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The effects of overnight nutrient intake on hypothalamic inflammation in a free-choice diet-induced obesity rat model

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ABSTRACT

Consumption of fat and sugar induces hyperphagia and increases the prevalence of obesity and diabetes type 2. Low-grade inflammation in the hypothalamus, a key brain area involved in the regulation of energy homeostasis is shown to blunt signals of satiety after long term high fat diet. The fact that this mechanism can be activated after a few days of hyperphagia before apparent obesity is present led to our hypothesis that hypothalamic inflammation is induced with fat and sugar consumption. Here, we used a free-choice high-fat high-sugar (fcHFHS) diet-induced obesity model and tested the effects of differential overnight nutrient intake during the final experimental night on markers of hypothalamic inflammation. Male Wistar rats were fed a control diet or fcHFHS diet for one week, and assigned to three different feeding conditions during the final experimental night: 1) fcHFHS-fed, 2) fed a controlled amount of chow diet, or 3) fasted. RT-qPCR and Western blot were utilized to measure hypothalamic gene and protein expression, of cytokines and intermediates of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. Lastly, we investigated the effects of acute fat intake on markers of hypothalamic inflammation in fat-naïve rats. fcHFHS-fed rats consumed more calories, increased adipose tissue, and showed elevated expression of hypothalamic inflammation markers (increased phosphorylation of NF-κB protein, *Nfkbia* and *Il6* gene expression) compared to chow-fed rats. These effects were evident in rats consuming relative high amounts of fat. Removal of the fat and sugar, or fasting, during the final experimental night ameliorated hypothalamic inflammation. Finally, a positive correlation was observed between overnight acute fat consumption and hypothalamic NF-κB phosphorylation in fat-naïve rats. Our data indicate that one week of fcHFHS diet, and especially the fat component, promotes hypothalamic inflammation, and removal of the fat and sugar component reverses these detrimental effects.

1. Introduction

The global prevalence of obesity and type 2 diabetes has reached epidemic properties that put social and financial pressure on modern society (Cowie et al., 2006; Hubert, Feinleib, McNamara, & Castelli, 1983; Janssen, Heymsfield, Allison, Kotler, & Ross, 2002; Ogden et al., 2006; Poirier et al., 2006). Easy access to fat and sugar rich foods and beverages in Western societies is tightly associated with obesity as it leads to hyperphagia beyond energy needs of the organism (Berg et al., 2009; Berteus Forslund, Torgerson, Sjostrom, & Lindroos, 2005; Chapelot, 2011). The hypothalamus is a key brain area involved in the regulation of energy homeostasis which integrates nutritional, neuronal and hormonal signals regulating energy intake and expenditure (Myers & Olson, 2012). During obesity, hypothalamic response to the adipokine leptin, an indicator of the body's energy reserves, is decreased (Morton, 2007). Recent studies in rodents demonstrated that snacking fat and sugar blunts leptin signaling which could

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contribute to further hyperphagia and obesity development (van den Heuvel et al., 2014). Several studies have now provided evidence that desensitization in leptin signaling results from diet-induced inflammation in the hypothalamus (Cai & Liu, 2012; De Souza et al., 2005; van den Heuvel et al., 2014; Milanski et al., 2009; Posey et al., 2009; Thaler & Schwartz, 2010; Zhang et al., 2008).

One of the first studies to investigate hypothalamic inflammation associated with obesity demonstrated that expression of pro-inflammatory cytokines, such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), was increased in the hypothalamus of obese rats after long-term consumption of a high-fat diet (De Souza et al., 2005). Furthermore, several important intracellular inflammatory intermediates, such as inhibitor of nuclear factor kappa-β kinase subunit beta (IKK-β), nuclear factor-κB (NF-κB), and c-Jun N-terminal kinase (JNK), all appear to play a key role in the hypothalamic inflammatory response to chronic high-fat diet consumption (Milanski et al., 2009; Zhang et al., 2008). In addition, functional disruption of these key players in the medial basal hypothalamus (MBH) of rodents prevented both diet-induced hypothalamic inflammation and diet-induced impaired central leptin signaling, and resulted in lower diet-induced body weight improving systemic insulin sensitivity (De Souza et al., 2005; Milanski et al., 2009; Posey et al., 2009; Zhang et al., 2008).

Several studies have demonstrated the onset of hypothalamic inflammation following the long-term consumption of high-caloric diets. However, other studies did not observe such diet-induced hypothalamic inflammation (de Git & Adan, 2015; Milanski et al., 2009; Posey et al., 2009; Zhang et al., 2008). In addition, Thaler and colleagues demonstrated that hypothalamic inflammation occurred after only a few days of high-caloric diet consumption, when mice were hyperphagic but had not yet gained any substantial body weight (Thaler et al., 2012). Collectively, these findings suggest that acute overload of nutrients, and not obesity itself, promotes hypothalamic inflammation, but the exact role of fat or sugar consumption in this process remains unclear.

Both fat and sugars are strong candidates to induce hypothalamic inflammation, as both can activate the hypothalamic IKKβ/NF-κB pathway or stimulate the production of cytokines following intracerebroventricular infusion in lean rodents (Zhang et al., 2008). In addition, long chain saturated fatty acids as well as palmitic acid can activate the Toll like receptor 4 (TLR4)/IKKβ/NF-κB pathway in lean healthy mice (Kleinridders et al., 2009; Milanski et al., 2009). To mimic the human diet more closely, which is characterized by choices of several healthy and unhealthy food items, we developed the free-choice high-fat high-sugar diet (fcHFHS) in which rats are provided with saturated fat and sugar water separately from their regular food (chow pellets) (la Fleur, Luijendijk, van der Zwaal, Brans, & Adan, 2014; la Fleur, van Rozen, Luijendijk, Groeneweg, & Adan, 2010). Animals on this diet are persistently hyperphagic, show snacking like behavior and they develop systemic leptin resistance rapidly (la Fleur et al., 2014). The direct effects of fat and sugars on hypothalamic inflammatory pathways, in combination with the inconsistent findings on hypothalamic inflammation following consumption of high-caloric diets, led us to the hypothesis that an (acute) overload of fat or sugars, which occurs during snacking, can promote hypothalamic inflammatory signaling. To test this hypothesis rats were fed the fcHFHS diet, or a low-caloric control diet, for one week and cytokine mRNA expression and (phospho)protein levels of the IKKβ/NF-κB pathway were measured in the hypothalamus following the 3 different feeding conditions during the final experimental night: 1) fcHFHS-fed, 2) fcHFHS replaced with controlled amount of chow diet, or 3) overnight fasted. Fat-naïve rats were used to investigate the effects of acute fat consumption on markers of hypothalamic inflammation.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Germany) weighing 250–280 g were habituated to the animal facility of the Academic Medical Center of the University of Amsterdam, The Netherlands, for one week before start of experiments. Animals were housed on a 12:12 h light: dark cycle, (lights on at 7:00), at 19 ± 1 °C, and had *ad libitum* access to tap water and a standard low-caloric diet (Teklad global diet 2918, 18.6% protein, 44.2% carbohydrate, and 6.2% fat, 3.1 kcal/g, Envigo). All experimental protocols were evaluated and approved by the Committee for Animal Experimentation at the Academic Medical Center of the University of Amsterdam, The Netherlands.

2.2. Experimental groups and metabolic measurements

Three different experiments were performed to study the effect of acute nutrient intake on markers of hypothalamic inflammation after 1-week consumption of fcHFHS diet. After habituation, rats were randomly assigned to control or fcHFHS conditions (n = 8/group) for each experiment and housed individually. Control rats were housed under the standard conditions, whereas fcHFHS rats had simultaneous *ad libitum* access to the standard low-caloric diet, a bottle of tap water, a bottle with 30% sugar water (1.0 M sucrose mixed from commercial grade sugar and water; 1.2 kcal/g) and a dish with pure saturated fat (beef tallow; Ossewit/Blanc de Boeuf, Vandermoortele, Belgium; 9 kcal/g). For all experimental groups, diet interventions lasted for one week. However, the three experiments differed in feeding regimen during the final (7th) night: starting at 19:00, animals kept *ad libitum* access to their respective diets (experiment 1, groups: chow/chow and fcHFHS/fcHFHS); fat and sugar water was removed and all animals received 10 g total of the standard low-caloric diet (experiment 2, groups: chow/10 gr chow and fcHFHS/10 gr chow); or animals were fasted, and only had *ad libitum* access to tap water (experiment 3, groups: chow/fasted and fcHFHS/fasted). We have previously shown that overnight removal of the lard and sugar water components from fcHFHS-fed rats results in consumption of approximately 10–15 g standard low-fat diet (Pandit, 2015). Therefore, we chose to provide 10 g of standard low-fat diet to achieve a comparable caloric intake between fcHFHS and chow rats.

A final experiment was performed to determine the effects of acute overnight fat consumption on activation of the hypothalamic NF-κB pathway. Control rats (n = 8) had *ad libitum* access to the standard low-fat diet and tap water. The experimental group (n = 8) received 5 g total of standard low-fat diet, while having *ad libitum* overnight access to a dish of pure saturated fat and tap water (fat consumption was determined by the difference in the weight of the fat component before and at the end of the experiment). The limited amount of standard low-fat diet was provided to limit potential malaise from fat overconsumption.

Body weight of animals and intake of all diet components was measured daily.

For all experiments, animals were anesthetized between 9:00 and 11:00 using asphyxia (30% CO_2/70% O_2) followed by rapid decapitation. Trunk blood was collected, epididymal, mesenteric, subcutaneous and peritoneal fat pads were weighed, and brains were quickly isolated, snap-frozen, and stored at −80 °C until further analysis. Plasma leptin concentrations were determined by radioimmunoassay (Linco Research, Inc., St. Charles, MO, USA).