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Dietary fat drives whole-body insulin resistance and promotes intestinal inflammation independent of body weight gain

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ABSTRACT

Background. The obesogenic potential of high-fat diets (HFD) in rodents is attenuated when the protein:carbohydrate ratio is increased. However, it is not known if intake of an HFD irrespective of the protein:carbohydrate ratio and in the absence of weight gain, affects glucose homeostasis and the gut microbiota.

Methods. We fed C57BL6/J mice 3 different HFDs with decreasing protein:carbohydrate ratios for 8 weeks and compared the results to a LFD reference group. We analyzed the gut microbiota composition by 16S rDNA amplicon sequencing and the intestinal gene expression by real-time PCR. Whole body glucose homeostasis was evaluated by insulin and glucose tolerance tests as well as by a hyperinsulinemic euglycemic clamp experiment.

Results. Compared with LFD-fed reference mice, HFD-fed mice, irrespective of protein:carbohydrate ratio, exhibited impaired glucose tolerance, whereas no differences were observed during insulin tolerance tests. The hyperinsulinemic euglycemic clamp revealed tissue-specific effects on glucose homeostasis in all HFD-fed groups. HFD-fed mice exhibited decreased insulin-stimulated glucose uptake in white but not in brown adipose tissue, and sustained endogenous glucose production under insulin-stimulated conditions. We observed no impairment of insulin-stimulated glucose uptake in skeletal muscles of different fiber type composition. HFD-feeding altered the gut microbiota composition paralleled by increased expression of pro-inflammatory cytokines and genes involved in gluconeogenesis in intestinal epithelial cells of the jejunum.

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Conclusions. Intake of a HFD profoundly affected glucose homeostasis, gut inflammatory responses, and gut microbiota composition in the absence of fat mass accretion.

1. Introduction

High fat diet-induced obesity is frequently used to study obesity and related metabolic disorders in rodents. Since such a model cannot be used to distinguish whether the observed metabolic dysfunctions result from the obese state or from the high fat feeding, the health consequences of a high fat intake, in the absence of weight gain, remain largely unknown. Using rodent models to elucidate the influence of dietary fat, while eliminating weight gain as confounder, is a challenging approach as high fat diet (HFD)-feeding tends to induce obesity to various degree depending on the protein:carbohydrate ratio [1]. Nevertheless, glucose intolerance induced by conventional HFD-feeding has been shown to precede weight gain in both humans [2] and mice [3], suggesting that a HFD per se affects glucose homeostasis. In support, we have previously demonstrated how exchanging sucrose with casein in an isoenergetic HFD protects against weight gain, but not glucose intolerance [1,4]. The mechanism behind the observed decrease in glucose tolerance remains elusive. Reduced adipose plasticity affecting both local and global insulin signaling [5], and further aggravated by accompanying adipocyte inflammation, may be involved [6]. Yet, whether adipocyte inflammation is a consequence [7] or a promoter [8,9] of insulin resistance remains to be established. Apart from changes in adiposity and adipocyte function, HFDs promote intestinal alterations such as increased intestinal permeability [10] and an elevated inflammatory milieu [11]. Both factors are believed to contribute to the progression of insulin resistance [12]. Interestingly, gut anti-inflammatory agents have been shown to protect against HFD-induced glucose intolerance and insulin resistance despite significant weight gain [13], indicating intestinal inflammation as key in the development of insulin resistance.

We hypothesized that dietary fat, independent of weight gain, would change the intestinal microenvironment translating to impaired metabolic homeostasis. To disentangle the influence of HFD-feeding on intestinal inflammation and whole-body glucose disposal, we fed mice 3 experimental isoenergetic HFDs with fixed fat content and a stepwise increase in the protein:carbohydrate ratio and compared the results to a low fat diet (LFD) reference group. We took advantage of an in-house observation where mice appeared less prone to diet-induced obesity (DIO) in 1 out of our 5 animal facilities, which reflects a recent report deciphering how mouse origin and housing conditions influence weight development [14]. Using the hyperinsulinemic euglycemic clamp technique, this experimental setting enabled acquisition of information on the extent to which macronutrient composition affects whole-body metabolism without possible confounding effects of weight gain and adipose tissue inflammation.

2. Materials and Methods

2.1. Mice and Ethical Statements

Wildtype C57BL/6J male mice (Taconic Biosciences, Denmark) were 6–8 weeks of age at delivery and single-housed for acclimatization one week prior to the start of the experiment. All experiments were conducted in accordance with national Danish guidelines (amendment #1306 of November 23, 2007) as approved by the Danish Animal Experiments Inspectorate (#2014-15-2934-01,027). Mice were kept under specific pathogen free conditions at 22 °C in 12 h light/dark cycle (7 AM–7 PM).

2.2. Experimental Outline and Diets

Twenty-four mice entered the experiment on a weekly basis for 3 consecutive weeks, and mice were kept on their respective diets for 8 weeks. Experimental diets were obtained from Ssniff (Germany) and kept at –20 °C until use. Mice were ad libitum fed 1 of 4 different diets (Table 1): 1) LFD – low fat control diet where sucrose and protein content mirrored the lowest amount in any of the HFDs; 2) HFHP – HFD with normal sucrose but high protein content; 3) HFIP – HFD with intermediate protein and sucrose content; 4) HFNP – HFD with normal protein but high sucrose content. We used corn oil, high in polyunsaturated and low in saturated fat, as fat source to minimize the likelihood of adipose tissue inflammation. Because both protein and carbohydrate sources affect host metabolism, we standardized our diets using casein as protein source and high-glycemic index sucrose as main carbohydrate source. After 5 and 6 weeks on the experimental diets mice were subjected to an insulin tolerance test (ITT) and a glucose tolerance test (GTT), respectively. All mice were MR-scanned prior to the ITT using EchoMRI 4in1 (USA). After 7 weeks, a subset of mice in each group underwent a surgical procedure (see Section 2.4. for

Table 1 – Diet composition.

MASS (g/kg)	LFD	HFHP	HFIP	HFNP
Casein	200	500	350	200
L-cystine	3	3	3	3
Corn starch	489.5	9.5	9.5	9.5
Cellulose	50	50	50	50
Sucrose	130	130	280	430
Corn oil	70	250	250	250
Choline bitartrate	2.5	2.5	2.5	2.5
Vitamine mix AIN-93-VX	10	10	10	10
Mineral mix AIN 93	45	45	45	45
t-Butylhydroquinone	0.014	0.014	0.014	0.014
Total	1000	1000	1000	1000
Energy content (kJ/kg)	16,972	20,572	20,572	20,572

Diet composition. Comparison between the LF reference diet and the 3 eucaloric HFDs.