Original Article

MOLECULAR METABOLISM

Dietary fat and gut microbiota interactions determine diet-induced obesity in mice

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ABSTRACT

Objective: Gut microbiota may promote positive energy balance; however, germfree mice can be either resistant or susceptible to diet-induced obesity (DIO) depending on the type of dietary intervention. We here sought to identify the dietary constituents that determine the susceptibility to body fat accretion in germfree (GF) mice.

Methods: GF and specific pathogen free (SPF) male C57BL/6N mice were fed high-fat diets either based on lard or palm oil for 4 wks. Mice were metabolically characterized at the end of the feeding trial. FT-ICR-MS and UPLC-TOF-MS were used for cecal as well as hepatic metabolite profiling and cecal bile acids quantification, respectively. Hepatic gene expression was examined by qRT-PCR and cecal gut microbiota of SPF mice was analyzed by high-throughput 16S rRNA gene sequencing.

Results: GF mice, but not SPF mice, were completely DIO resistant when fed a cholesterol-rich lard-based high-fat diet, whereas on a cholesterol-free palm oil-based high-fat diet, DIO was independent of gut microbiota. In GF lard-fed mice, DIO resistance was conveyed by increased energy expenditure, preferential carbohydrate oxidation, and increased fecal fat and energy excretion. Cecal metabolite profiling revealed a shift in bile acid and steroid metabolites in these lean mice, with a significant rise in 17β-estradiol, which is known to stimulate energy expenditure and interfere with bile acid metabolism. Decreased cecal bile acid levels were associated with decreased hepatic expression of genes involved in bile acid synthesis. These metabolic adaptations were largely attenuated in GF mice fed the palm-oil based high-fat diet. We propose that an interaction of gut microbiota and cholesterol metabolism is essential for fat accretion in normal SPF mice fed cholesterol-rich lard as the main dietary fat source. This is supported by a positive correlation between bile acid levels and specific bacteria of the order *Clostridiales* (phylum *Firmicutes*) as a characteristic feature of normal SPF mice fed lard.

Conclusions: In conclusion, our study identified dietary cholesterol as a candidate ingredient affecting the crosstalk between gut microbiota and host metabolism.

Keywords Germfree; Energy balance; Diet-induced obesity resistance; High-fat diet

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Abbreviations: Abcg5, ATP-binding cassette sub-family G member 5; Abcg8, ATP-binding cassette sub-family G member 8; Actb, beta actin; Akr1d1, aldo-keto-reductase family member 1; ANOVA, analysis of variance; BMR, basal metabolic rate; CD, control diet; CA, cholic acid; CDCA, chenodeoxycholic acid; CIDEA, cell death inducing DFFA-like effector; COX4, cytochrome c oxidase subunit 4; Cyp7a1, cholesterol 7 alpha-hydroxylase; Cyp27a1, cholesterol 27 alpha-hydroxylase; DCA, deoxycholic acid; Dhcr7, 7-dehydrocholesterol reductase; DIO, diet-induced obesity; DEE, daily energy expenditure; Eef2, eukaryotic elongation factor 2; FT-ICR-MS, Fourier transform-Ion Cyclotron Resonance-Mass Spectrometry; FT-IR, Fourier transform-infrared spectroscopy; GF, germfree; GUSB, beta-glucuronidase; HDCA, hyodeoxycholic acid; Hmgcr, 3-hydroxy-3-methylglutaryl Coenzyme A reductase; Hmgcs, 3-hydroxy-3-methylglutaryl Coenzyme A synthase 1; HP, heat production; Hprt1, hypoxanthine guanine phosphoribosyl transferase; Hsd11b1, hydroxysteroid (11-β) dehydrogenase 1; Hsp90, heat shock protein 90; Ldlr, low density lipoprotein receptor; LHFD, high-fat diet based on lard; MCA, muricholic acid; Nr1h2, nuclear receptor subfamily 1, group H, member 2 (liver X receptor β); Nr1h3, nuclear receptor subfamily 1, group H, member 3 (liver X receptor α); Nr1h4, nuclear receptor subfamily 1, group H, member 4 (farnesoid X receptor α); PHFD, high-fat diet based on palm oil; PRDM16, PR domain containing 16; qPCR, quantitative real-time polymerase chain reaction; SPF, specific pathogen free; Srebf1, sterol regulatory element binding transcription factor 1; TCA, taurocholic acid; Tf2b, transcription factor II B; TMCA, Tauromuricholic acid; UCP1, uncoupling protein 1; UDCA, ursodeoxycholic acid; UPLC-TOF-MS, ultraperformance liquid chromatography-time of flight-mass spectrometry

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1. INTRODUCTION

Obesity and associated comorbidities are major health problems in all Westernized societies. Although obesity is the expression of an imbalance between energy intake and energy expenditure, it has been known for decades that the gut microbial ecosystem, positioned at the interface between diet and host energy metabolism, can affect energy balance [1,2]. Recent studies revealed that changes in gut colonization in response to diet result in altered energy balance and contribute to obesity and metabolic disorders, such as diabetes. The need to better define the molecular mechanisms governed by diet—microbiota—host interactions has been addressed [3—12], in particular since studies on energy balance in germfree (GF) mice revealed conflicting results [13,14]. Controversial findings may have their origin in the obesogenic diets used. 'Western diets', high-sucrose or high-fat diets, and the respective control diets all vary in caloric density, texture, amount and composition of macronutrients and micronutrients, and, quite frequently, their composition is not sufficiently documented. In fact, the initial description that GF mice are resistant to diet-induced obesity [13] turned out to be strongly dependent on diet composition [14] but the dietary constituents responsible for this differential response have not been identified. Among many other variables diets utilized in these studies differed in fat sources, which affected the responses of host energy balance and microbial composition in conventional mice with a pronounced effect of saturated fat. Compared to olive or safflower oil, palm oil induced body mass gain, lowered microbial diversity, and increased *Firmicutes* to *Bacteroidetes* ratio [15]. Additionally, lard rather than fish oil aggravated white adipose tissue inflammation and promoted a higher degree of obesity, which was partly attributed to distinct microbiota composition [16]. In a subsequent study, dietary lipid composition using lard or fish oil also affected gut microbiota-induced regulation of hepatic cholesterol metabolism [17]. These results emphasize the notion that the interaction between gut microbiota and diet composition, and not the gut microbiota *per se*, merits further investigation to determine the proximate mechanisms affecting host metabolism with respect to obesity development.

Microbial communities are related to changes in gut morphology, physiology, and biochemistry [18]. Microbes ferment polysaccharides and proteins, produce vitamins, and metabolize bile acids, thereby affecting enterohepatic circulation and nutrient absorption [19,20]. However, the implication of gut microbiota on host energy homeostasis remains elusive [3,21], partly due to inappropriate analysis of energy expenditure data and spurious data interpretation. In obesity studies, normalization of energy expenditure for variation in body size and composition requires proper statistical methods, since lean mass is metabolically more active than fat mass, and simple body mass-specific ratios do not account for such differences [22—24]. Hence, the influence of diet and host microbiome on energy balance must be analyzed carefully.

In the present study, we assessed the impact of dietary fat source and the gut microbiota on diet-induced obesity (DIO) by performing comprehensive phenotyping of the host combined with metabolite profiling. This was achieved by feeding GF and specific pathogen free (SPF) male C57BL/6N mice high-fat diets either based on lard (LHFD) or palm oil (PHFD). Analyses included mouse energy expenditure, fecal fat and energy excretion, cecal bacterial diversity and composition, as well as gene expression pathways and metabolite profiling with particular focus on gut and hepatic bile acid and steroid metabolism. Hence, by using state-of-the-art molecular and physiological methods, the present work brings light to an ambiguous array of literature data on the interaction of diet and gut microbiota in mouse models of DIO. Our work assessed host energy balance in response to different dietary fat sources, in combination with metabolite profiling, qPCR, and high-throughput sequencing approaches, providing novel insights into the physiological relevance of gut microbiota and cholesterol-derived metabolites interactions.

2. MATERIALS AND METHODS

2.1. Animals

Studies were performed in SPF and GF male C57BL/6N mice housed at 22 ± 1 °C and 50—60% relative humidity with a 12 h light—dark cycle. Food and water were provided *ad-libitum*. SPF mice were kept in individually ventilated cages, and GF mice were housed in open cages within flexible film isolators ventilated via HEPA-filtered air. At 8 wks of age, mice were adapted for 4 wks to a purified control diet (CD; 5 wt% soybean oil corresponding to 12 kcal% fat; Ssniff, Soest, Germany). At 12 wks of age, mice were switched from CD to a high-fat diet (48 kcal % fat) based on palm oil (PHFD) or lard (LHFD) or they were maintained on CD for further 4 wks (Ssniff, Soest, Germany) (Table 1, Table S1). The sterol contents of the diets were determined via lipid extraction, saponification, and capillary gas chromatography (Table 2). Body mass was recorded weekly while food intake and feces production of group-housed mice were recorded during the first and the last week of the feeding trial. Body composition was determined at the end of the experiment by quantitative time domain NMR spectroscopy (MiniSpec, Bruker, Billerica, MA, USA). Mice were killed using CO_2 in the fed or in the fasted state due to basal metabolic rate recordings. Sterility was confirmed as described in the SI and as shown in Figure S1, respectively. Further details on sampling are explained in SI. Animal experimentation and procedures were approved by the German animal welfare authorities at the district government (approval no. 55.2-1-54-2532-103-2014).

2.2. Energy expenditure

Indirect calorimetry was based on an open respirometer system (LabMaster System; TSE Systems, Bad Homburg, Germany) and was performed as described previously [25]. In the morning of the third measurement day, basal metabolic rate was determined for 6 h at thermoneutrality (30 ± 0.5 °C). The variation in heat production (HP) ($HP_{adj.,22\ °C,ad\text{-}lib.}$, $HP_{adj,30\ °C,pa}$) due to individual differences in lean and fat mass was adjusted by ANCOVA. Further details on indirect

Table 1 — Compositions of the diets used in the present study.

	CD	PHFD	LHFD
		wt%	
Casein	24.0	24.0	24.0
Corn starch	45.9	26.7	26.7
Sucrose	5.0	5.0	5.0
Maltodextrin	5.6	5.6	5.6
Soy oil	5.0	5.0	5.0
Palm oil	—	20.0	—
Lard	—	—	20.0
Cellulose	5.0	5.0	5.0
Mineral mixture	6.0	6.0	6.0
Vitamin mixture	1.2	1.2	1.2
		kJ%	
Protein	23.0	18.0	18.0
Fat	12.0	48.0	48.0
Carbohydrates	65.0	34.0	34.0
Energy content [kJ*g^{-1}][a]	15.5	22.7	22.7

[a] Gross calorific value according to bomb calorimetry.

