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Modification of the fatty acid composition of an obesogenic diet improves the maternal and placental metabolic environment in obese pregnant mice

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ABSTRACT

Peri-conceptional exposure to maternal obesogenic nutrition is associated with *in utero* programming of later-life overweight and metabolic disease in the offspring. We aimed to investigate whether dietary intervention with a modified fatty acid quality in an obesogenic high-calorie (HC) diet during the preconception and gestational phases can improve unfavourable effects of an adipogenic maternal environment. In NMRI mice, peri-conceptional and gestational obesity was induced by feeding a HC diet (controls), and they were compared with dams on a fat-modified (Fat-mod) HC diet of the same energy content but enriched with medium-chain fatty acids (MCFAs) and adjusted to a decreased ratio of n-6 to n-3 long-chain polyunsaturated fatty acids (LC-PUFAs). Effects on maternal and placental outcomes at delivery (day 17.5 post coitum) were investigated. Despite comparable energy assimilation between the two groups of dams, the altered fatty acid composition of the Fat-mod HC diet induced lower maternal body weight, weights of fat depots, adipocyte size, and hepatic fat accumulation compared to the unmodified HC diet group. Further, there was a trend towards lower fasting glucose, insulin and leptin concentrations in dams fed the Fat-mod HC diet. Phenotypic changes were accompanied by inhibition of transcript and protein expression of genes involved in hepatic *de novo* lipogenesis comprising PPARG2 and its target genes *Fasn*, *Acaca*, and *Fabp4*, whereas regulation of

Abbreviations: Abca1, ATP-binding cassette, sub-family A, member 1; ABCA1, ATP-binding cassette, sub-family A, member 1; *Acaca*, acetyl-Coenzyme A carboxylase alpha; ACACA, acetyl-CoA carboxylase 1; *Adipoq*, adiponectin, C1Q and collagen domain containing; ANOVA, analysis of variance; AUC, area under the curve; BAT, brown adipose tissue; CD, control diet; *Cd36*, Cd36 antigen; *Cpt1*, carnitine palmitoyltransferase 1; *Dgat1*, diacylglycerol O-acyltransferase 1; DHA, docosahexaenoic acid; dpc, day post coitum; EPA, eicosapentaenoic acid; FA, fatty acid; *Fabp4*, fatty acid binding protein 4; FABP4, fatty acid-binding protein, adipocyte; *Fasn*, fatty acid synthase; FAS, fatty acid synthase; Fat-mod HC diet, fat-modified high-calorie diet; FFPE, formalin-fixed, paraffin-embedded; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; *Got2*, glutamatic-oxaloacetic transaminase 2 (also known as *Fabp-pm*, plasma membrane fatty acid binding protein); HC diet, high-calorie diet; HDL, high-density lipoprotein; HE, hematoxylin-eosin; HFD, high-fat diet; ipGTT, intraperitoneal glucose tolerance test; LCFA, long-chain fatty acid; LC-PUFA, long-chain polyunsaturated fatty acid; LDL, low-density lipoprotein; *Lep*, leptin; LEP, leptin; MCFA, medium-chain fatty acid; MCT, medium-chain triglyceride; *Me1*, malic enzyme 1; *Mest*, mesoderm-specific transcript/imprinted paternally expressed gene 1 (also known as *Peg1*); MEST, mesoderm-specific transcript homolog protein; MRI, magnetic resonance imaging; NEFA, non-esterified fatty acid; NMRI, Naval Medical Research Institute; *Nr1h3*, nuclear receptor subfamily 1, group H, member 3 (also known as *Lxra*, liver X receptor alpha); NR1H3, oxysterols receptor LXR-alpha; PFA, paraformaldehyde; *Plin2*, perilipin 2; *Pnpla2*, patatin-like phospholipase domain containing 2 (also known as *Atgl*, adipose triglyceride lipase); *Ppara*, peroxisome proliferator activated receptor alpha; *Pparg*, peroxisome proliferator activated receptor gamma; PPARG, peroxisome proliferator-activated receptor gamma; *Ppargc1a*, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; *Ppib*, peptidylprolyl isomerase B; *Rxra*, retinoid X receptor alpha; *Scd2*, stearoyl-coenzyme A desaturase 2; S.E.M., standard error of the mean; *Slc27a1*, solute carrier family 27 (fatty acid transporter), member 1 (also known as *Fatp1*, fatty acid transport protein 1); *Slc27a4*, solute carrier family 27 (fatty acid transporter), member 4 (also known as *Fatp4*, fatty acid transport protein 4); *Srebf1*, sterol regulatory element binding transcription factor 1; n-SREBP1, sterol regulatory element-binding protein 1, nuclear; p-SREBP1, sterol regulatory element-binding protein 1, precursor; *Sry*, sex determining region of Chr Y; *Tbp*, TATA box binding protein; TUBA1A, tubulin alpha-1A; *Ube2d2*, ubiquitin-conjugating enzyme E2D 2.

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other lipogenic factors (*Srebf1*, *Nr1h3*, *Abca1*) appeared to be more complex. The modified diet led to a sex-specific placental response by upregulating PPARG-dependent fatty acid transport gene expression in female versus male placentae. Qualitative modification of the fatty acid spectrum of a high-energy maternal diet, using a combination of both MCFAs and n-3 LC-PUFAs, seems to be a promising interventional approach to ameliorate the adipogenic milieu of mice before and during gestation.

1. Introduction

To date, up to two thirds of women in the reproductive age are overweight or obese in the United States [1]. Among the various perinatal risk factors of later-life overweight and metabolic disease in the offspring including gestational diabetes and excessive gestational weight gain, peri-conceptional maternal obesity confers the strongest risk in humans [2]. An adipogenic maternal environment during pregnancy and lactation is considered to induce such adverse later-life outcomes [3,4] via mechanisms referred to as "fetal programming". Even during the earliest developmental stages peri-conceptionally, an obesogenic milieu can exert sex-specific adverse effects on adult offspring, as shown in mice [5].

To counteract these early-life risks predisposing to childhood obesity, effective primary preventive strategies are needed [6]. The nutritional composition of the maternal diet during gestation has been suggested to have an impact on fetal development in mice and humans [3,7]. Specifically, the consumption of a high-fat (60% kcal fat) diet (HFD) had strong effects on offspring outcomes in mice, irrespective of maternal obesity *per se* [8]. Compared to dietary fat consisting of mainly long-chain fatty acids (LCFAs, ≥14 carbon atoms), the use of medium-chain triglycerides (MCTs) containing medium-chain fatty acids (MCFAs, 8-12 carbon atoms) has been supposed to result in a reduction of body weight and fat mass in animals and humans [9,10]. A recent study in rats, consuming either an LCFA- or MCFA-rich diet during pregnancy, suggested an adiposity-lowering effect in offspring of MCFA-fed dams when they were exposed to HFD later in life [11].

Nutritional supplementation with omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) and thereby reducing the ratio of n-6 to n-3 LC-PUFAs has also been suggested to have a beneficial impact on important health issues including improvements of dyslipidemia, insulin resistance, and inflammation [12]. Despite advantageous effects also on body weight reduction and lowering of fat accumulation, as observed in *in vitro* and animal experiments [13,14], data on n-3 LC-PUFA supplementation during pregnancy on both maternal and offspring outcomes are scarce and inconsistent, from both animal and human studies [15,16].

In previous studies, we found that female Naval Medical Research Institute (NMRI) mice fed a high-calorie (HC) diet with 60% of energy from mainly saturated fat and a high sugar-to-starch-ratio presented with an increased weight at conception, an impaired glucose tolerance in pregnancy, and higher placental and offspring weights compared to control diet (CD)-fed dams [5]. We hypothesized that such adverse effects of a maternal obesogenic HC diet might be ameliorated or even prevented if the fat composition is altered via a combination of both an enrichment with MCFAs and a reduction of the n-6:n-3 LC-PUFA ratio. The aim was to evaluate the impact of a modification of the fatty acid quality of a HC diet during the peri-conceptional and gestational period of NMRI dams, by assessing maternal and placental outcomes at delivery.

2. Materials and methods

2.1. Experimental schedule

The experimental design with NMRI dams was reported previously by our group [5]; details are presented in the Supplementary Materials and Methods section. NMRI outbred dams were chosen because we aimed to generate a model of mild diet-induced maternal adiposity in pregnancy that develops less severe metabolic consequences of HFD feeding than standard inbred strains [17,18], and thus shows closer correspondence to the considerable share of overweight and obese women without metabolic complications during pregnancy. Briefly, 30 female NMRI mice at 3 weeks of age were randomly distributed into two groups. One group received the HC diet, an unmodified obesogenic diet (E15741, Ssniff, Soest, Germany) and served as a control group. Apart from a high-saturated fat content, the HC diet was enriched with sugar (Supplementary Table 1). The intervention group received a diet with the same high energy density and identical protein and carbohydrate composition but with a modified fat quality consisting of an increased amount of MCFAs and a reduced n-6:n-3 LC-PUFA ratio (termed "Fat-mod HC diet"; S8379-E012, Ssniff) (Supplementary Table 1). To provide the respective fatty acid patterns, beef tallow and soybean oil was used for the HC diet, whereas for the Fat-mod HC diet, hydrogenated coconut oil, marine oil (n-3), walnut oil, and lower amounts of beef tallow were applied (Supplementary Table 1). Walnut oil was used as a source of essential fatty acids, because it contains slightly higher concentrations of linolenic acid (C18:3 n-3) and lower concentrations of palmitic acid (C16:0) than soybean oil (Supplementary Table 1). The fatty acid compositions of coconut oil and marine oil (n-3) are presented in the Supplementary Table 2; additional details on stability analyses of fatty acids in the diet are provided in the Supplementary Materials and Methods section.

Body weight and body composition were determined weekly. At age 5 weeks, individual food intake and fecal excretion were recorded over 4 days. At age 12 weeks, mice were mated and screened for vaginal plugs every 12 h. Besides screening for vaginal plugs, mating success was determined by abdominal palpation of fetuses at 7.5 days post coitum (dpc). Mice remained on their respective experimental diets during pregnancy, and food intake, body weight, and body composition were assessed every three days. At 17.5 dpc, animals were anesthetized following fasting for 12 h, bled from the retro-orbital plexus, and killed by cervical dislocation. Organs including 6 different fat depots, placentae, and fetuses were promptly dissected, blotted dry, and weighed to the nearest mg. Tissue samples were immediately processed and either frozen at −80 °C, fixed in 4% paraformaldehyde (pH 7.4), or fixed in RNALater (Qiagen, Hilden, Germany) and frozen at −20 °C. Experiments were approved by the Animal Ethics Committee (Bavaria, Germany) and are in accordance with the Council of Europe Convention ETS 123 (2010/63/EU).

2.2. Determination of body composition, energy intake, and energy assimilation

Body composition (% fat and lean mass) was assessed via whole animal body composition measurement using a time domain nuclear magnetic resonance imaging (MRI) analyzer (Minispec LF50; Bruker Optics, Ettlingen, Germany) without anesthesia as reported previously [5]. Details on analyses of energy intake, energy assimilation, and feces composition are outlined in the Supplementary Materials and Methods section.

2.3. Analysis of serum parameters of lipid and glucose metabolism in dams

To verify that the functional compounds of the Fat-mod HC diet reached the systemic circulation, serum samples of two subgroups of