SABiosciences online PCR array analysis software. Comparisons were based on average of duplicate

assays. Refer to Appendix B for complete results of PCR array analyses. Refer to Table 6 for a list of those genes that were 'significant.'

Additionally, data were not included for subsequent analyses if mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples were too low for consideration. Subsequent individual verification PCR was not performed

Adiponectin (ADIPOQ)

ANOVA performed on PCR array analyses did not reveal any significant differences among treatments (ANOVA $F=4.23$, $df= 4$, $P=0.0729$). Duncan's new multiple range test however revealed a significant upregulation of ADIPOQ message with glucose consumption (374%). In glucose fed animals ADIPOQ was significantly upregulated when compared to control as was sucrose and HFCS. Though upregulated 236%, the fructose group did not differ from the other experimental groups ($p<0.05$). Data are presented in Table 7.

ANOVA was not applied to the pooled PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples was too low for consideration. Because of this, subsequent individual verification PCR was not performed.

Adrenergic 2b receptor (ADRA2b)

Analysis of variance applied to the PCR array data revealed that access to a sugar solution significantly affected ADRA2b message (ANOVA $F=20.09$, $df=4$,

p=0.003). Post hoc analyses of group means found that ADRA2b in the hypothalamus was significantly downregulated by fructose by 52% and HFCS by 65% (Duncan's new multiple range test, $p<0.05$). The mean fold change of the glucose group was significantly higher than that of the sucrose group, but neither differed from control. Data are presented in Table 8.

ANOVA applied to the verification assay replicated the observation that sugar access significantly affected ADRA2b message (ANOVA F=163.38, df=4, $p<0.0001$). Duncan's new multiple range tests revealed that glucose and sucrose significantly upregulated ADRA2b message by 1461% and 1180% respectively ($p<0.05$). The two groups were significantly different from each other and both were different from the remaining experimental groups. No other statistically significant differences were observed. Data are presented in Table 8.

ANOVA was not applied to the individual PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples was too low for consideration.

Agouti related peptide (Agrp)

ANOVA applied to the array data revealed that sugar access had a significant effect on AGRP message in the hypothalamus (ANOVA F=18.73, df=4, p=0.0033). HFCS promoted a 199% upregulation of AGRP message when compared to control ($p<0.05$). No other differences were observed. Data are presented in Table 9.

ANOVA applied to the pooled data failed to reveal any effect of sugar access to hypothalamic AGRP message (ANOVA F=1.36, df=4, p=0.3661). Results from

Duncan's new multiple range test verified that there were no differences between group ($p>0.05$). Data are presented in Table 9.

Similarly, ANOVA of individual verification assays revealed no significant effect of sugar access on AGRP message (ANOVA $F=1$, $df=3$, $p=0.4659$). Duncan's new multiple range test revealed that there was a 3222% upregulation in AGRP message with consumption of HFCS ($p<0.05$). Data are presented in Table 9.

Apolipoprotein A-IV (APOA4)

ANOVA applied to the PCR array data revealed that there are differences in APOA4 expression with consumption of different sugars (ANOVA $F=18.8$, $df= 4$, $P=0.0032$). Duncan's new multiple range test revealed that the only significant changes were the downregulation of APOA4 message ($p<0.05$). The greatest change was a downregulation of 70% that resulted from sucrose intake. Also significant was a 39% downregulation of APOA4 by fructose consumption. Data are presented in Table 10.

ANOVA was not applied to the pooled PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples was too low for consideration. Because of this, subsequent individual verification PCR was not performed.

Cholecystokinin (CCK)

Variations in sugar solutions have significant effects on the regulation of CCK in the hypothalamus, as revealed by a significant group effect (ANOVA $F=566.95$,

df= 4, $p<0.0001$). The PCR array revealed that glucose promoted a 198% upregulation of CCK in the hypothalamus. HFCS and sucrose upregulated CCK expression by 139% and 123% respectively. By contrast, fructose promoted a 37% reduction in CCK expression as compared to control. Duncan's new multiple range tests that were used to determine differences between group mean fold changes revealed that all groups were statistically different from one another and control ($p<0.05$). Data are presented in Table 11.

For the most part, results from the PCR array were replicated in the pooled verification analyses. Glucose was markedly upregulated, 187%, and fructose was markedly downregulated, 60% in the pooled analyses. The Duncan's new multiple range test revealed that these differences were statistically different from each other ($p<0.05$) as well as the remaining experimental groups. No differences were observed between the control group and the sucrose and HFCS groups. Data are presented in Table 11.

Due to variability between animals, results from the individual verification analyses were not significant using ANOVA ($F=164.45$, df= 4, $p=0.0984$). However, subsequent Duncan's new multiple range test confirmed the observation for the pooled verification that glucose caused the greatest upregulation (637%) in CCK message ($p<0.05$). Data are presented in Table 11.

Colipase (CLPS)

ANOVA applied to the PCR array data revealed that there are no differences in CLPS expression with consumption of different sugars (ANOVA $F=0.56$, df= 4,

P=0.7). Duncan's new multiple range tests further revealed that there were not significant changes in CLPS. Data are presented in Table 12.

ANOVA was not applied to the pooled PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples was too low for consideration. Because of this, subsequent individual verification PCR was not performed.

Corticotropin Releasing Hormone (CRH)

Results from ANOVA of PCR array data revealed that different sugars result in significant changes in the expression of CRH in the hypothalamus (ANOVA $F=20.09$, $df=4$, $p=0.0028$). Subsequent post hoc tests revealed HFCS induced a 51% downregulation in CRH expression ($p<0.05$). The remaining groups did not differ from control. Data are presented in Table 13.

Results from the array were replicated in the pooled verification. Sugar consumption caused significant changes in CRH expression (ANOVA $F=163.38$, $df=4$, $p<0.0001$). Fructose was upregulated by 124% (Duncan's new multiple range test $p<0.05$). Also, both glucose and HFCS ingestion resulted in downregulation of CRH message in the hypothalamus, by 28% and 73% respectively. Sucrose did not significantly affect expression. The remaining groups significantly differed from control. Data are presented in Table 13.

In both the array and the pooled verification, HFCS caused a significant downregulation in CRH expression. Data are presented in Table 13.

Because of time and resource limitations individual verification was not performed for CRH.

Dopamine Receptor 1a (DRD1a)

Results from ANOVA revealed a significant effect of sugar consumption on expression in DRD1a using the PCR array system (ANOVA $F=52.33$, $df=4$, $p=0.0003$). Duncan's new multiple range test determined that both HFCS and sucrose caused changes in DRD1a expression that was significantly different from control ($p<0.05$). HFCS was upregulated 207% and sucrose was downregulated 30%. Remaining groups did not differ from control. Data are presented in Table 14.

The subsequent pooled verification assay also revealed differences between experimental groups (ANOVA $F=5.38$, $df=4$, $p=0.0467$). Duncan's new multiple range test found that glucose and HFCS did not differ from control ($p<0.05$). Fructose caused a 415% upregulation of DRD1a message ($p<0.05$). Sucrose also caused a significant upregulation in DRD1a expression, 332% ($p<0.05$). Data are presented in Table 14.

ANOVA was not applied to the individual PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples was too low for consideration. Data are presented in Table 14.

Growth hormone (GH1)

Different sugar solutions caused significant changes in expression of GH1 as revealed by a group effect (ANOVA F=12.96, df= 4, p=0.0075). Using the PCR array system we found that glucose causes a 332% upregulation in expression of the GH1 gene when compared to controls (Duncan's new multiple range test $p<0.05$). No other significant differences were observed between groups. Data are presented in Table 15.

The pooled verification analysis also revealed a significant group effect of sugar solutions on GH1 expression (ANOVA F=31.7, df= 4, p=0.001). Using Duncan's new multiple range test, it was again observed that glucose consumption results in a significant upregulation of GH1, 393% ($p<0.05$). Fructose fed animals also expressed upregulation of hypothalamic GH1 by 254%. Upregulation was also observed, though less robust, in the sucrose group with a 167% increase in the expression of GH1. Data are presented in Table 15.

ANOVA was not applied to the individual PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples was too low for consideration. Data are presented in Table 15.

Islet amyloid polypeptide (IAPP)

Although results from the PCR array do not reveal any differences between treatments for IAPP expression (ANOVA F=1.4, df= 4, p=0.3552), ANOVA of pooled verification did (ANOVA F=55.13, df= 4, p=0.0003). In the pooled

verification, there was a 483% upregulation of hypothalamic IAPP message induced by glucose consumption (Duncan's new multiple range test $p<0.05$). Fructose also induced upregulation of IAPP message with a mean fold change of 2.98. No other significant changes were observed. Data are presented in Table 16.

ANOVA was not applied to the pooled verification PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples was too low for consideration. Data are presented in Table 16.

Neuropeptide Y (NPY)

Results from the PCR array revealed that HFCS had a significant impact on NPY expression (ANOVA $F=24.08$, $df= 4$, $p=0.0018$). Duncan's New Multiple range tests that were used to determine differences between group means revealed that HFCS promoted a 227% upregulation in the expression of NPY in the hypothalamus when compared to controls ($p<0.05$). No other significant differences between group means were observed. Data are presented in Table 17.

ANOVA applied to the subsequent pooled verification analyses revealed a significant treatment effect (ANOVA $F=36.07$, $df= 4$, $P=0.0007$). The Duncan's new multiple range tests failed to replicate the upregulation of NPY observed with the array analyses. No statistically significant difference between the HFCS fed animals and controls was found. On the other hand, glucose fed animals had a 49% reduction in NPY expression as compared to control ($p<0.05$). Data are presented in Table 17.

Individual verification analyses replicated the array results (ANOVA F=16.33, df= 4, $p<0.001$). Hypothalamic NPY in HFCS fed rats was upregulated by 352% compared to control. The downregulation of NPY message in glucose fed animals, as observed in the pooled verification analysis, failed to replicate. Data are presented in Table 17.

In both the pooled verification as well as the individual verification, no differences were observed between sucrose and fructose fed animals. Both groups showed slight downregulation in the pooled verification (approximately 25% less then control, and in individual verification assay results showed approximately a 25% upregulation as compared to control.

Receptor (G protein-coupled) activity modifying protein 3 (RAMP3)

Results from the PCR array analysis revealed that sugars caused significant changes in the expression of RAMP3 (ANOVA F=36.03, df= 4, P=0.0007). Results from Duncan's new multiple range tests revealed specific differences in RAMP3 regulation by different sugar solutions. Glucose resulted in the greatest upregulation, (by 132% as compared to control, $p<0.05$). Fructose and HFCS however promoted downregulation in hypothalamic RAMP3 expression, by 47% and 56% respectively (both $p<0.05$). RAMP3 expression in animals consuming sucrose did not differ from control ($p>0.05$). Data are presented in Table 18.

Results from the pooled verification analysis replicated the results from the PCR array. ANOVA revealed that there were significant differences between the treatment groups and the control (ANOVA F=304.63, df= 4, P<0.0001). As was in

the PCR array results, glucose promoted the greatest upregulation of RAMP3 expression, by170% ($p<0.05$). HFCS and fructose downregulated RAMP3 message by 45% and 43% respectively (both $p<0.05$). Sucrose led to an upregulation of RAMP3 expression. RAMP3 in the sucrose group was significantly higher than the other treatment groups as well as the control, 147% (Duncan's new multiple range test $p<0.05$). Data are presented in Table 18.

Differences in treatment groups were not significant in the individual verification experiment (ANOVA F=19.63, df= 4, P=0.4603). However, Duncan's new multiple range test revealed animals fed HFCS had a 243% upregulation in RAMP3 expression. Sucrose and control did not differ. Glucose and fructose fed animals both showed significant downregulation by 35% and 93% respectively. Differences between glucose and fructose were significant ($p<0.05$). Data are presented in Table 18.

Tumor Necrosis Factor (TNF-α)

Results from the PCR array did not reveal any significant differences in TNF-α expression when analyzed with ANOVA (F=3.87, df= 4, P=0.085). Duncan's new multiple range test however did reveal significant differences between glucose and HFCS and control ($p<0.05$). Glucose, upregulated TNF-α by 165%, differed from HFCS and control. Sucrose and fructose did not differ from any other experimental groups. Data are presented in Table 19.

Results from the pooled verification were significant when analyzed with ANOVA (F=15.96, df= 4, P=0.0047). Glucose, sucrose and fructose significantly

upregulated hypothalamic TNF-α as compared to control though they were not significantly different amongst themselves (glucose 148%, sucrose 167% and fructose 167%) (Duncan's new multiple range test $p<0.05$). TNF-α expression in rats fed HFCS however did not differ from control ($p>0.05$). Data are presented in Table 19.

ANOVA was not applied to the individual verification PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples was too low for consideration. Data are presented in Table 19.

Thyrotropin Releasing Hormone (TRH)

ANOVA applied to the PCR array data revealed significant differences in TRH expression in experimental groups (ANOVA $F=32.89$, $df= 4$, $P=0.0009$). Subsequent post-hoc analyses (Duncan's new multiple range test) further revealed the greatest change in TRH expression was from consumption of HFCS that promoted a 57% downregulation as compared to control ($p<0.05$). Glucose and sucrose also downregulated TRH by 45% and 23% respectively. By contrast, fructose consumption promoted an upregulation of 122% ($p<0.05$). Data are presented in Table 20.

Pooled verification ANOVA analysis also revealed differences in experimental groups (ANOVA $F=37.56$, $df= 4$, $P=0.0006$). All treatment groups with the exception of HFCS were upregulated when compared to control. The greatest upregulation in TRH expression was that in animals given access to sucrose solutions by 504%. This is in conflict to the results observed from the PCR array in which

sucrose fed rats had a 23% downregulation in TRH expression. Glucose and fructose also significantly upregulated TRH message by 386% and 372% respectively. Lastly, HFCS consumption resulted in the least change in message of 122% ($p<0.05$). Data are presented in Table 20.

Because of time and resource limitations individual verification was not performed for TRH.

Urocortin (UCN)

Results from the ANOVA of PCR array data revealed that there were significant differences in treatment groups (ANOVA $F=116.76$, $df= 4$, $P<0.0001$). Further post-hoc analyses revealed that the only significant difference was an 111% upregulation of hypothalamic UCN by glucose consumption ($p<0.05$). The remaining groups did not differ from control. Data are presented in Table 21.

ANOVA was not applied to the pooled or individual PCR data due to fact that the mean thresholds were higher than 36 cycles, suggesting that the existing message concentrations in these samples were too low for consideration. Data are presented in Table 21.

Discussion

It is well accepted that there is an alarming obesity epidemic in this country. The obesity problem is particularly worrying not only in terms of human health and quality of life but also in terms of dollars spent on healthcare in the treatment of obesity related comorbidities. Obesity has become the nation's most preventable health problem (1, 2). Despite it's prevalence, there is no known cure for this disease or consensus about how it develops. One hypothesis for the cause of obesity is that dietary intake patterns of Americans have significantly shifted over the past fifty years by including unprecedented amounts of refined sugar (4).

The purpose of this experiment was to examine this hypothesis by evaluating how sugars affect the appetite regulatory center of the brain, the hypothalamus. Animals were given 24 hour access to either a dilute glucose, sucrose, HFCS, or fructose sugar solution. Intake of both chow and sugar was measured. Hypothalamic tissue was collected and changes in gene expression were measured using a RT-PCR array as well as traditional RT-PCR on pooled tissue samples (pooled verification) and individual rat samples (individual verification).

Intake

Previous studies have shown that initial presentation of a sugar solution will result in rats consuming excess calories from sugar. Over time however this result washes out and total caloric intake returns to a level that resembles control (70, 74). These findings may suggest an explanation for our results that sugar fed animals all consumed increased calories as compared to control animals. All sugar-fed animals

consumed the same amount of total calories and there were no differences in percent kcal from sugar for groups consuming fructose containing sugars (sucrose, HFCS, fructose).

The rats in this experiment did not increase intake of glucose-fructose disaccharides (sucrose and HFCS) in such a way as to match the amount of fructose consumed in the fructose group. In other words rats did not have a threshold for fructose consumption and compensate by consuming 50% more sucrose or 45% more HFCS to match the total consumption of fructose in the fructose fed group. We applied the same model as Collier and Bolles did in their 1968 sugar dilution studies. Looking at intake of various sugar dilutions in rats, Collier and Bolles found that regardless of the type of sugar presented, rats will increase their intake of a dilute sugar so as to take in a certain threshold amount of total grams of sugar (75). In the current study, this model was applied to dilutions of fructose intake. For example, sucrose fed animals had a solution that was 50% less fructose than the fructose animals. Replicating Collier and Bolles' findings, there was not set level for fructose intake as animals consuming sucrose and HFCS, which have less fructose, consumed the same amount of total kcal from sugar. These findings also show that fructose having a sweeter taste than glucose did not impact intake.

Plasma

Fructose caused a significant and robust increase in triglycerides with only 24 hours of access. This phenomenon has been previously reported in longer term

fructose feeding studies in rats and in humans (76, 77). The importance of this finding is that hypertriglyceridemia occurred with only 24 hour access to fructose.

No differences in plasma insulin or glucose were observed, as all blood samples were collected under ad libitum feeding conditions.

Gene expression

By using the PCR array technique we were able to evaluate the effects of different sugars on multiple hypothalamic genes with a very fast turn over. As seen in Table 6, significant changes for glucose fed animals were only upregulations.

We had also expected to see sucrose and HFCS fed animals to have similar results. Surprisingly, there were no genes with significant changes observed in both groups. Given that HFCS and sucrose only slightly vary in composition, it is unclear why results from intake of these sugars were not similar. The lack of a similar effect or a dose dependent effect demonstrates a limitation of the present analysis. In the future, this problem may be resolved with increasing the number of animals in each group and also running more replicates of the array assay.

We selected genes for subsequent analyses (pooled and individual verifications) based on those with significant up or downregulation (1.5 fold change and above, or 0.5 fold change and below). From this list of significant genes we were able to select genes that had a known role in the regulation of CHO intake and metabolism or those with very robust changes to further explore and verify.

Cholecystokinin

Cholecystokinin (CCK) is an anorexogenic peptide that is expressed in both the small intestine and the brain. Expression of CCK results in short duration reduction of meal size (23). CCK is also important in the regulation of insulin.

As shown here, glucose consumption results in significant upregulation of CCK. This has been observed in previous work in animals maintained on a high fat diet (27). The impact of glucose on CCK expression may be as a result of the interaction of CCK with insulin. Lo (2011) using CCK-KO mice, found that these animals had reduced insulin response. CCK is required for regulating both insulin secretion and glucose tolerance in mice on a high fat diet. Considering these findings, it is proposed that CCK is upregualated by glucose such that CCK can in turn stimulate insulin release.

Alternatively, fructose consumption resulted in significant downregulation of CCK (if you exclude information from the individual analysis). This is a novel finding and has yet to be discussed in the literature. It is known that fructose and glucose are metabolized differently (Figure 2). Because CCK is anorectic and is upregulated by glucose but down regulated by fructose it illustrates how the differences the two sugars interact metabolically. It is also interesting to note that CCK is an anorectic peptide and fructose fails to upregulate it. This point suggests that fructose may fail to induce satiation and thus allow for overeating.

Corticotrophin Releasing Hormone

Corticotropin-releasing hormone (CRH), also known as Corticotropin-releasing factor (CRF) is an anorectic peptide secreted by neurosecretory cells of the parvocellular region of the periventricular nucleus (PVN) of the hypothalamus and carried via the portal system to the anterior lobe of the pituitary where it promotes ACTH production (30).

Ulrich-Lai and colleagues evaluated the effect of sucrose on CRF expression. They found that daily limited access to a sucrose sweetened drink resulted in decreased CRH mRNA expression (33). In the present study however, sucrose did not differ from control in either the PCR array or the pooled verification. On the other hand, HFCS (the other disaccharide in this study) intake did significantly downregulate CRH message. Though the aforementioned study did not measure changes to CRH with HFCS consumption, it is unclear why in this study HFCS replicates the finding while sucrose does not.

The literature also shows that when glucose decreases, CRH expression increases (21, 31, 32). However in the present study the effects of increased glucose rather than reduced glucose were measured. Although no changes in CRH expression in glucose fed animals were observed, in the future this could be examined by comparing animals that had been fasted and then given a dose of glucose, to animals that had only been fasted. Utilizing different doses of glucose would allow the examination of the amount of glucose required to reduce CRH expression back to normal levels.

Also, fructose, though not different from control, prevented the downregulation observed with HFCS. This is an interesting finding because it suggests a role of insulin in CRH expression. It should be mentioned that fructose does not initiate an insulin response, but HFCS does.

In the future it will be interesting to evaluate changes in CRH expression in animals consuming fructose that have been administered insulin. This experiment will provide interesting insight into connections between CRH and sugars as they relate to insulin.

Growth Hormone

GH is thought to be an anorexogenic peptide that not only contributes to the regulation of energy expenditure but also growth. However, there is limited literature surrounding the effects of Growth Hormone (GH) on the regulation of energy balance and intake.

Growth hormone is another anorectic protein whose expression was upregulated by glucose intake. As was discussed in the intake sections, giving rats access to sugar solutions and chow results in increased total caloric intake, and at the same time a significant reduction in daily chow intake. Given the upregulation of GH it could be suggested that GH is playing a role in suppressing chow intake but not sugar intake. Sugar solution intake might be spared the suppression as the result of increased dopamine activation of the reward pathway.

We did not find any significant effects of fructose on GH and thus our results did not replicate the findings of Strauch that fructose stimulates GH secretion (51).

One possible explanation may be that in the Strauch study, fructose was administered IV.

There has been interesting work done on the effects of fasting and obesity states on GH secretion (50). In the future it would be interesting to further explore the effects of both glucose and fructose on obese animals given only 24 hour access to these sugars. It could be predicted that glucose would be less able to increase GH expression due to changes in metabolic flexibility resulting from obesity.

Receptor Activity Modifying Protein 3

Receptor activity modifying protein 3 (RAMP3) is a G protein-coupled receptor (GPCR) accessory protein in the family of RAMP. One function of RAMP3 is to interact with the calcitonin receptor to produce amylin receptor complexes. Once formed, these complexes allow binding of amylin to the receptor. Another function of RAMP3 is to complex with calcitonin receptor-like receptor (CLR) to form adrenomedullin receptors (15, 58). Because the RAMP family plays such an important role in the transport and modulation of GPCRs, there have been extensive pharmacological studies aimed at examining RAMPs as potential sites for modulating GPCRs for the sake of disease treatment. Though much is known about the function of this protein, little has been reported on the specific impact of RAMP3 in the control of intake and energy balance.

In the present study, if individual analyses are excluded, RAMP3 was found to be upregulated by glucose and alternatively downregulated by HFCS and fructose. Although not much is known about the role of RAMP3 in sugar metabolism, it is

known that RAMP3 is involved in amylin processing. It is also known that CCK and amylin have similar metabolic actions such as slowing gastric emptying. Because the trends in gene expression for RAMP3 are similar to those observed with CCK, upregulation by glucose and downregulation by fructose, it is reasonable to assume that sugars have the same implications on RAMP3 expression as they do on CCK. That is to say perhaps RAMP3, upstream on the amylin pathway, triggers the same metabolic consequences as CCK.

Tumor Necrosis Factor

Tumor Necrosis Factor-α (TNF-α) is an inflammatory cytokine found in the hypothalamus. TNF plays in active role in inflammation and obesity and is involved in lipid metabolism regulation, adipocyte differentiation, and insulin sensitivity (60). TNF has been shown to interact with well-known satiety signals including leptin. Huypens has proposed a model for TNF that suggests the gene has a role in the regulation of the inverse relationship between ADIPOQ and leptin (61).

Glucose, sucrose, and fructose consumption all resulted in an upregulation of TNF expression in the hypothalamus. Recently much of the work surrounding possible causes of obesity has been centered on inflammation and the impact sugar has on inflammation (62). TNF is an important inflammatory cytokine and because sugar has been shown to cause inflammation, these results are as expected. However, it is unclear as to why HFCS did not result in upregulation as well.

Wang suggests that once animals that have been made obese with a high fat diet are switched to a chow diet, the resulting elevated TNF expression does not

return to levels of control (62). The importance of this finding here is that even if these animals were removed from the sugar diet they may still suffer from elevations of this inflammatory peptide. In the future a longer term study which examines TNF levels following removal of sugar access from sugar-induced obese rats may provide insight into this subject.

Thyrotropin Releasing Hormone

Thyrotropin- releasing hormone (TRH), also called Thyrotropin- releasing factor (TRF), is produced in the PVN of the hypothalamus and functions to stimulate the release of thyroid stimulating hormone and prolactin from the anterior pituitary. TRH is an anorectic hormone.

The mechanism of TRH action on appetite regulation is not yet fully understood (63). Leptin has been found to have direct and indirect (through activating POMC neurons that in turn release αMSH to stimulate TRH) effects on stimulation of TRH synthesis (65).

Compared to control, TRH gene expression is upregulated in animals given access to fructose. These results are consistent with the long-recognized role of fructose promoting increases in uric acid, leading to increased risk of gout (a disease associated with hypothyroidism) (78).

NPY also acts on TRH as an inhibitor through activation of NPY receptors on the TRH neurons (66). Here however this association could not be evaluated because results for NPY were not replicated between the array and verification analyses. In

the future it may be interesting to further explore the connection between NPY and TRH.

Conclusion

Obesity has become such a significant public health concern that there is urgency to better understand its causes. Due to sugars' ability to alter hypothalamic appetite regulation, this research attempted to evaluate the hypothesis that increased intake of added sugars may be contributing to the obesity epidemic. This was a very well designed protocol as it looked at common disaccharides while also having the monosaccharaide controls. Many other studies examining HFCS fail to have fructose and/or glucose control groups. This research has shed light onto many important effects of sugar consumption and also presented the opportunity for many other follow up experiments.

It is curious that the only significant changes in gene expression that were verified in all performed assays were changes in anorectic genes. However, it should be pointed out that there are fewer known orexogenic genes. Another interesting trend in gene expression is that in the glucose fed group, the only significant changes were those of upregulations. In other words, glucose intake only resulted in the upregulation of anorectic gene expression. This might be an indicator of how glucose metabolism evolved and how glucose, being a good source of calories, tells the body that enough calories have been taken in and eating can stop.

This research laid the groundwork for many other follow up studies. First of all, a longer term sugar feeding study would allow for the examination of changes in intake following adaptation to the novelty of the sugar solutions. Laboratory rats will over eat sugar initially but will subsequently restore control-level total caloric intake. A longer duration study would examine the novelty effect of initial access to sugar

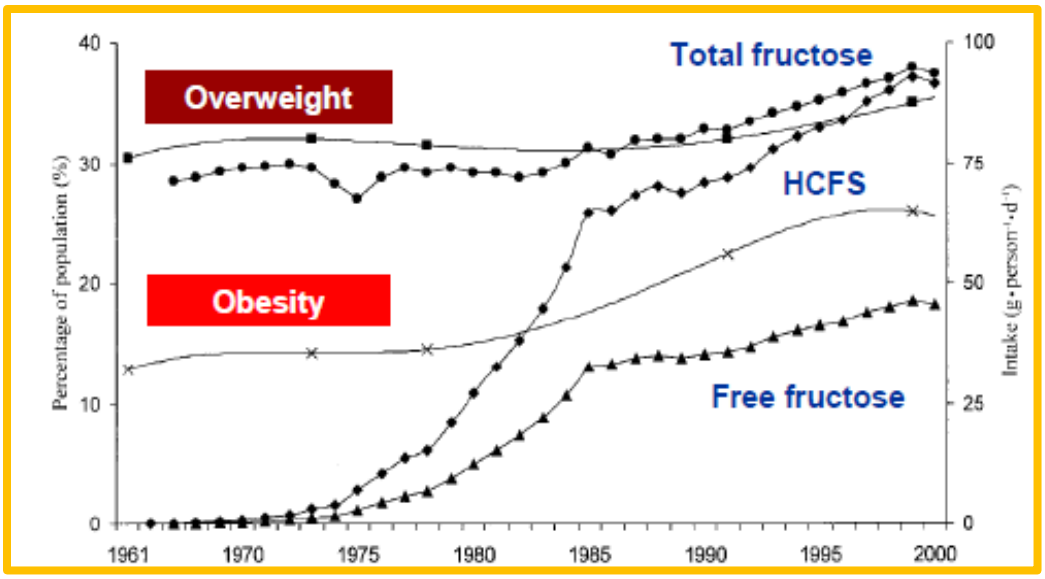
solutions and should show that intake of treatment groups will, over time, more closely resemble controls. Once intake is 'leveled off,' evaluation of gene expression in the hypothalamus will provide insight into how gene expression changes with longer term access. Also, depending on the length of access, by the end of an extended feeding study the animals may become obese. It would be interesting to compare changes in hypothalamic gene expression between both the long term study obese animals with the present study lean animals.

With a longer term study it might also be interesting to provide animals with different doses of sugars and also take samples at more time intervals. Assuming animals are sacrificed and hypothalamic tissue analyzed throughout the study, the varied doses will allow for the examination of thresholds at which changes become significant. This would apply to humans who consume sugar sweetened beverages at different levels of consumption.

While doing this long term study, or even in a short term study as this was, sampling plasma continuously would allow for a more real time analysis of change in plasma TG as well as glucose. In the present study, there were no changes observed in insulin and glucose. Continuous sampling would allow for the measure of these in as they respond to intake.

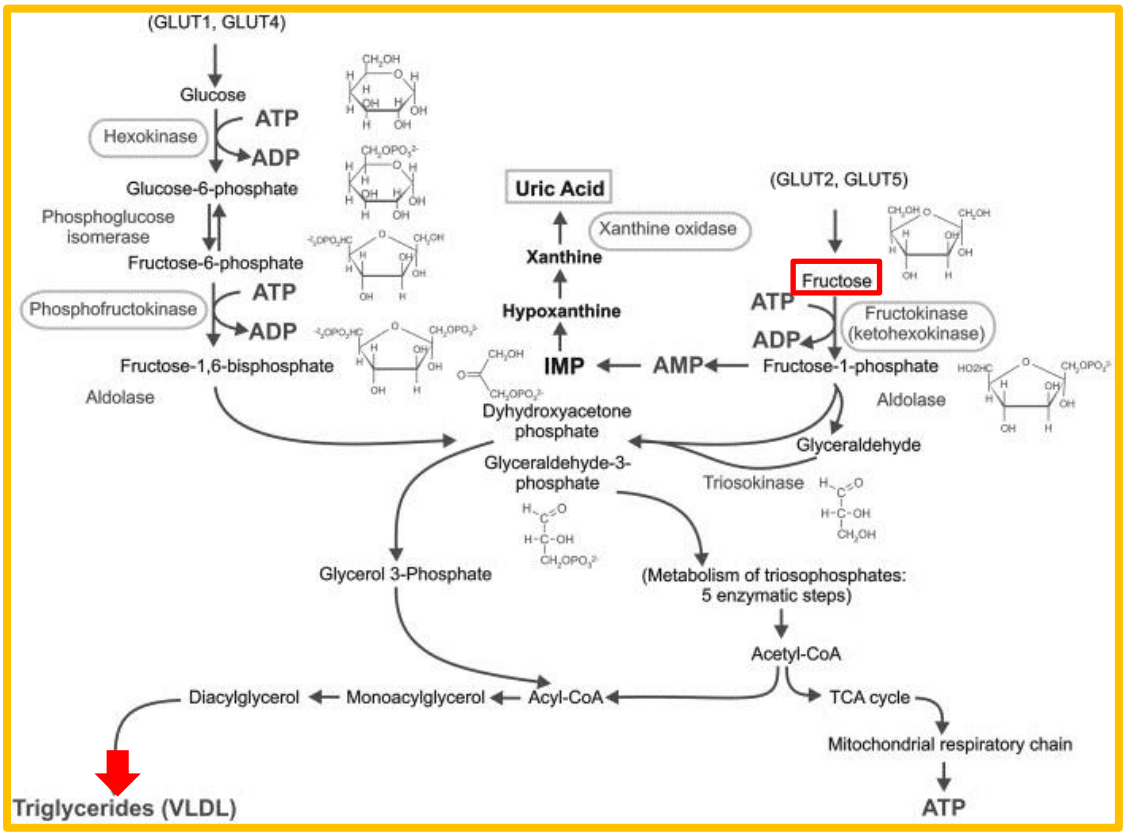
Finally in future studies it would be interesting to examine changes in protein levels of the hypothalamic genes of interest. The fact that a gene is upregulated does not necessarily mean the protein will be expressed in greater amounts. Measuring changes in protein will allow for a complete examination of the impact of sugars on gene expression in the appetite center of the brain, the hypothalamus.

Figure 1. Relationship between fructose intake and prevalence of overweight and obesity.



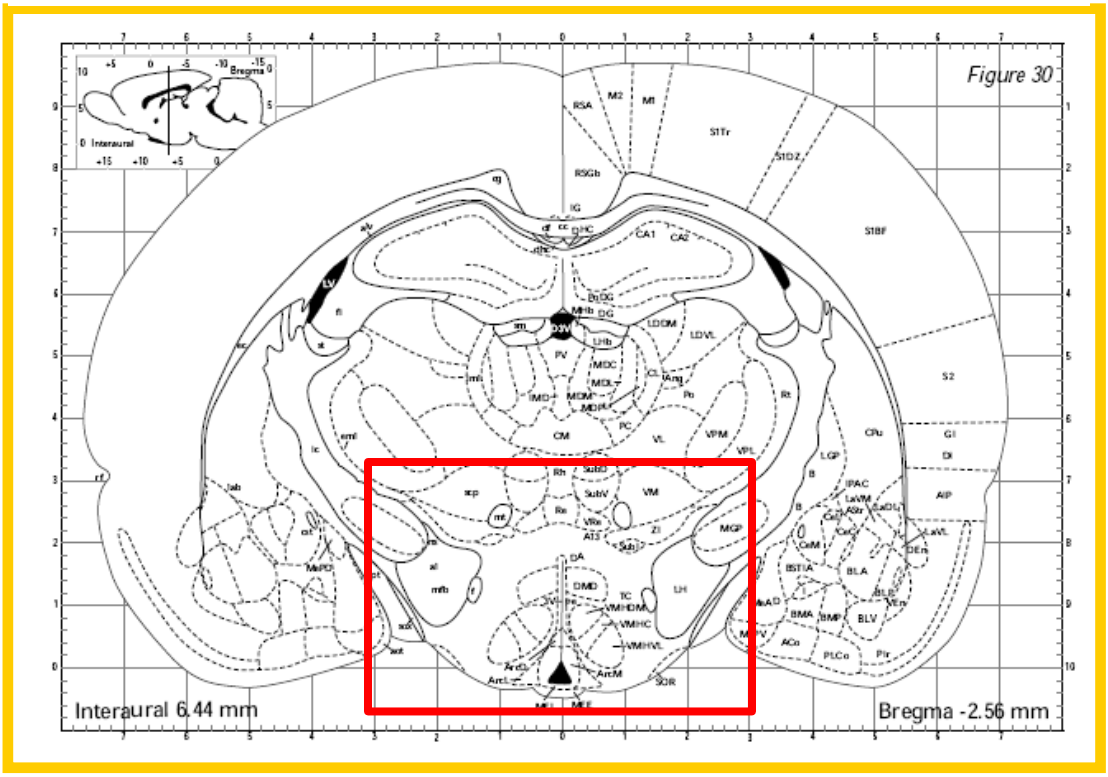
Plotted above are the average changes in intake of fructose (free and total) and HFCS over time (in years) as well as the average changes in the % of the population classified as either overweight or obese during that same period. (Adapted from Bray et al., 2004)

Figure 2. Metabolism of glucose and fructose



Comparison of the metabolism of fructose and glucose. The red arrow indicates where fructose enters the liver cell via the GLUT5 transporter and feeds into the glycolysis pathway. Fructose has less control points and feeds directly into G3P (glucose-3-phosphate). The red box surrounds the results of the TCA cycle which is the formation of TG. Adapted from Johnson et al., 2009 (79).

Figure 3. Hypothalamic sectioning



Sectioned rat brain showing region of tissue collected for measure of changes in gene expression. Area of collection is boxed in red. Regions include VMN, DMN, PVN, arcuate nucleus and median eminence. Adapted from Paxinos and Watson, 1986 (80).

Table 1. Primers used for pooled and individual verification RT-PCR analyses.

Gene	Accession Number	Product Length (BP)	TaOpt (°C)	Sense Primer	Anti-sense Primer
ADIPOQ	NM_144744	194	51	TTGAGAGGTTGATTGTATT	AGTGGCAATATAGTTGTAAT
ADRA2b	NM_138505	114	53.5	TACTCTCATCATCCCTTTC	ATGGAGGAGGTACAGAAG
AGRP	XM_574228	97	57.6	GAGTTCTCAGGTCTAAGTCT	GTGGATCTAGCACCTCTG
APOA4	NM_012737	129	58.9	CTCCATACCAAAGTCTCC	CTTGTTCAGGTCTTCCAG
B ACTIN	NM_031144	165	56.5	TGTCACCAACTGGGACGATA	GGGGTGTTGAAGGTCTCAAA
CCK	NM_012829	133	53	GCGTTTATTTATTAAGTCC	ATAGCATAGCAACATTAG
CLPS	NM_013139	83	58.9	CCTTGTTGTTCTGCTTGTA	CGTCCTCCAGGTTGATAA
CRH	NM_031019	158	51	TGGAGATTATCGGGAAAT	TACATCTTCTATGCTTCAAG
DRD1A	NM_012546	75	54.5	CTCAACCCTCTTAGGATGCTACA	TTGTTGTTAATGCTCACCGTCTC
GAPDH	NM_017008	92	56	TCCCATTCTTCCACCTTT	TAGCCATATTCATTGTCATACC
GH1	NM_001034848	88	51	CAAGCAAACCTATGACAA	CTTCTTGAAGCAGGAGAG
IAPP	NM_012586	92	56.2	AACTTCTTGGTTCGCTCC	TCTGCCACATTCCTCTTC
LDHA	NM_017025	112	54.2	TTGTTGATGTCATAGAAGA	GCAGTCACACTATAATCT
NPY	NM_012614	89	53.9	AATGAGAGAAAGCACAGAAA	AAGTCAGGAGAGCAAGTT
RAMP3	NM_020100	109	54.6	CAAGGTCATCTGGAAGGT	GACTCCTAACAACTCCATTC
RPLP1	NM_001007604	81	53.5	GAAGAATCCGAGGATGACA	CAGGTTCAGCTCTTTATTGG
TNF	NM_012675	318	61	GGCAGGTCTACTTTGGAGTCATTGC	ACATTCGGGGATCCAGTGAGCTCCG
TRH	NM_013046	75	59.7	AAAGACATTGAAGCTGAAGAGAGG	GGGGTGCTGTCGTTTGTG
UCN	NM_019150	75	60.6	AGCAGAACCGCATCATAT	AAAGGGTCAAGGCTTTCT

Primers were designed using Beacon Designer 7 unless otherwise noted in Methods section. TaOpt determined empirically.

Table 2. Average body weight of rats following 24 hour sugar access

	Control	Glucose	Sucrose	HFCS	Fructose
Average BW (g)	319.4[a]	323.6[a]	319.1[a]	314.5[a]	321.8[a]

Average body weight in grams. Rats were weighed just prior to sacrifice. Averages that share a common superscript are not different from one another ($p>0.05$).

Table 3. Mean chow and sugar intake following 24 hour access to sugar solutions

Grams	Control	Glucose	Sucrose	HFCS	Fructose
Fructose			6.20^{b}	7.28^{b}	10.96^{a}
Glucose		9.83^{a}	6.20^{b}	5.96^{b}	
Total sugar		9.83^{b}	12.41^{ab}	13.23^{a}	10.96^{ab}
Chow	28.32^{a}	21.36^{b}	19.26^{bc}	17.90^{c}	21.41^{b}
kCal					
Chow	96.57^{a}	72.85^{b}	65.69^{bc}	61.04^{c}	73.02^{b}
Sugar		39.32^{b}	49.62^{ab}	52.94^{a}	43.83^{ab}
Total	96.57^{b}	112.17^{a}	115.31^{a}	113.98^{a}	116.85^{a}
% kCal as sugar		$33.65\%^{b}$	$43.00\%^{a}$	$46.17\%^{a}$	$37.31\%^{ab}$

Rats were given 24 hour access to a sugar solution as well as water and chow. Control animals had access to chow and water only. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 4. Results of plasma analyses

	Group				
	Control	**Glucose**	**Sucrose**	**HFCS**	**Fructose**
Triglyceride (mg/dL)	148.21[b]	154.11[b]	175.80[b]	224.02[a]	283.88[ab]
Glucose (mg/dL)	166.58[a]	163.72[a]	157.80[a]	167.84[a]	161.86[a]
Insulin (ng/mL)	4.51[a]	3.42[a]	4.27[a]	4.01[a]	4.65[a]

Changes in plasma TG, glucose and insulin following 24 hour access to sugar solutions. Plasma was collected from trunkal blood at the time of sacrifice. Triglycerides were measured with the Dimension clinical chemistry system with a Flex reagent cartridge (Siemens Healthcare Diagnostics, Newark, DE) using a Dade Behring Dimension Xpand automated system. Glucose was measured enzymatically (Smith-Kline Beecham Laboratories, Brentford, Middlesex, UK). Insulin was measured by ELISA (LINCOplex; LINCO Research, St. Charles, MO). Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 5. Cycle threshold (Ct) of housekeepers

	Control	Glucose	Sucrose	HFCS	Fructose
β-Actin	19.11^{a}	16.08^{cd}	15.74^{d}	17.60^{b}	16.47^{c}
GAPDH	16.90^{ab}	16.95^{ab}	16.55^{b}	17.01^{ab}	17.05^{a}
RPLP1	17.57^{a}	17.88^{a}	17.53^{a}	17.55^{a}	17.56^{a}
N	5	8	5	5	8

Multiple housekeepers were analyzed and the one with the least variation between experimental groups and the lowest Ct was selected. Presented are the cycle thresholds (Ct) of RT-PCR using each housekeeper primer set and pooled hypothalamic cDNA from each experimental group. Primer sequence can be found in Table 1. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 6. List of significant fold changes

Gene		Group			
Symbol	**Name**	**Glucose**	**Sucrose**	**HFCS**	**Fructose**
Adipoq	Adiponectin	3.54	1.01	1.33	2.33
Adra2b	Adrenergic, alpha-2B-, receptor	1.16	0.86	0.35	0.48
Agrp	Agouti related protein homolog (mouse)	1.26	0.96	1.98	1.05
Apoa4	Apolipoprotein A-IV	0.86	0.28	1.15	0.58
Cck	Cholecystokinin	1.98	1.23	1.39	0.63
Clps	Colipase, pancreatic	1.56	1.48	1.56	0.98
Crh	Corticotropin releasing hormone	0.87	0.76	0.48	1.21
Drd1a	Dopamine receptor D1A	0.83	0.70	2.07	1.08
Gh1	Growth hormone 1	3.27	1.01	1.20	1.09
Iapp	Islet amyloid polypeptide	2.00	1.11	1.20	2.28
Il1a	Interleukin 1 alpha	1.19	1.75	0.95	1.01
Il6	Interleukin 6	1.12	1.51	0.83	0.92
Ins2	Insulin 2	0.66	0.97	0.47	0.80
Nmu	Neuromedin U	1.11	0.38	1.30	1.08
NPY	Neuropeptide Y	1.13	1.03	2.27	1.01
Ntrk1	Neurotrophic tyrosine kinase, receptor, 1	1.20	0.94	1.20	1.85
Ramp3	Receptor activity modifying protein 3	1.32	1.09	0.44	0.52
Tnf	Tumor necrosis factor	1.65	1.39	1.04	1.25
Trh	Thyrotropin releasing hormone	0.55	0.77	0.43	1.22
Ucn	Urocortin	4.08	1.01	1.44	1.09

Presented in the right hand columns of this table are the fold changes in appetite regulating genes. Highlighted in yellow or red are those changes that were considered "significant." In order to be considered "significant" a fold change needed to be downregulated (red) by 50% or more (fold change ≤ 0.5) or upregulated (yellow) by 150% or more (fold change ≥ 1.5) following sugar access. Fold change was calculated Fold change was calculated as 2^(-ddCt).

Table 7. Summary of analysis used to determine the effect of sugar solution access on hypothalamic ADIPOQ.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	37.12	33.54	36.01	34.88	33.90
	Fold change	1.00^{b}	3.74^{a}	1.02^{b}	1.34^{b}	2.36^{ab}
	N	5	8	5	5	8

Results of PCR array analysis of ADIPOQ expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain estimates of changes to ADIPOQ. PCR array was performed on pooled hypothalamic tissue from all animals in each group. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 8. Summary of analyses used to determine the effect of sugar solution access on hypothalamic ADRA2b expression

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	32.69	32.84	32.93	34.48	33.87
	Fold change	1.00^{ab}	1.17^{a}	0.86^{b}	0.35^{c}	0.48^{c}
	N	5	8	5	5	8
Pooled verification	**Ct**	33.14	29.59	29.55	33.67	32.43
	Fold change	1.00^{c}	14.61^{a}	11.80^{b}	0.69^{c}	1.63^{c}
	N	5	8	5	5	8

Results of two analyses of ADRA2b expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to ADRA2b. Pooled verification was then performed using RT-PCR and primers designed in house. Primer sequences for pooled verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 9. Summary of the three analyses used to determine the effect of sugar solution access on hypothalamic AGRP.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	26.59	26.62	26.67	25.87	26.64
	Fold change	1.00[b]	1.27[b]	0.96[b]	1.99[a]	1.06[b]
	N	5	8	5	5	8
Pooled verification	**Ct**	27.72	27.35	28.43	26.87	26.62
	Fold change	1.00[a]	1.66[a]	0.62[a]	1.80[a]	2.53[a]
	N	5	8	5	5	8
Individual verification	**Ct**	34.84	35.93	35.94	31.04	39.72
	Fold change	1.00[b]	1.37[b]	0.74[b]	32.22[a]	*
	N	4	4	4	4	4

Results of three analyses of AGRP expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to AGRP. Pooled verification, followed by individual verification, was then performed using RT-PCR and primers designed in house. Primer sequences for pooled and individual verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Individual verification was performed on individual hypothalamic tissue from four rats from each group. Averages of the four individual assays are presented. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

* Mean threshold cycle was above cutoff value of 36 cycles. As a result, data from this group was not included.

Table 10. Summary of analysis used to determine the effect of sugar solution access on hypothalamic APOA4.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	31.81	32.39	33.68	31.87	32.73
	Fold change	1.00^{a}	0.86^{ab}	0.30^{c}	1.15^{a}	0.59^{b}
	n	5	8	5	5	8

Results of PCR array analysis of APOA4 expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain estimates of changes to APOA4. PCR array was performed on pooled hypothalamic tissue from all animals in each group. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 11. Summary of the three analyses used to determine the effect of sugar solution access on hypothalamic CCK.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	24.19	23.57	23.92	23.98	24.98
	Fold change	1.00^{d}	1.98^{a}	1.23^{c}	1.39^{b}	0.63^{e}
	N	5	8	5	5	8
Pooled verification	**Ct**	21.86	21.27	21.78	21.82	23.17
	Fold change	1.00^{b}	1.87^{a}	1.06^{b}	1.01^{b}	0.40^{c}
	N	5	8	5	5	8
Individual verification	**Ct**	28.75	27.10	24.13	28.46	29.06
	Fold change	1.00^{c}	6.38^{a}	2.83^{b}	1.34^{c}	1.12^{c}
	N	4	4	4	4	4

Results of three analyses of CCK expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to CCK. Pooled verification, followed by individual verification, was then performed using RT-PCR and primers designed in house. Primer sequences for pooled and individual verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Individual verification was performed on individual hypothalamic tissue from four rats from each group. Averages of the four individual assays are presented. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 12. Summary of analysis used to determine the effect of sugar solution access on hypothalamic CLPS.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	32.77	32.92	32.49	32.39	32.22
	Fold change	1.00[a]	1.09[a]	1.57[a]	1.59[a]	1.62[a]
	N	5	8	5	5	8

Results of PCR array analysis of CLPS expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain estimates of changes to CLPS. PCR array was performed on pooled hypothalamic tissue from all animals in each group. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 13. Summary of analyses used to determine the effect of sugar solution access on hypothalamic CRH.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	27.03	27.59	27.45	28.34	26.88
	Fold change	1.00^{ab}	0.87^{b}	0.76^{bc}	0.49^{c}	1.21^{a}
	N	5	8	5	5	8
Pooled verification	**Ct**	30.40	31.22	30.49	32.29	30.12
	Fold change	1.00^{b}	0.72^{c}	0.94^{b}	0.27^{d}	1.24^{a}
	N	5	8	5	5	8

Results of two analyses of CRH expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to CRH. Pooled verification was then performed using RT-PCR and primers designed in house. Primer sequences for pooled verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 14. Summary of the three analyses used to determine the effect of sugar solution access on hypothalamic DRD1a.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	28.87	29.51	29.41	28.08	28.87
	Fold change	1.00^{b}	0.83^{bc}	0.70^{c}	2.07^{a}	1.09^{b}
	n	5	8	5	5	8
Pooled verification	**Ct**	30.80	29.65	29.11	29.32	28.74
	Fold change	1.00^{b}	2.76^{ab}	3.32^{a}	2.75^{ab}	4.15^{a}
	n	5	8	5	5	8
Individual verification	**Ct**	38.96	39.32	39.22	38.27	39.02
	n	4	4	4	4	4

Results of three analyses of DRD1a expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to DRD1a. Pooled verification, followed by individual verification, was then performed using RT-PCR and primers designed in house. Primer sequences for pooled and individual verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Individual verification was performed on individual hypothalamic tissue from four rats from each group. Averages of the four individual verification assays are presented. Fold change is not presented for individual verification because Ct was greater than 35 and thus not 'significant.' Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 15. Summary of the three analyses used to determine the effect of sugar solution access on hypothalamic GH1.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	36.78	33.66	36.64	36.90	35.77
	Fold change	1.00^{b}	3.32^{a}	1.02^{b}	1.20^{b}	1.11^{b}
	n	5	8	5	5	8
Pooled verification	**Ct**	34.01	32.35	32.73	33.25	32.68
	Fold change	1.00^{d}	3.93^{a}	2.37^{bc}	1.67^{cd}	2.54^{b}
	n	5	8	5	5	8
Individual verification	**Ct**	38.95	33.66	38.80	37.56	38.29
	n	4	4	4	4	4

Results of three analyses of GH1 expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to GH1. Pooled verification, followed by individual verification, was then performed using RT-PCR and primers designed in house. Primer sequences for pooled and individual verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Individual verification was performed on individual hypothalamic tissue from four rats from each group. Averages of the four individual verification assays are presented. Fold change is not presented for individual verification because Ct was greater than 35 and thus not 'significant.' Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 16. Summary of analyses used to determine the effect of sugar solution access on hypothalamic IAPP.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	35.82	34.37	35.42	35.75	33.93
	Fold change	1.00[a]	2.24[a]	1.13[a]	1.20[a]	2.39[a]
	n	8	8	8	8	8
Pooled verification	**Ct**	34.82	32.86	34.36	34.27	33.24
	Fold change	1.00[c]	4.83[a]	1.36[c]	1.49[c]	2.98[b]
	n	5	8	5	5	8

Results of two analyses of IAPP expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to IAPP. Pooled verification was then performed using RT-PCR and primers designed in house. Primer sequences for pooled verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 17. Summary of the three analyses used to determine the effect of sugar solution access on hypothalamic NPY.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	25.80	25.98	25.77	24.88	25.90
	Fold change	1.00^{b}	1.14^{b}	1.03^{b}	2.27^{a}	1.02^{b}
	n	5	8	5	5	8
Pooled verification	**Ct**	24.69	26.03	25.10	24.72	25.13
	Fold change	1.00^{a}	0.49^{c}	0.73^{b}	0.96^{a}	0.74^{b}
	n	5	8	5	5	8
Individual verification	**Ct**	27.01	26.80	26.25	25.03	27.06
	Fold change	1.00^{c}	1.61^{b}	1.24^{c}	3.52^{a}	1.11^{c}
	n	4	4	4	4	4

Results of three analyses of NPY expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to NPY. Pooled verification, followed by individual verification, was then performed using RT-PCR and primers designed in house. Primer sequences for pooled and individual verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Individual verification was performed on individual hypothalamic tissue from four rats from each group. Averages of the four individual assays are presented. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 18. Summary of the three analyses used to determine the effect of sugar solution access on hypothalamic RAMP3.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	25.99	25.95	25.88	27.43	27.05
	Fold change	1.00^{b}	1.32^{a}	1.09^{ab}	0.44^{c}	0.53^{c}
	N	5	8	5	5	8
Pooled verification	**Ct**	22.20	21.75	21.61	23.04	23.02
	Fold change	1.00^{c}	1.70^{a}	1.47^{b}	0.55^{d}	0.57^{d}
	N	5	8	5	5	8
Individual verification	**Ct**	30.29	32.57	31.00	29.85	35.80
	Fold change	1.00^{b}	0.65^{c}	1.06^{b}	2.43^{a}	0.07^{d}
	N	4	4	4	4	4

Results of three analyses of RAMP3 expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to RAMP3. Pooled verification, followed by individual verification, was then performed using RT-PCR and primers designed in house. Primer sequences for pooled and individual verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Individual verification was performed on individual hypothalamic tissue from four rats from each group. Averages of the four individual assays are presented. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 19. Summary of analyses used to determine the effect of sugar solution access on hypothalamic TNF-α.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	32.76	32.40	32.30	32.96	32.55
	Fold change	1.00^{b}	1.65^{a}	1.40^{ab}	1.06^{b}	1.26^{ab}
	n	5	8	5	5	8
Pooled verification	**Ct**	34.48	34.24	33.71	34.90	33.74
	Fold change	1.00^{b}	1.48^{a}	1.67^{a}	0.74^{b}	1.67^{a}
	n	5	8	5	5	8

Results of two analyses of TNF-α expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to TNF-α. Pooled verification was then performed using RT-PCR and primers designed in house. Primer sequences for pooled verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 20. Summary of analyses used to determine the effect of sugar solution access on hypothalamic TRH.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	25.23	26.46	25.63	26.71	25.07
	Fold change	1.00^{b}	0.55^{d}	0.77^{c}	0.43^{d}	1.22^{a}
	n	5	8	5	5	8
Pooled verification	**Ct**	23.20	21.57	20.84	22.89	21.30
	Fold change	1.00^{c}	3.86^{b}	5.04^{a}	1.22^{c}	3.72^{b}
	n	5	8	5	5	8

Results of two analyses of TRH expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to TRH. Pooled verification was then performed using RT-PCR and primers designed in house. Primer sequences for pooled verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Table 21. Summary of analyses used to determine the effect of sugar solution access on hypothalamic UCN.

		Control	Glucose	Sucrose	HFCS	Fructose
PCR Array	**Ct**	35.83	35.50	33.34	34.74	35.78
	Fold change	1.00^{b}	1.11^{a}	4.08^{b}	1.44^{b}	1.02^{b}
	n	5	8	5	5	8
Pooled verification	**Ct**	37.01	33.99	36.95	36.48	36.45
	n	5	8	5	5	8

Results of two analyses of UCN expression in the hypothalamus of animals given 24 hour access to sugar solutions. Rat Obesity RT² Profiler™ PCR Array (PARN-017A, SABiosciences) was used to obtain initial estimates of changes to UCN. Pooled verification was then performed using RT-PCR and primers designed in house. Primer sequences for pooled verification assays are found in Table 1. PCR array and pooled verification assays were performed on pooled hypothalamic tissue from all animals in each group. Fold change is not presented for pooled verification because Ct was greater than 35 and thus not 'significant.' Averages of each measurement that share a common superscript are not different from one another ($p>0.05$).

Appendices

Appendix A. List of genes on RT^2 PCR array

Unigene	GeneBank	Symbol	Description	Gene Name
Rn.202559	NM_016989	Adcyap1	Adenylate cyclase activating polypeptide 1	Pacap
Rn.88408	NM_133511	Adcyap1r1	Adenylate cyclase activating polypeptide 1 receptor 1	PACAP-R1A, PACAPR1, PACAPR1A
Rn.24299	NM_144744	Adipoq	Adiponectin, C1Q and collagen domain containing	Acdc, Acrp30
Rn.104556	NM_207587	Adipor1	Adiponectin receptor 1	-
Rn.101984	NM_001037979	Adipor2	Adiponectin receptor 2	-
Rn.10296	NM_138505	Adra2b	Adrenergic, alpha-2B-, receptor	-
Rn.87064	NM_012701	Adrb1	Adrenergic, beta-1-, receptor	B1AR, RATB1AR
Rn.137597	NM_033650	Agrp	Agouti related protein homolog (mouse)	-
Rn.15739	NM_012737	Apoa4	Apolipoprotein A-IV	Apo-AIV, ApoA-IV, apoAIV
Rn.53846	NM_031351	Atrn	Attractin	-
Rn.11266	NM_012513	Bdnf	Brain-derived neurotrophic factor	MGC105254
Rn.86415	NM_152845	Brs3	Bombesin-like receptor 3	-
Rn.11378	NM_016994	C3	Complement component 3	-
Rn.90085	NM_017338	Calca	Calcitonin-related polypeptide alpha	CAL6, CGRP, Cal1, Calc, RATCAL6, calcitonin
Rn.10062	NM_053816	Calcr	Calcitonin receptor	-
Rn.89164	NM_017110	Cartpt	CART prepropeptide	Cart
Rn.9781	NM_012829	Cck	Cholecystokinin	-
Rn.10184	NM_012688	Cckar	Cholecystokinin A receptor	Cck-ar
Rn.6714	NM_013139	Clps	Colipase, pancreatic	COLQ

Appendix A. List of genes on RT^2 PCR array (continued)

Unigene	GeneBank	Symbol	Description	Gene Name
Rn.89774	NM_012784	Cnr1	Cannabinoid receptor 1 (brain)	SKR6R
Rn.6067	NM_013166	Cntf	Ciliary neurotrophic factor	-
Rn.55036	NM_001003929	Cntfr	Ciliary neurotrophic factor receptor	-
Rn.10349	NM_031019	Crh	Corticotropin releasing hormone	CRF
Rn.10499	NM_030999	Crhr1	Corticotropin releasing hormone receptor 1	-
Rn.24039	NM_012546	Drd1a	Dopamine receptor D1A	D1a, Drd-1, Drd1
Rn.87299	NM_012547	Drd2	Dopamine receptor D2	-
Rn.8929	NM_033237	Gal	Galanin prepropeptide	Galn
Rn.10213	NM_012958	Galr1	Galanin receptor 1	Galnr1
Rn.54383	NM_012707	Gcg	Glucagon	GLP-1
Rn.11225	NM_172092	Gcgr	Glucagon receptor	MGC93090
Rn.146351	NM_001034848	Gh1	Growth hormone 1	Gh, RNGHGP
Rn.2178	NM_017094	Ghr	Growth hormone receptor	GHR, BP, MGC124963, MGC156665
Rn.42103	NM_021669	Ghrl	Ghrelin/obestatin prepropeptide	-
Rn.74241	NM_032075	Ghsr	Growth hormone secretagogue receptor	-
Rn.11408	NM_012728	Glp1r	Glucagon-like peptide 1 receptor	Glip, RATGL1RCP
Rn.138127	NM_139193	Prlhr	Prolactin releasing hormone receptor	Gpr10, Uhr-1
Rn.10822	NM_031758	Mchr1	Melanin-concentrating hormone receptor 1	Gpr24, Mch-1r, Slc1
Rn.10930	NM_133570	Grp	Gastrin releasing peptide	-
Rn.10316	NM_012706	Grpr	Gastrin releasing peptide receptor	-
Rn.7628	NM_013179	HcRt	Hypocretin	orexin-A
Rn.88262	NM_013064	Hcrtr1	Hypocretin (orexin) receptor 1	Hctr1

Appendix A. List of genes on RT^2 PCR array (continued)

Unigene	GeneBank	Symbol	Description	Gene Name
Rn.81032	NM_017018	Hrh1	Histamine receptor H 1	Hisr
Rn.9935	NM_012765	Htr2c	5-hydroxytryptamine (serotonin) receptor 2C	5-HT2C, 5-HTR2C, 5HT-1C
Rn.11394	NM_012586	Iapp	Islet amyloid polypeptide	-
Rn.12300	NM_017019	Il1a	Interleukin 1 alpha	IL-1 alpha
Rn.9869	NM_031512	Il1b	Interleukin 1 beta	-
Rn.9758	NM_013123	Il1r1	Interleukin 1 receptor, type I	-
Rn.9873	NM_012589	Il6	Interleukin 6	ILg6, Ifnb2
Rn.1716	NM_017020	Il6r	Interleukin 6 receptor	IL6R1, Il6ra
Rn.962	NM_019129	Ins1	Insulin 1	-
Rn.989	NM_019130	Ins2	Insulin 2	-
Rn.9876	NM_017071	Insr	Insulin receptor	-
Rn.44444	NM_013076	Lep	Leptin	OB, obese
Rn.9891	NM_012596	Lepr	Leptin receptor	Fa
Rn.215838	NM_001025270	Mc3r	Melanocortin 3 receptor	-
Rn.130371	XM_218815	Nmb	Neuromedin B	RGD1562710
Rn.89709	NM_012799	Nmbr	Neuromedin B receptor	NMB-R
Rn.47720	NM_022239	Nmu	Neuromedin U	-
Rn.54457	NM_023100	Nmur1	Neuromedin U receptor 1	Gpr66
Rn.9714	NM_012614	Npy	Neuropeptide Y	NPY02, RATNPY, RATNPY02
Rn.11642	NM_001113357	Npy1r	Neuropeptide Y receptor Y1	MGC109393, NPY-1
Rn.90070	NM_012576	Nr3c1	Nuclear receptor subfamily 3, group C, member 1	GR, Gcr, Grl
Rn.39098	NM_021589	Ntrk1	Neurotrophic tyrosine kinase, receptor, type 1	Trk
Rn.60814	NM_001102381	Nts	Neurotensin	-
Rn.200149	NM_001108967	Ntsr1	Neurotensin receptor 1	Ntsr
Rn.89571	NM_017167	Oprk1	Opioid receptor, kappa 1	-

Appendix A. List of genes on RT^2 PCR array (continued)

Unigene	GeneBank	Symbol	Description	Gene Name
Rn.10118	NM_013071	Oprm1	Opioid receptor, mu 1	MORA, Oprm, Oprrm1
Rn.1129	NM_030996	Sigmar1	Sigma non-opioid intracellular receptor 1	Oprs1
Rn.108195	NM_139326	Pomc	Proopiomelanocortin	Pomc1, Pomc2
Rn.9753	NM_013196	Ppara	Peroxisome proliferator activated receptor alpha	PPAR
Rn.23443	NM_013124	Pparg	Peroxisome proliferator-activated receptor gamma	-
Rn.19172	NM_031347	Ppargc1a	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	Ppargc1
Rn.11317	NM_012637	Ptpn1	Protein tyrosine phosphatase, non-receptor type 1	MGC93562, Ptp
Rn.13173	NM_001034080	Pyy	Peptide YY (mapped)	GHYY, RATGHYY, Yy, peptide-YY
Rn.48672	NM_020100	Ramp3	Receptor (G protein-coupled) activity modifying protein 3	-
Rn.11286	NM_031767	Sort1	Sortilin 1	Nt3, Nts3
Rn.34418	NM_012659	Sst	Somatostatin	SS-14, SS-28, Smst
Rn.42915	NM_012719	Sstr1	Somatostatin receptor 1	Gpcrrna
Rn.88692	NM_012672	Thrb	Thyroid hormone receptor beta	C-erba-beta, ERBA2, Nr1a2, RATT3REC, T3rec, TRbeta
Rn.2275	NM_012675	Tnf	Tumor necrosis factor (TNF superfamily, member 2)	MGC124630, RATTNF, TNF-alpha, Tnfa

Appendix A. List of genes on RT^2 PCR array (continued)

Unigene	GeneBank	Symbol	Description	Gene Name
Rn.22	NM_013046	Trh	Thyrotropin releasing hormone	THR, TRH01
Rn.9962	NM_013047	Trhr	Thyrotropin releasing hormone receptor	-
Rn.10190	NM_019150	Ucn	Urocortin	-
Rn.10281	NM_012682	Ucp1	Uncoupling protein 1 (mitochondrial, proton carrier)	MGC108736, Ucp, Ucpa, Uncp
Rn.973	NM_001007604	Rplp1	Ribosomal protein, large, P1	MGC72935
Rn.47	NM_012583	Hprt1	Hypoxanthine phosphoribosyltransferase 1	Hgprtase, Hprt, MGC112554
Rn.92211	NM_173340	Rpl13a	Ribosomal protein L13A	-
Rn.107896	NM_017025	Ldha	Lactate dehydrogenase A	Ldh1
Rn.94978	NM_031144	Actb	Actin, beta	Actx
N/A	U26919	RGDC	Rat Genomic DNA Contamination	RGDC
N/A	SA_00104	RTC	Reverse Transcription Control	RTC
N/A	SA_00104	RTC	Reverse Transcription Control	RTC
N/A	SA_00104	RTC	Reverse Transcription Control	RTC
N/A	SA_00103	PPC	Positive PCR Control	PPC
N/A	SA_00103	PPC	Positive PCR Control	PPC
N/A	SA_00103	PPC	Positive PCR Control	PPC

List of genes on RT^2 PCR array (SABioscience, PARN-017A). Utilizing RT-PCR analysis, each array profiles the expression of 84 genes related to obesity, including genes that code for orexigenic peptides, hormones, and receptors, anorectic peptides, hormones, and receptors, and central and peripheral signaling molecules related to energy expenditure. Array also includes 5 housekeepers and 7 controls.

Appendix B. Results of RT^2 PCR array

	Fold Change (comparing to control group)			
Symbol	**Fructose**	**Glucose**	**HFCS**	**Sucrose**
Adcyap1	1.0182	1.0345	1.0852	1
Adcyap1r1	0.7479	0.7366	0.6704	0.7873
Adipoq	2.3311	3.5406	1.3315	1.014
Adipor1	0.97	1.1439	0.9781	0.9727
Adipor2	0.9801	1.0526	0.8111	0.9428
Adra2b	0.48	1.1559	0.347	0.8586
Adrb1	1.0324	1.1599	1.2167	0.8322
Agrp	1.0541	1.2649	1.9834	0.9593
Apoa4	0.5767	0.8639	1.1511	0.2784
Atrn	0.97	0.9069	0.8055	0.8526
Bdnf	1.0468	1.2388	1.2861	1.0497
Brs3	1.0989	0.9356	1.0056	1.0905
C3	0.9019	1.2218	0.9679	1.2702
Calca	0.8712	0.76	0.694	1.1134
Calcr	1.1736	0.76	1.0777	1.1408
Cartpt	0.8213	0.9389	1.1157	1.0497
Cck	0.6289	1.9779	1.3928	1.2269
Cckar	1.1736	0.7366	0.8111	0.8766
Clps	0.9767	1.5572	1.5616	1.4794
Cnr1	1.1181	0.8061	1.2596	0.917
Cntf	0.9835	1.0063	0.8456	0.9526
Cntfr	1.0578	1.0203	0.8876	0.9044
Crh	1.2066	0.873	0.484	0.7552
Crhr1	1.0253	1.0417	0.9221	0.8265
Drd1a	1.0837	0.8259	2.0677	0.6974
Drd2	1.2449	1.3186	1.4671	0.9862
Gal	1.08	0.8202	1.1794	1.0353
Galr1	1.08	0.7895	0.8397	0.9593
Gcg	1.0875	1.287	1.2	1.3708
Gcgr	1.1736	0.9324	0.935	0.9862
Gh1	1.0875	3.2693	1.2	1.014
Ghr	1.0725	0.852	1.0231	1.0353
Ghrl	1.0147	0.9292	0.7361	0.8351
Ghsr	1.2492	0.855	1.0126	0.8858
Glp1r	1.0762	0.9069	1.1551	0.8675
Prlhr	0.95	0.6547	0.5657	0.9395
Mchr1	1.0007	0.9356	0.9382	0.933
Grp	1.1258	0.8882	1.0852	1.1647

Appendix B. Results of RT^2 PCR array (continued)

	Fold Change (comparing to control group)			
Symbol	**Fructose**	**Glucose**	**HFCS**	**Sucrose**
Grpr	1.19	0.8699	0.8693	1.1212
HcRt	0.6139	0.7392	1.1392	0.8888
Hcrtr1	1.0725	0.8287	0.7889	0.9013
Hrh1	1.0147	0.8033	0.7972	0.8467
Htr2c	1.1142	0.9356	0.8111	1.014
Iapp	2.2831	1.9986	1.2	1.1096
Il1a	1.0147	1.1925	0.9546	1.7471
Il1b	1.2932	0.9487	0.8426	1.1975
Il1r1	1.1655	1.0563	0.9781	1.0317
Il6	0.9176	1.1243	0.8282	1.5105
Il6ra	1.2277	1.1802	1.2083	1.3472
Ins1	0.9938	1.0973	0.7621	0.9363
Ins2	0.7989	0.6616	0.4707	0.9727
Insr	0.9767	0.8005	0.8938	0.9202
Lep	1.0875	1.287	1.2	1.014
Lepr	0.7906	1.2959	0.9849	1.0981
Mc3r	1.0762	0.8944	1.0629	0.9559
Nmb_predicted	1.08	1.0168	1.0592	0.9593
Nmbr	0.7797	1.06	0.6988	0.8858
Nmu	1.0837	1.105	1.3041	0.3776
Nmur1	1.0989	1.0822	1.0852	1.0461
NPY	1.0112	1.1321	2.2705	1.0317
NPY1r	0.9801	0.9653	0.831	1.1057
Nr3c1	0.905	0.9389	0.8139	0.9559
Ntrk1	1.8545	1.1966	1.2041	0.9428
Nts	1.2932	0.9586	1.1511	1.244
Ntsr1	1.0951	1.4379	0.9382	1.0943
Oprk1	0.9633	0.76	0.8282	0.9298
Oprm1	0.8386	0.989	0.5795	0.9931
Oprs1	1.1455	0.952	0.9	0.9526
Pomc	1.0837	0.8639	1.1471	0.8066
Ppara	1.1416	1.0822	0.7889	1.0681
Pparg	1.0504	1.3604	0.726	1.1567
Ppargc1a	0.9733	1.0098	0.9	0.895
Ptpn1	1.0762	1.0238	1.0446	1.0681
Pyy	1.0182	0.5963	0.632	1.0461
Ramp3	0.5216	1.3186	0.4407	1.0943
Sort1	1.0288	1.0747	1.089	1.007

Appendix B. Results of RT^2 PCR array (continued)

	Fold Change (comparing to control group)			
Symbol	**Fructose**	**Glucose**	**HFCS**	**Sucrose**
Sst	1.1181	0.8287	0.831	1.0035
Sstr1	1.1219	0.8316	1.0303	0.8039
Thrb	1.0147	0.7732	1.1672	0.8123
Tnf	1.2535	1.646	1.041	1.3899
Trh	1.2193	0.5506	0.4302	0.7711
Trhr	1.0913	1.105	0.9849	0.9931
Ucn	1.0875	4.0812	1.4369	1.014
Ucp1	0.9633	0.784	0.8633	0.8438
Rplp1	0.9801	0.9993	0.9986	0.9965
Hprt1	0.9369	1.0028	1.1119	1.0389
Rpl13a	1.0651	0.9686	0.9063	1.0792
Ldha	0.9337	1.0133	1.0021	0.8766
Actb	1.0951	1.0168	0.9917	1.021
RGDC	1.0875	1.287	1.2	1.014
RTC	0.9467	0.8491	0.9917	0.9862
RTC	0.9938	0.8852	1.0056	0.9965
RTC	1.0504	0.876	1.0629	1.0389
PPC	1.0432	1.2825	1.2816	1.057
PPC	1.2406	1.3232	1.35	1.0497
PPC	1.1818	1.305	1.2466	1.0943

Complete results of findings from RT^2 PCR array (SABioscience, PARN-017A). Presented is the average fold change from two replicate experiments. PCR array was performed on pooled hypothalamic tissue from all animals in each group. Fold change was obtained from manufactures online analysis tool and calculated as 2^(-ddCt).

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