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A free-choice high-fat diet modulates the effects of a sucrose bolus on the expression of genes involved in glucose handling in the hypothalamus and nucleus accumbens.

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ABSTRACT

The consumption of saturated fat and sucrose can have synergistic effects on the brain that do not occur when either nutrient is consumed by itself. In this study we hypothesize that saturated fat intake modulates glucose handling in the hypothalamus and nucleus accumbens, both brain areas highly involved in the control of food intake. To study this, male Wistar rats were given a free-choice high fat diet (fCHFD) or a control diet for two weeks. During the last seven days rats were given a daily bolus of either a 30% sucrose solution or water. Rats were sacrificed on day eight, 30 minutes after the onset of drinking. mRNA and protein levels of genes involved in glucose handling were assessed in the hypothalamus and nucleus accumbens. We found increased *Glut3* and *Glut4* mRNA in the hypothalamus of fCHFD-fed rats without an additional effect of the sucrose bolus. In the nucleus accumbens, the sucrose bolus increased *Glut3* mRNA and decreased *Glut4* mRNA independent of prior diet exposure. The ATP-sensitive potassium channel subunit Kir6.1 in the nucleus accumbens tended to be affected by the synergistic effects of a fCHFD and a sucrose bolus. These data suggest that acute glucose handling in the hypothalamus and nucleus accumbens may be affected by prior high fat exposure.

1. Introduction

A Western style diet, typically defined as a diet high in saturated fat and simple carbohydrates, is one of the major contributors to the current obesity epidemic. The consumption of sugar-sweetened beverages in addition to a Western diet further increases the risk for weight gain and metabolic disease [1]. This might be explained by synergistic effects of combined sugar and fat consumption on overall food intake. In line, we earlier showed that a high-fat high-sucrose hypercaloric diet induced changes in the brain serotonergic system in humans while a matched high-sucrose hypercaloric diet did not, suggesting that combining fat and sugar elicits differential effects on brain circuitries involved in food intake that sugar alone does not. [2]. In addition, subjects are willing to pay more for a high-fat high-sucrose treat compared to a high-fat or a high-sucrose treat, and this effect is associated with a

larger activation of brain areas involved in reward assessment [3]. In rat studies, we have found that the consumption of saturated fat combined with a sucrose solution in addition to chow pellets and water, induced persistent hyperphagia and increased motivation to work for a sucrose reward, resulting in an obese phenotype [4,5]. Remarkably, addition of either saturated or a sucrose solution alone to a chow diet did not induce these effects. The concurrent consumption of saturated fat and a sucrose solution also has unique interacting effects on dopamine receptor availability in the nucleus accumbens [6], neuropeptide Y (NPY) and proopiomelanocortin (POMC) gene expression in the arcuate nucleus [4] and central NPY sensitivity [7]. However, in these studies, rats had *ad libitum* access to all dietary components. Thus, we could not assess whether these molecular and functional changes in the brain resulted directly from consumption of the nutrients, or from the multitude of metabolic disturbances that accompany the obese

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phenotype induced in the rats by the consumption of a high-fat high sucrose diet [8].

We therefore studied the direct effect of a daily oral sucrose bolus in rats on a chow or free-choice high-fat diet (fCHFD) on the hypothalamus and nucleus accumbens, both highly involved in the control of food intake [9,10]. Consumption of a sucrose bolus will have multiple physiological effects, and can affect brain glucose handling through two different mechanisms. Firstly, a change in peripheral glycaemia levels will alter extracellular glucose levels in the hypothalamus and nucleus accumbens, which can affect the activity of neurons directly. This phenomenon is referred to as glucose-sensing. For example, low extracellular glucose concentrations alter the activity of orexin neurons in the lateral hypothalamus [11]. Secondly, the hedonic properties of sucrose drinking will activate neuronal pathways, which will cause an increase in fuel utilization of glucose in these neurons. This is shown in the nucleus accumbens where glucose utilization is increased after sugar drinking, likely due to the involvement of accumbal neurons in motivated behavior [12]. Interestingly, a high fat diet can alter glucose handling through both mechanisms. Glucose utilization is affected by a high fat diet through altering brain glucose consumption and metabolism [13]. In addition to glucose utilization, the neuronal sensitivity to sensing extracellular glucose in the arcuate nucleus of the hypothalamus can also be affected by the presence of fatty acids [14]. In this exploratory study, we first focused on the machinery involved in glucose handling, which thus involves mRNA and protein expression of genes involved in both glucose sensing and glucose utilization. More specifically, we hypothesize that exposure to a high fat diet alters sucrose bolus induced expression of genes in glucose handling in the hypothalamus and nucleus accumbens.

2. Methods

2.1. Animals

All experiments were performed in male Wistar rats (Charles River Breeding Laboratories, Sulzfeld, Germany) weighing 240-280 grams at arrival to the animal facility of The Netherlands Institute for Neuroscience (Amsterdam, The Netherlands). Rats were housed in temperature- ($21 \pm 2^\circ\text{C}$), humidity- ($60 \pm 5\%$) and light-controlled (12:12hr light/dark; lights on 07:00-19:00) rooms with background noise (radio) during the entire experiment. During acclimatization, rats had free access to a container with nuggets of a nutritionally-complete high-carbohydrate control diet (chow; Teklad global diet 2918; 24% protein, 58% carbohydrate, and 18% fat, 3.1 kcal/g, Envigo, Horst, The Netherlands) and a bottle of tap water. The animal ethics committee of the Netherlands Institute for Neuroscience approved all experiments according to Dutch legal ethical guidelines.

2.2. Diet paradigm

Experiments were performed in two cohorts (cohort 1: $n=34$, cohort 2: $n=32$), both receiving the same experimental treatment. During the first week of the experiment, half of the experimental rats continued consuming the control diet and a bottle of water *ad libitum*. The other half of the experimental rats consumed a fCHFD (consisting of a container of standard chow pellets, a dish of beef tallow (Ossenwit/Blanc de boeuf, Belgium) and a bottle of tap water). During the second week of the experiment, the diet groups were further split into four groups, resulting in the following experimental groups: chow water (chow W), chow sucrose (chow S), fCHFD W and fCHFD S. The chow W and fCHFD W groups daily received a second bottle of tap water for five minutes (between 16:00-16:05), whereas the chow S and fCHFD S groups received a 30% sucrose solution during the same timeframe. However, the chow S group was restricted to a shorter time period to match the sucrose intake of the fCHFD S group (also starting at 16:00). Food, water intake and body weight were daily measured. On the 8th consecutive

day of water or sucrose consumption, rats were sacrificed 30 minutes after the onset of sucrose/water drinking by decapitation under carbon dioxide sedation. 30 minutes is sufficient to study the effect of changes in extracellular glucose in the brain, as these glucose levels peak at 20 minutes after the onset of sucrose drinking [12] and mRNA levels rise within 5-10 minutes after induction [15]. Unilateral epididymal white adipose tissue (eWAT) pads were dissected and weighed. Brains were quickly dissected, frozen rapidly on dry ice, and stored at -80°C until analysis.

2.3. Brain dissection

Coronal brain slices of 200 μm were obtained using a cryostat and put directly in RNA stabilizing solution (Ambion, Thermofisher Scientific, United Kingdom). For the nucleus accumbens, 9 slices were punched with a small punching needle (diameter 1100 μm), starting at Bregma 3,00 (Rat atlas, George Paxinos, and Charles Watson). Bilateral punches were combined for analysis. The hypothalamus was dissected from 12 slices starting at Bregma -0,48. The hypothalamus in each slice was bilaterally cut into two parts along the third ventricle. One part was used for quantitative real-time PCR (RT-qPCR; cohort 1) or protein analysis (cohort 2).

2.4. Selection of genes

To measure the response to a sucrose bolus, we selected a panel of genes that are involved in glucose handling. These include all known brain glucose transporters, glucokinase (GCK), representing the first step in intracellular glucose metabolism and Kir6.1, Kir6.2 that form channels together with SUR1 and SUR2 and that are controlled by changes in the ATP/ADP ratio. To assess intracellular energy status, we measured downstream targets of phosphorylated AMP-activated protein kinase (AMPK), i.e. nitric oxide synthase (NOS), and the nitric oxide receptors soluble guanylyl cyclase a (SGCa) and b (SGCb) [16].

2.5. mRNA extraction and RT-qPCR

Hypothalamus and nucleus accumbens samples from cohort 1 were used to assess mRNA expression. Tissue was placed in TRIreagent (Ambion, Thermofisher Scientific, United Kingdom) and homogenized using an ULTRA THURRAX homogenizer (IKA, Germany). The Isolate II RNA Mini Kit was used to isolate RNA, according to the manufacturer's instructions (Bioline, United Kingdom). 300 ng of total RNA was used per cDNA synthesis reaction using the "Transcriptor First Strand cDNA synthesis kit" for RT-PCR with oligo(dT) primers (Roche Molecular Biochemicals, Germany). RT-qPCR was performed using the primers and temperatures listed in Table 1. PCR efficiency was checked for all samples individually using LinRegPCR [17]. The starting RNA concentration expressed in arbitrary fluorescence units, is calculated using the mean PCR efficiency per sample and the Cq value per sample. The starting RNA concentration was then normalized for by the gene expression of housekeeping genes. For the mRNA expression in the nucleus accumbens, all gene expression was normalized by the geometric mean of *Cyclophilin*, *Hprt* and *UbiquitinC* expression. In the hypothalamus, only *Cyclophilin* was used, as all other tested housekeeping genes were affected by the diet or sucrose consumption.

2.6. Protein analysis using protein simple

Hypothalamus and nucleus accumbens samples from cohort 2 were homogenized in RIPA buffer (50 mM Tris HCL, pH = 7.6, 150 mM NaCl, 1% Triton \times 100, 0.5% Sodium Desoxycholate, and 0.1% SDS, 2 mM EDTA), enriched with protease (Complete EDTA-free protease inhibitor cocktail tablets, Roche) and phosphatase (PhosSTOP, Roche, Mannheim, Germany) inhibitors according to manufacturer's instructions. Total protein levels were measured using BioRad Protein Assay

Table 1

Primers used for RT-qPCR and temperature of RT-qPCR reaction. Official gene name, when different from protein name, is listed between brackets. Abbreviations used: Hypoxanthine-guanine phosphoribosyltransferase (*Hprt*), Sulfonylurea receptor (*Sur*), Glucose transporter (*Glut*), Glucokinase (*Gck*), Sodium-glucose transporter (*Sglt*), Nitric oxide synthase (*Nos*), Soluble guanylyl cyclase (*SGC*).

Gene	Forward primer	Reverse primer	Temperature of RT-qPCR reaction
<i>Hprt</i>	CCATCACATTGTGGCCCTCT	TATGTCCCCGTTGACTGGT	65°C
<i>Cyclophilin</i>	TGTTCTTCGACATCACGGCT	CCGTAGATGGACTTGCCACC	65°C
<i>UbiquitinC</i>	TCGTACCTTTCTCACCACAGTATCTAG	GAAAACCTAAGACACCTCCCATCA	65°C
<i>Kir6.1</i> (KCNJ8)	AGCTGGCTGCTCTTCGCTATCA	CCCTCCAAACCCAATGGTCACT	65°C
<i>Kir6.2</i> (KCNJ11)	CAACGTCGCCACAGAAGACATC	CCAGCTGCACAGGAAGGACATG	65°C
<i>Sur1</i> (ABCC8)	CAGGACCAAGAGCTGGAGAAGGA	CATCCAGCAGAAGGCCATCTCT	65°C
<i>Sur2</i> (ABCC9)	TTACCACTATCCGGGCCTTC	AATGCAAGCTCCCAGGTAG	65°C
<i>Glut1</i> (SLC2A1)	ACGTCCATTCTCCGTTTCAC	TCCCACGGCCAACATAAG	65°C
<i>Glut2</i> (SLC2A2)	GTCAGAAAAGCCCAGATAAC	TGCCCTTAGTCTTTTCAAGCT	65°C
<i>Glut3</i> (SLC2A3)	GCTCTGGTGTATGTGTGG	TCAACCGACTCCGCTATCTT	65°C
<i>Glut4</i> (SLC2A4)	GGGCTGTGAGTGAGTGCTTTC	CAGCGAGGCAAGGCTAGA	65°C
<i>Glut6</i> (SLC2A6)	CACCTGGCTCCTCATGTCTG	ACATCCTGCGAGTGTGGAAG	65°C
<i>Gck</i>	CAAGCTGCACCCGAGCTT	TGATTTCGATGAAGGTGATTTTC	70°C
<i>Sglt1</i> (SLC5A1)	GCCTACGGAAGTGGAAAGCTG	GACGGTGACGACGCTGATAG	70°C
<i>Glut8</i> (SLC2A8)	TGGTTGTCAGTGGCATCCTC	GTAAGGGTTCGGGTACTTT	65°C
<i>Nos</i>	CGACCATCTCTACGCCACA	AAACAGGCAATATCGGGGCA	65°C
<i>SGCa</i> (GUCY1A2)	TCATACCATGCTCAAGCT	ACGCACATTAGGCTGTCAAC	65°C
<i>SGCb</i> (GUCY1B2)	GGAGCCAATGCAAGTCTGGT		

for spectrophotometry (BioRad, USA). Using an automated size resolving capillary electrophoresis system called 'WES' (Protein Simple, USA), protein levels were measured according to manufacturer's instructions. For GLUT3, samples were diluted in 1x sample buffer to a concentration of 0,25 µg/µL of total protein. GLUT3 antibody (ab125465, abcam, USA) was diluted 1:10. For GLUT4, samples were diluted in 1x sample buffer to a concentration of 0,4 µg/µL of total protein. Samples were denaturated at 55°C for 15 minutes. GLUT4 antibody (sc53566, Santa Cruz Biotechnology, USA) was diluted 1:4. For Kir6.1, samples were diluted in 0,1x sample buffer to a concentration of 1 µg/µL of total protein. Samples were denaturated at 70°C for 10 minutes. Kir6.1 antibody (PA5-41028, ThermoFisher Scientific, USA) was diluted 1:8. Area under the curves (AUCs) were calculated for the correct peak in the capillary electrophoresis results. Peaks were manually selected, in a blind fashion, to obtain the best-fitting peak of interest. All samples were measured in duplicates, results were removed if variation between 2 duplicate samples exceeded 20%.

2.7. Statistics

Data are shown as mean ± SEM. In addition, individual data points are shown in the graphs. SPSS (SPSS Inc., USA) and Graphpad Prism (GraphPad Software, USA) were used to perform statistical analyses. Assessment of effects in experiments involving diet and drink conditions was performed using two-way analysis of variance (ANOVA), followed, when appropriate, by Tukey HSD post hoc tests, adjusting for multiple comparisons. Statistical outliers were determined using a Grubb's test for statistical outliers (GraphPad Software, USA), and removed if they were statistically an outlier. For all cases, a p-value < 0.05 was considered significant.

3. Results

3.1. A fCHFD increases body weight, adiposity and total caloric intake independent of daily sucrose bolus

During two weeks of access to a fCHFD, rats showed an average higher total caloric intake, resulting in a significantly greater body weight (in cohort 1) and adiposity (in both cohorts), compared to chow fed controls (Table 2.). fCHFD-fed rats in cohort 2 showed a trend for a higher final body weight (p=0,05). Total average sucrose intake during the daily 5 minutes timeframe was similar between chow-fed controls and fCHFD-fed animals. The daily ingested calories from the sucrose solution did not alter total caloric intake in both chow and fCHFD-fed

groups (Table 2). See supplemental figure 1 for the body weight, food intake and sucrose bolus consumption throughout the course of the experiment.

3.2. *Glut3*, *Glut4* and *Kir6.1* are differentially affected by the fCHFD and the sucrose bolus

We tested a panel of genes involved in glucose uptake, metabolism and subsequent changes in membrane potential, in the hypothalamus and nucleus accumbens. *Glut1*, *Glut2*, *Glut6*, *Glut8*, *Nos*, *Sgca*, *Sgcb*, *Kir6.2*, *Sur1* and *Sur2* mRNA expression was not significantly different between the four experimental groups (Table 3). *Sglt1* was expressed at such a low level, that it could not be accurately measured using RT-qPCR. *Gck* was not altered by the diet or sucrose drinking and was undetectable in the nucleus accumbens (Table 3).

In the hypothalamus *Glut3* and *Glut4* mRNA expression was increased by the fCHFD (Fig. 1A, 1B). Notably, consumption of sucrose solution increased expression of *Glut3* in the nucleus accumbens, whereas it downregulated *Glut4* expression, both independent of diet consumption (Fig. 1C, 1D). We subsequently measured GLUT3 and GLUT4 protein in the hypothalamus and nucleus accumbens. Unfortunately, the GLUT3 assay showed large inter-plate variability and was therefore deemed not reliable enough. GLUT4 protein was similar in the four experimental groups (Fig. 2), both in the hypothalamus and the nucleus accumbens.

An interacting effect of the diet and sucrose drink was found for *Kir6.1* mRNA in the hypothalamus and a trend for this interaction effect was found in the nucleus accumbens (Fig. 3A, 3C). The post-hoc test revealed no significant differences in *Kir6.1* mRNA in the hypothalamus between the chow W and chow S group, or HF W of HF S group. In the hypothalamus, no differences in *Kir6.1* protein levels were found between the groups, whereas the nucleus accumbens again showed a strong trend (Fig. 3B, 3D).

4. Discussion

In this study, we investigated the effects of a fCHFD on sucrose bolus induced expression of genes involved in glucose handling in the hypothalamus and nucleus accumbens. Remarkably, only the expression of *Glut3* and *Glut4* was modulated by the concurrent consumption of fCHFD and a sucrose solution, whereas *Glut1*, *Glut2*, *Glut6* and *Glut8* expression remained unaltered. This suggests that early diet induced changes involve specific subtypes of the glucose transporter family. Moreover, we found differential effects of consumption of a fCHFD or a

Table 2
Overview of final body weight, average chow intake, fat intake and sucrose intake during the course of the experiment, and adiposity in cohorts 1 and 2. Data are shown as mean \pm SEM. Cohorts were split into 4 experimental groups (chow W, chow S, HF W, and HF S).

	Cohort 1				Cohort 2					
	Chow W (n=8)	Chow S (n=9)	fcHFD W (n=8)	fcHFD S (n=9)	Diet or drink effect	Chow W (n=8)	Chow S (n=8)	fcHFD W (n=8)	fcHFD S (n=8)	Diet or drink effect
Final body weight (gr)	341,88 \pm 4,93	341,67 \pm 3,86	351,50 \pm 3,45S	348,56 \pm 3,69	Diet p<0,05	350,88 \pm 8,98	351,63 \pm 2,97	361,25 \pm 5,10	365,63 \pm 5,10	Diet p=0,050
Chow intake (kcal/day)	67,41 \pm 1,50	65,18 \pm 1,08	43,42 \pm 1,53	44,27 \pm 1,87	Diet p<0,05	69,07 \pm 1,78	66,32 \pm 1,41	51,47 \pm 2,08	49,41 \pm 0,83	Diet p<0,05
Fat intake (kcal/day)			34,75 \pm 5,46	35,15 \pm 3,85				37,72 \pm 3,91	40,30 \pm 2,00	
Sucrose bolus (kcal/5 min)		5,05 \pm 0,23		4,87 \pm 0,57			3,73 \pm 0,05		3,88 \pm 0,29	
Total intake (kcal/day)	67,41 \pm 1,50	70,23 \pm 1,04	78,17 \pm 5,97	84,29 \pm 4,73	Diet p<0,05	69,07 \pm 1,78	70,05 \pm 1,42	89,19 \pm 3,75	93,60 \pm 2,30	Diet p<0,05
eWAT (gr/100grBW)	0,43 \pm 0,02	0,45 \pm 0,01	0,62 \pm 0,03	0,60 \pm 0,02	Diet p<0,05	0,52 \pm 0,02	0,52 \pm 0,02	0,59 \pm 0,04	0,70 \pm 0,02	Diet p<0,05

sucrose solution on *Glut3* and *Glut4* expression in the hypothalamus and nucleus accumbens. GLUT3 is predominantly expressed in neurons, and is the main transporter that facilitates glucose uptake by neurons [18]. GLUT4 is less abundantly expressed in the brain, but similar its mode of action in the periphery, is regulated by insulin signaling [19]. In this study, we found that hypothalamic *Glut3* and *Glut4* mRNA were increased by consumption of a fCHFD, independent of the addition of a sucrose solution. Little is known about the direct effects of saturated fat on the expression of glucose transporters. Moreover, a fCHFD will not only increase the presence of triglycerides in the blood, but will also induce an obese phenotype that is associated with dysregulation of multiple metabolic hormones [8,20]. Whereas only a very few studies have investigated the effect of these metabolic factors on hypothalamic glucose transporter gene expression, a handful of studies have shown that metabolic factors can affect glucose transporter translocation to the membrane. For example, insulin and leptin can both induce GLUT4 translocation [19]. Specifically, leptin is interesting in this regard, as we have previously shown that a fCHFD increases leptin levels [8,20]. Leptin is less likely to induce changes on *Glut3*, as mice lacking leptin receptors show unaltered GLUT3 expression [21]. GLUT3 translocation is enhanced by insulin [22] and ghrelin [23]. However, whether these metabolic factors influence not only translocation, but mRNA expression as well is unknown.

In contrast to the observations in the hypothalamus, consumption of a sucrose solution increased *Glut3* expression and lowered *Glut4* expression in the nucleus accumbens, whereas no effects of the fCHFD were observed. As mentioned previously, changes in glucose handling can be due to fluctuations in extracellular glucose or to altered neuronal activity and subsequent fuel utilization. As GLUT3 is one of the main glucose transporters responsible for providing fuel to neurons due to its high affinity for glucose [18], the increase in *Glut3* expression likely reflects an increase in neuronal activity and a subsequent increased demand for glucose. In line with this hypothesis, neuronal utilization of glucose has previously been shown to be increased in the nucleus accumbens upon sucrose consumption [12]. Lower *Glut4* expression following the consumption of a sucrose solution could be an insulin-dependent effect. Studies have shown that *Glut4* mRNA is decreased in adipocytes upon insulin stimulation [24,25]. The insulin receptor is abundantly expressed in the nucleus accumbens [26], highlighting the possibility that a similar decrease in *Glut4* mRNA could also occur in neurons upon stimulation with insulin. As shown in the study by Flores-Rivera *et al.*, a decrease in GLUT4 protein followed the decreases *Glut4* expression, but this did occur with a delay [24]. As gene and protein expression were both assessed 30 minutes after the onset of sucrose/water drinking, this potentially explains why we did not observe changes in GLUT4 levels following consumption of a sucrose solution.

Moreover, both Kir6.1 mRNA as well as protein expression showed a strong tendency to be synergistically affected by the consumption of a fCHFD and a sucrose bolus. Most interestingly, a sucrose bolus tended to increase Kir6.1 protein expression in the nucleus accumbens of rats fed a chow diet, but this effect was not seen in rats fed a fCHFD. While Kir6.1, a channel sensitive to changes in cellular energy status, is predominantly expressed on astrocytes throughout the brain, it is also found on cholinergic interneurons in the nucleus accumbens [27]. These neurons only make up a very small proportion (1-2%) of the neurons in the nucleus accumbens [28], but they widely project to medium spiny neurons in the nucleus accumbens [29], thereby influencing dopamine release [30]. The tendency for increased expression of Kir6.1 after the consumption of a sucrose solution might thus reflect increased excitability of cholinergic interneurons, indirectly implying an activation of the reward system. As this effect is not present in rats fed a fCHFD, we could speculate that this alters reward processing of sucrose when rats are fed a fCHFD. Lastly, it is worth noting that similar to the effects on *Glut4* expression, the effects on Kir6.1 could also be mediated by insulin and not merely by the sucrose itself. It has been demonstrated that insulin can also increase the excitability of

Table 3
 An overview of all measured genes using RT-qPCR. Data are shown as mean ± SEM in an arbitrary unit (gene of interest expression normalized by housekeeping gene expression). Not detected (N.D.) indicates genes that were expressed at such a low level that they could not be reliable measured using the RT-qPCR approach.

	Hypothalamus				Nucleus accumbens				Diet or drink effect
	Chow W (n = 8)	Chow S (n = 9)	fcHFD W (n = 8)	fcHFD S (n = 8)	Chow W (n = 8)	Chow S (n = 9)	fcHFD W (n = 8)	fcHFD S (n = 8)	
<i>Glut1</i>	6,02E-03 ± 3,00E-04	6,11E-03 ± 3,53E-04	6,11E-03 ± 3,64E-04	6,83E-03 ± 2,92E-04	1,02E-02 ± 1,00E-03	1,02E-02 ± 8,15E-04	9,90E-03 ± 7,00E-04	1,03E-02 ± 4,39E-04	
<i>Glut2</i>	3,12E-04 ± 3,72E-05	2,97E-04 ± 3,79E-05	3,46E-04 ± 4,62E-05	3,12E-04 ± 2,69E-05	7,72E-04 ± 1,35E-04	7,93E-04 ± 8,38E-05	7,71E-04 ± 9,86E-05	6,31E-04 ± 5,51E-05	
<i>Glut3</i>	4,14E-03 ± 2,67E-04	3,84E-03 ± 1,22E-04	4,47E-03 ± 2,20E-04	4,45E-03 ± 2,10E-04	1,56E-02 ± 3,39E-04	1,63E-02 ± 7,89E-04	1,50E-02 ± 3,96E-04	1,67E-02 ± 6,10E-04	Drink effect p < 0,05
<i>Glut4</i>	1,43E-03 ± 1,21E-04	1,35E-03 ± 9,01E-05	1,75E-03 ± 1,08E-04	1,61E-03 ± 1,33E-04	2,60E-03 ± 2,78E-04	2,52E-03 ± 2,30E-04	2,80E-03 ± 1,65E-04	1,96E-03 ± 2,10E-04	Drink effect p < 0,05
<i>Glut6</i>	1,33E-02 ± 8,07E-04	1,32E-02 ± 6,03E-04	1,27E-02 ± 9,18E-04	1,33E-02 ± 5,35E-04	2,59E-02 ± 2,30E-03	2,95E-02 ± 3,72E-03	2,38E-02 ± 1,55E-03	2,65E-02 ± 1,29E-03	
<i>Glut8</i>	7,79E-03 ± 5,53E-04	7,82E-03 ± 3,62E-04	6,81E-03 ± 9,49E-04	8,14E-03 ± 1,99E-04	2,40E-02 ± 1,82E-03	2,36E-02 ± 2,06E-03	2,22E-02 ± 1,33E-03	2,25E-02 ± 9,34E-04	
<i>Sglt1</i>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
<i>Gck</i>	2,50E-02 ± 3,26E-03	2,58E-02 ± 3,65E-03	2,21E-02 ± 2,75E-03	2,93E-02 ± 3,49E-03	N.D.	N.D.	N.D.	N.D.	
<i>Nos</i>	4,41E-04 ± 8,19E-05	4,30E-04 ± 5,49E-05	4,27E-04 ± 6,12E-05	5,49E-04 ± 8,75E-05	2,84E-03 ± 2,92E-04	4,17E-03 ± 6,38E-04	3,11E-03 ± 5,20E-04	3,64E-03 ± 5,11E-04	
<i>Sgca</i>	2,90E-02 ± 1,09E-03	2,81E-02 ± 1,39E-03	2,51E-02 ± 3,67E-03	2,86E-02 ± 1,37E-03	6,46E-01 ± 2,79E-02	5,83E-01 ± 3,13E-02	5,99E-01 ± 3,09E-02	6,20E-01 ± 3,49E-02	
<i>Sgcb</i>	4,50E-02 ± 2,10E-03	4,33E-02 ± 2,64E-03	4,10E-02 ± 5,85E-03	4,70E-02 ± 1,40E-03	2,85E-01 ± 1,10E-02	2,75E-01 ± 1,74E-02	2,73E-01 ± 7,54E-03	2,97E-01 ± 1,06E-02	
<i>Kfr6.1</i>	8,80E-04 ± 6,43E-05	9,85E-04 ± 3,43E-05	1,00E-03 ± 1,25E-04	8,00E-04 ± 3,87E-05	3,15E-03 ± 2,83E-04	3,01E-03 ± 2,30E-04	3,66E-03 ± 2,38E-04	2,84E-03 ± 2,94E-04	Diet*drink effect p = 0,0728
<i>Kfr6.2</i>	1,51E-03 ± 1,92E-04	1,42E-03 ± 8,33E-05	1,51E-03 ± 8,39E-05	1,57E-03 ± 1,77E-04	8,96E-03 ± 9,97E-04	9,57E-03 ± 1,02E-03	7,09E-03 ± 1,15E-03	8,38E-03 ± 5,82E-04	
<i>Sur1</i>	1,91E-03 ± 1,62E-04	1,85E-03 ± 1,07E-04	1,64E-03 ± 1,00E-04	2,18E-03 ± 1,49E-04	7,24E-03 ± 4,66E-04	7,89E-03 ± 8,25E-04	6,87E-03 ± 7,62E-04	7,04E-03 ± 5,00E-04	
<i>Sur2</i>	2,24E-03 ± 1,46E-04	2,25E-03 ± 1,72E-04	2,24E-03 ± 1,82E-04	2,23E-03 ± 1,01E-04	4,19E-03 ± 4,78E-04	4,54E-03 ± 4,54E-04	4,00E-03 ± 3,54E-04	4,12E-03 ± 3,21E-04	

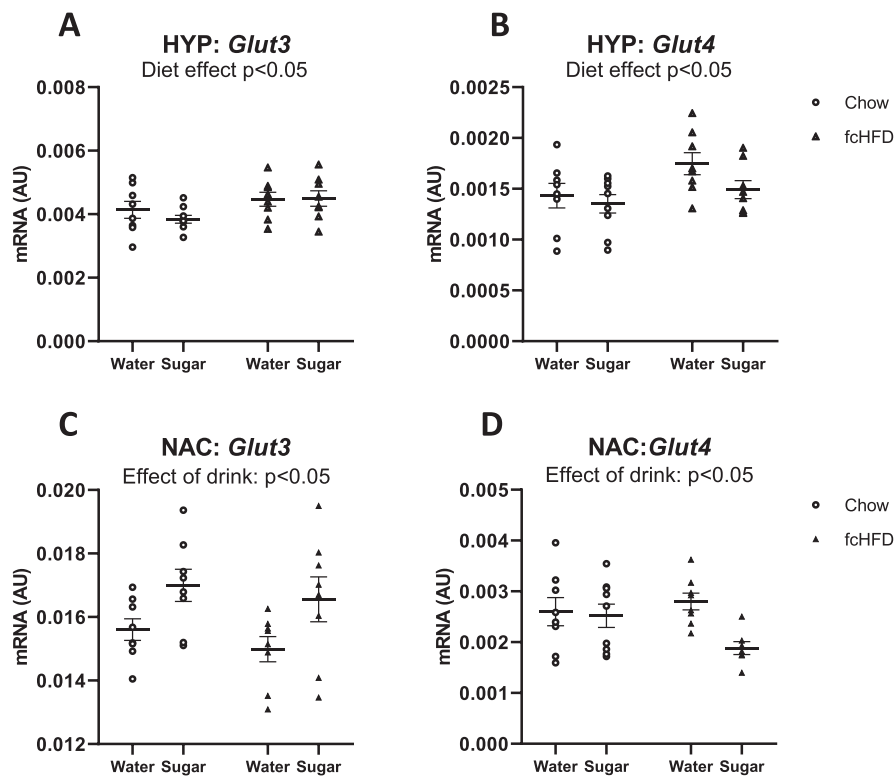


Fig. 1. A,B. Hypothalamic mRNA expression of (A) *Glut3* and (B) *Glut4*, as measured using RT-qPCR. C,D. Nucleus accumbens mRNA expression of (C) *Glut3* and (D) *Glut4*, as measured using RT-qPCR.

cholinergic interneurons, thereby altering reward processing [31]. Thus, it would be interesting to further investigate whether the changes in Kir6.1 observed in our paradigm are due to the nutrients consumed or to other metabolic factors, such as insulin secretion.

While we found changes in the expression of glucose transporters involved in glucose uptake, and in channels sensitive to cellular energy status, the enzymes that are part of the glucose metabolism pathway were unaltered by the consumption of a fCHFD or a sucrose solution. *Gck* was not affected in the hypothalamus, and not detected in the nucleus accumbens. This suggests that other hexokinases are involved in metabolizing glucose in this brain area. On the other hand, while mRNA levels of the enzymes measured were not affected, we cannot rule out that their protein expression and enzymatic activity might have altered. Assessment of mRNA and protein levels only is a limitation of this study since these remain indirect measures of glucose uptake and utilization. Diet-induced changes in translocation of glucose transporters, or in the phosphorylation of enzymes involved in glucose

metabolism that would affect overall nutrient sensing, should be assessed in future studies.

In summary, in this exploratory study, we found that the expression of *Glut3* and *Glut4* in the hypothalamus and nucleus accumbens is differentially affected by the consumption of a fCHFD and a sucrose solution. In addition, Kir6.1 tended to be increased in the nucleus accumbens after the consumption of a sucrose bolus, but this effect was blunted by prior high fat exposure. This points to possible differences in the processing of reward values associated with sucrose during consumption of a fCHFD.

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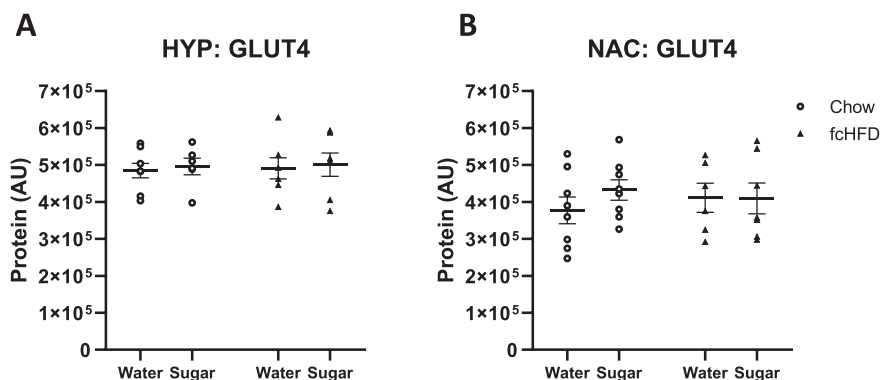


Fig. 2. A. GLUT4 protein levels in the hypothalamus (HYP), as measured using Protein Simple. B. GLUT4 protein levels in the nucleus accumbens (NAC), as measured using Protein Simple.

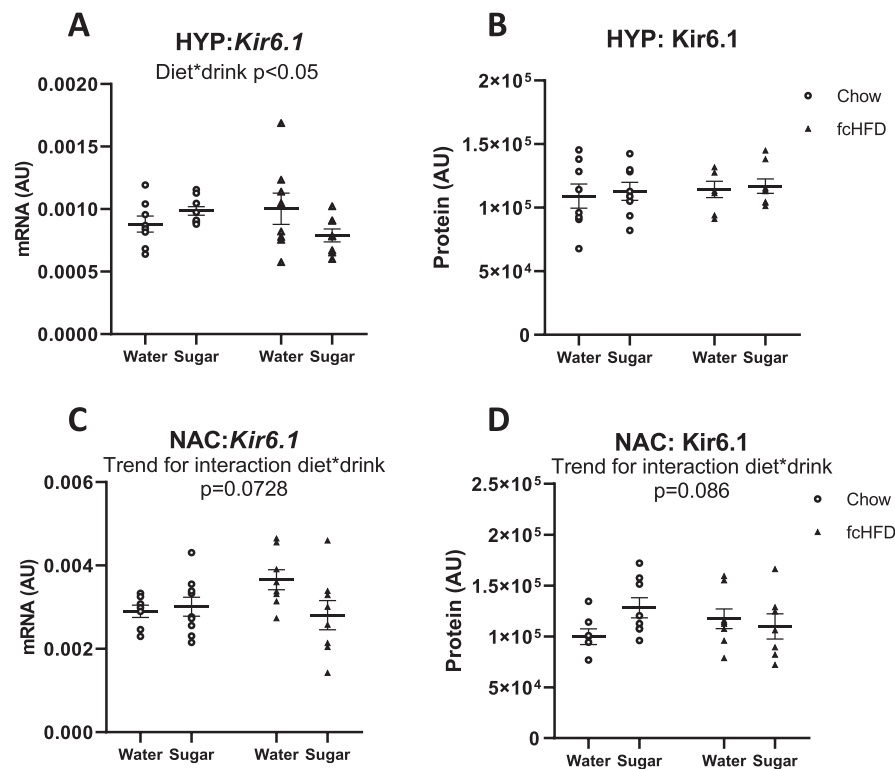


Fig. 3. A,B. Hypothalamic Kir6.1 (A) mRNA and (B) protein expression, as measured using RT-qPCR and Protein Simple, respectively. C,D. Nucleus accumbens Kir6.1 (C) mRNA and (D) protein expression, as measured using RT-qPCR and Protein Simple, respectively. * $P < 0.05$.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.physbeh.2020.112936](https://doi.org/10.1016/j.physbeh.2020.112936).

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