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Excess intake of fat and sugar potentiates epinephrine-induced hyperglycemia in male rats

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ABSTRACT

Aims: Over the past five decades, *per capita* caloric intake has increased significantly, and diet- and stress-related diseases are more prevalent. The stress hormone epinephrine stimulates hepatic glucose release during a stress response. The present experiment tested the hypothesis that excess caloric intake alters this ability of epinephrine to increase blood glucose.
Methods: Sprague–Dawley rats were fed a high-energy cafeteria-style diet (HED). Weight gain during the first 5 days on the diet was used to divide the rats into an HED-lean group and HED-obese group. After 9 weeks, the rats were injected with epinephrine, and blood glucose was measured.
Results: HED-obese rats gained body and fat mass, and developed insulin resistance (IR) and hepatic steatosis. HED-lean and control rats did not differ. Epinephrine produced larger increases in blood glucose in the HED-obese rats than in the HED-lean and control rats. Removing the high-energy components of the diet for 4 weeks reversed the potentiated effects of epinephrine on glucose and corrected the IR but not the steatosis or obesity.
Conclusions: Consumption of a high-energy cafeteria diet potentiates epinephrine-induced hyperglycemia. This effect is associated with insulin resistance but not adiposity or steatosis and is reversed by 4 weeks of standard chow.

1. Introduction

Over the past five decades, *per capita* caloric intake has increased by approximately 28% in the United States (USDA, 2011). Many Americans now consume what is referred to as the "western" diet, which is characterized by excess intake of energy sources from saturated fat and refined carbohydrates (Grotto & Zied, 2010; Iqbal et al., 2008; Odermatt, 2011). This excessive caloric intake is associated with an obesity epidemic (Heinonen et al., 2014); over 35% of the population in the United States is considered obese (Flegal, Carroll, Kit, & Ogden, 2012). Obesity is an extremely serious health concern. It predisposes individuals to a variety of harmful illnesses including insulin resistance (Bezerra et al., 2000; Panchal et al., 2011; Tobey, Mondon, Zavaroni, & Reaven, 1982; Zavaroni, Sander, Scott, & Reaven, 1980), cardiovascular disease (Despres, 2012; Juonala et al., 2011; Ritchie & Connell, 2007; Shah, Mehta, & Reilly, 2008; Sowers, 2003; Wilson, D'Agostino, Sullivan, Parise, & Kannel, 2002), and non-alcoholic fatty liver disease (NAFLD) (Ackerman et al., 2005; Fu et al., 2009). NAFLD is characterized by an accumulation of lipids in the liver (>5% wet weight; a.k.a. steatosis) in the absence of excessive alcohol intake (Neuschwander-Tetri & Caldwell, 2003). It is the most common form of liver disease in the United States, affecting at least 30% of the American population (Lazo & Clark, 2008). The presence of NAFLD contributes to other disease states, including diabetes mellitus and cirrhosis (Anstee, Targher, & Day, 2013). Also, obesity and NAFLD are associated with impaired insulin regulation of hepatic glucose production (i.e. hepatic insulin resistance; Deivanayagam et al., 2008; Kotronen, Vehkavaara, Seppala-Lindroos, Bergholm, & Yki-Jarvinen, 2007; Lomonaco et al., 2012; Roden, 2008) and disrupted cellular glucose uptake (peripheral insulin resistance; Deivanayagam et al., 2008; Lomonaco et al., 2012).

In addition to the increase in caloric intake, the prevalence of stress and stress-related diseases has also increased significantly in the United States (Caruso, 2006; Djindjic, Jovanovic, Djindjic, Jovanovic, & Jovanovic, 2012; Xu et al., 2011). In humans and rodents, one consequence of acute stress is increased secretion of epinephrine from the sympathoadrenal axis (Frankenhaeuser, Dunne, & Lundberg, 1976; Gerra et al., 2001; Kvetnansky et al., 1987). Sympathetic nervous system activation produces acute hyperglycemia that helps meet the energetic demands of a stressor, such as increased glucose uptake in muscles and elevated heart rate and respiration. The liver is the principal organ that regulates glucose homeostasis; it stores glucose in the form of glycogen and releases glucose either by glycogen metabolism (glycogenolysis) or by *de novo* glucose synthesis (gluconeogenesis). Acute increases in epinephrine elevate blood

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glucose levels by promoting glycogenolysis and gluconeogenesis (Berg, Tymoczko, & Stryer, 2002; Sacca, Vigorito, Cicala, Corso, & Sherwin, 1983; Yajima & Ui, 1974) and by inhibiting insulin-mediated glucose uptake (Aslesen & Jensen, 1998; Chiasson, Shikama, Chu, & Exton, 1981; James, Burleigh, & Kraegen, 1986; Jensen, Aslesen, Ivy, & Brors, 1997).

It is likely that stress has a different impact on the health and functioning of obese versus lean individuals. For example, obese individuals have a more difficult time coping with work stress compared to their lean coworkers; job-related stressors are associated with body weight gain (Kivimaki et al., 2006), high blood pressure (Harada, Karube, Saruhara, Takeda, & Kuwajima, 2006), and type 2 diabetes in obese (Heraclides, Chandola, Witte, & Brunner, 2012), but not lean, individuals. In addition, obesity may increase the glycemic response to stress because simultaneous injections of the stress hormones epinephrine and B-endorphin produce severe hyperglycemia in obese individuals but not in their lean counterparts (Giugliano et al., 1988). Stress reactions are different in obese versus lean animals as well; obese rats display a blunted sympathetic response to footshock stress (Nyakas, Balkan, Steffens, & Bohus, 1995) and an increased corticosterone response to restraint stress (South, Westbrook, & Morris, 2012).

Based on the evidence reviewed above, the present research tested the hypothesis that elevated consumption of fat and sugar disrupts the ability of epinephrine to increase blood glucose concentrations. On one hand, high-energy diets could potentiate the effects of epinephrine on blood glucose levels given that consumption of such diets impairs glucose regulatory mechanisms (Barella et al., 2015; Knauf et al., 2008; Panchal et al., 2011; Song, Wang, Ren, & Zhao, 2014; Tobey et al., 1982; Zavaroni et al., 1980). Alternatively, high-energy diets could diminish the effects of the epinephrine, which is possible given the finding that high fat diets blunt adrenergic signaling in adipocytes (Gaidhu, Anthony, Patel, Hawke, & Ceddia, 2010). In the present study, rats were fed a cafeteria-style diet composed of animal lard, a 32% sucrose solution, standard chow and water. This cafeteria-style diet is physiologically relevant to human consumption patterns and the western diet because it provides rats with nutritional and non-nutritional diet choices. This allows the rat, rather than the investigator, to determine the amount of calories consumed and the macronutrient composition of calories. We have demonstrated that rats that gain the most weight during the first 5 days on this diet will become obese and develop hepatic steatosis 9 weeks later; in contrast, rats that gain the least amount of weight during the first 5 days on this diet will not differ from control rats (Darling, Ross, Bartness, & Parent, 2013). Rats were fed this cafeteria diet for 9 weeks, and then the effects of epinephrine on blood glucose concentrations were determined. The effects of insulin administration on blood glucose concentrations were used as a measure of insulin sensitivity, and the amount of fat in the liver was characterized and quantified to determine the presence of hepatic steatosis. As the present results demonstrate, the high-energy diet potentiated the ability of epinephrine to increase blood glucose concentrations. To attempt to isolate the mechanism underlying this effect, we removed the high-energy components of the diet for 4 weeks. Specifically, we repeated these measures in rats that were fed the high-energy cafeteria diet for 9 weeks and then standard chow and water for 4 weeks. We reasoned that this short duration on standard chow might be sufficient to correct only some of the effects of the diet and that the pattern of results could then be used to dissociate whether insulin resistance, obesity or steatosis contributed to the potentiated glucose response to epinephrine.

2. Materials and methods

2.1. Animals and housing

Male Sprague–Dawley rats (Charles River, Wilmington, MA), aged 53 days, were housed individually in an OptiRat® cage system (Animal Care Systems, Centennial, CO) and maintained on a 12 h light cycle (lights on at 7:00 am). They were weighed upon arrival to the lab and allowed to acclimate for 1 week. All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with PHS guidelines.

2.2. Diets

All rats were fed standard chow (3.01 kcal/g; LabDiet 5001, Purina Mills, Gray Summit, MO) during the acclimation week. On the seventh day, they were reweighed, matched on body mass and percent change in body mass, and assigned to either the control diet (C; n = 41) or the high-energy diet (HED; n = 117) group. The high-energy diet consisted of a bottle of tap water, a bottle of 32% sucrose solution (1.13 kcal/g), standard rat chow, and a glass petri dish containing animal lard (9.0 kcal/g; Armour, Omaha, NE; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004). The control diet consisted of two bottles of tap water and standard rat chow, and an empty glass petri dish was placed in the cage. Both the control and high-energy diets were fed *ad libitum*. After 5 days of consuming the control or high-energy diet, the rats were reweighed. Rats in the bottom 33% (i.e., those with the smallest percent change in body mass) were defined as high energy diet (HED)-lean (n = 28) and those in the top 33% were labeled HED-obese (n = 39). Sixty-four rats from the middle 33% were removed from the study. The remaining rats were maintained on their respective diets for 9 weeks, and then the effects of epinephrine on blood glucose levels were tested. Although we typically measure food intake in our diet studies (Darling et al., 2013; Ross, Bartness, Mielke, & Parent, 2009; Ross, Bruggeman, Kasumu, Mielke, & Parent, 2012), food intake was not measured in the present experiment in order to avoid any potential stress associated with the wire-bottomed cages needed to measure intake accurately. All injections and measures were performed by an experimenter blind to the diet condition of each rat.

2.3. Epinephrine injections/blood glucose sampling

During the 2 days before the epinephrine injections, the rats were acclimated to the blood collection procedure by placing them into a folded towel and stroking their tails for 2 min once per day. On the injection day, the rats were placed into the folded towel, the tail vein was nicked, and a drop of blood was collected. Previous research has shown that this tail blood sampling protocol is minimally stressful in rats as indicated by basal levels of stress hormones during the sampling period (Fluttert, Dalm, & Oitzl, 2000). Blood was collected immediately before the rats were given a subcutaneous injection of epinephrine (0.05 or 0.1 mg/kg; Amphastar Pharmaceuticals, So. El Monte, CA) or saline (0.9% w/v NaCl) and then again 5, 10, 30, 60, 120 and 180 min after the injection. These doses of epinephrine were selected based on previous research showing that they increased blood glucose concentrations in a dose-dependent manner (Morris, Chang, Mohler, & Gold, 2010; Talley, Kahn, Alexander, & Gold, 2000). The amount of glucose in each drop of blood was measured using an Accu-Chek glucose meter (Roche, Indianapolis, IN).

2.4. Insulin tolerance test

The insulin tolerance test was conducted 3 days after the epinephrine injections. Following a 4 hr fast, a baseline blood glucose measurement was obtained as above, and then all of the rats were given an insulin injection (1.25U/kg IP; Boehringer Ingelheim, St. Joseph, MO). Blood glucose concentrations were measured 5, 10, 30, 60, 120 and 180 min after the injection.