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Cafeteria diet-induced obesity causes oxidative damage in white adipose

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ABSTRACT

Obesity continues to be one of the most prominent public health dilemmas in the world. The complex interaction among the varied causes of obesity makes it a particularly challenging problem to address. While typical high-fat purified diets successfully induce weight gain in rodents, we have described a more robust model of diet-induced obesity based on feeding rats a diet consisting of highly palatable, energy-dense human junk foods – the "cafeteria" diet (CAF, 45–53% kcal from fat). We previously reported that CAF-fed rats became hyperphagic, gained more weight, and developed more severe hyperinsulinemia, hyperglycemia, and glucose intolerance compared to the lard-based 45% kcal from fat high fat diet–fed group. In addition, the CAF diet-fed group displayed a higher degree of inflammation in adipose and liver, mitochondrial dysfunction, and an increased concentration of lipid-derived, pro-inflammatory mediators. Building upon our previous findings, we aimed to determine mechanisms that underlie physiologic findings in the CAF diet. We investigated the effect of CAF diet-induced obesity on adipose tissue specifically using expression arrays and immunohistochemistry. Genomic evidence indicated the CAF diet induced alterations in the white adipose gene transcriptome, with notable suppression of glutathione-related genes and pathways involved in mitigating oxidative stress. Immunohistochemical analysis indicated a doubling in adipose lipid peroxidation marker 4-HNE levels compared to rats that remained lean on control standard chow diet. Our data indicates that the CAF diet drives an increase in oxidative damage in white adipose tissue that may affect tissue homeostasis. Oxidative stress drives activation of inflammatory kinases that can perturb insulin signaling leading to glucose intolerance and diabetes.

1. Introduction

Obesity poses an alarming global public health concern [1,2]. Despite efforts aimed at ameliorating obesity, recent projections have estimated that 51% of the United States population will be obese by 2030 [3]. While typical high-fat purified pellet diets are successful in promoting obesity in mice and rats, we have described a diet-induced obesity model that incorporates a variety of highly palatable, energy-dense foods regularly consumed by humans – the "cafeteria" style (CAF) diet [4,5] that recapitulates obesity-like findings in humans. The CAF diet included a smorgasbord style offering of standard chow pellets, plus 3 human food choices offered daily. We previously reported that rats who ate the CAF diet developed hyperphagia and displayed significantly increased weight gain compared to other diet groups, including a commonly used lard-based high fat diet, consisting of 45% kilocalories-derived

Abbreviations: CAF, cafeteria diet; FDR, false discovery rate; SAM, significance analysis of microarrays; SC, standard control diet; WAT, white adipose tissue.

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from fat and added sucrose. CAF-fed rats developed severe hyperinsulinemia, hyperglycemia, and glucose intolerance. Using a combination of metabolomic strategies and histological tissue analysis, we previously observed a higher degree of inflammation in white and brown adipose and liver compared to rats fed a traditional, purified high-fat diet, low-fat diet, and SC-fed rats [5]. In addition, concentrations of mitochondrial-derived lipid mediators that promote obesity-associated inflammation were found to be significantly elevated in CAF fed rats [4,5]. Metabolomic and immunohistologic measures suggested dramatic adipose tissue dysfunction however, underlying mechanisms remained unclear. Thus, we sought to investigate mechanistic underpinnings of CAF diet-induced inflammation and adipose dysfunction using global gene expression profiling to identify relevant genes and pathways altered with ingestion of the CAF diet. We now report that CAF diet-induced obesity resulted in significant alterations in white adipose tissue gene expression profiles that were associated with blunted protection from oxidative stress and an increase in oxidative damage. Thus, oxidative damage is one CAF diet-mediated pathway that could be responsible for greater adipose inflammation and systemic metabolic dysfunction in this model of diet induced obesity.

2. Materials and methods

2.1. Animals and diet treatments

All procedures were performed with the approval of the Duke University Institutional Animal Care and Use Committee. Male Wistar rats (~200 g, 7–8 weeks old), purchased from Harlan Laboratories (Dublin, VA), were housed 2 rats/cage in the Duke University animal housing facility. Rats were maintained on a 12 h light/dark cycle and given *ad libitum* access to standard chow (SC, Harlan Teklan 7001, Dublin, VA) for 2 weeks before being randomized onto experimental diets. At 9–10 weeks of age (~300 g body weight, N = 5 for CAF and N = 4 for SC), rats were either maintained on the SC diet, or were switched to a CAF diet consisting of human snack food provided along with the SC pellet diet (Table 1). For a detailed description of the CAF diet components, refer to Sampey et al. [5]. Briefly, the CAF diet included human food purchased at a supermarket and was provided in excess, including cookies, cereals, cheese, processed meats, crackers, etc. [5]. To estimate total gram and overall caloric intake of the CAF diet, items were weighed prior to and after consumption, and corrected for drying. The snack food items varied daily according to the fat, protein, and carbohydrate content as listed in Supplementary Table S2 of Sampey et al. [5]. Fat intake was the most drastically altered macronutrient with an estimated intake of 55% kcal from fat per day in CAF-fed rats. In addition, kilocalories from simple carbohydrate consumption was increased 500% (from 36 kcal to 180 kcal) in the CAF-fed group compared to the SC-fed groups. There were no added sugars to the SC diet; any sugars present were derived from whole-grains (corn, oats, etc.). Simple sugars were <5% of total carbohydrates in SC7001 (see Supplementary Table S3 from Ref. [5]). After 15 weeks on diet, rats were fasted for 6 h and epididymal white adipose tissue (WAT) was collected. A portion of the WAT was isolated for mRNA isolation and another portion was fixed and paraffin-embedded for IHC.

2.2. RNA isolation

QIAzol Lysis Reagent was used to isolate mRNA from 100 mg WAT samples (Qiagen, Valencia, CA, USA and [5,6]). mRNA quantity and quality were analyzed by Nanodrop (Thermoscientific, Wilmington, DE) and Bioanalyzer 2100 (Agilent, Wilmington, DE), respectively. For microarray analyses, cDNA was synthesized at the Functional Genomics Core at UNC-Chapel Hill. N = 5 for CAF diet group and 4 for SC diet group.

2.3. Gene expression microarrays

Gene expression quantification was conducted at the Functional Genomics Core Facility at UNC-Chapel Hill using genome-wide transcript microarrays (Affymetrix Rat Gene 1.0 ST, Santa Clara, CA). Microarrays where subjected to quality assessment and processed by robust multi-array average to produce transcript-level expression estimates using the aroma.affymetrix R package [7]. Transcripts were annotated with rat gene symbols using Affymetrix's annotation (RaGene-1_0-st-v1.na28.rn4.transcript.csv). Expression values were then log2 transformed. See Supplemental table ("S") Tables 1 and 2 for the complete gene expression data sets. Full gene names are in S Table 3.

2.4. 4-Hydroxynonenal immunohistochemistry

Sections of WAT from rats fed SC or CAF diets were stained for 4-hydroxynonenal (4-HNE), a marker of cellular lipid peroxidation [8]. 5 μm sections were stained using an anti-4-HNE primary antibody (1:800 dilution in Dako antibody diluent, Abcam, Cambridge, MA) and biotinylated goat anti-mouse secondary antibody

Table 1
Composition of standard chow (SC) and cafeteria (CAF) diets.

	SC7001	Cafeteria
Name	Standard chow (SC)	Cafeteria (CAF)
Manufacturer	Harlan Teklad	Misc
Catalog number	SC7001	3 items + SC7001
Fat		
kcal/gm	3.83	Varies daily
% fat/weight	4%	
% fat/kcal	12%	45–53%
Fat sources	Porcine fat Linoleic acid (1%/wt)	See Table 2 of ref. [3]
Protein		
% protein/wt	25%	~20%
% prot/kcal	34%	
Protein sources	Dehulled soybean meal, porcine meat, dehydrated alfalfa meal, dried whey, casein, purified amino acids	See Table 2 of ref. [3]
Carbohydrate		
% carb/wt	66%	~35%
% carb/kcal	54%	
Carbohydrate sources	Corn, wheat, barley, oats	See Table 2 of ref. [3]

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