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Original article

Interesterified fat or palm oil as substitutes for partially hydrogenated fat in maternal diet can predispose obesity in adult male offspring

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SUMMARY

Background & aims: Palm oil (PO) and interesterified fat (IF) have been used to replace partially hydrogenated fat (PHF), which is rich in *trans* isomers, in processed foods. The purpose of this study was to investigate whether normolipidic diets containing PHF, IF, or PO consumed during pregnancy and lactation affect total body adiposity and adipose tissue morphology of adult offspring mice.
Methods: Four groups of female C57BL/6 mice were fed, during pregnancy and lactation, a control diet (control group, CG), a PHF diet (*trans* group, TG), a PO diet (PG group), or an IF diet (IG group). After weaning (at 21 days), male pups received the control diet for 70 days. Food intake and body weight were monitored in all groups throughout the experimental period. At 3 months of age, mice were sacrificed and the inguinal (IWAT), epididymal (EWAT), retroperitoneal (RPWAT), and mesenteric (MWAT) adipose fat pads were removed and weighed. Adiposity was quantified by micro computed tomography (micro-CT), and adipocyte areas and cell number were analyzed by histology.
Results: PG and IG offspring gained more weight than CG and TG groups ($p < 0.01$) during the first 10 weeks after weaning, resulting in higher final body weights ($p < 0.05$). IG mice and PG mice had respectively heavier EWAT and IWAT than TG and CG mice. Micro-CT scanning revealed that the total volumes of internal, external, and total fat depots were greater in IG animals, as compared to the other groups. Larger adipocyte areas were observed in EWAT and IWAT in IG and TG, respectively, in comparison to CG and PG mice. PG mice showed increased adipocyte numbers in IWAT.
Conclusions: Maternal intake of IF and/or PO during pregnancy and lactation predisposes the offspring to the development of obesity in adult life in mice.

1. Introduction

The increasing prevalence of obesity and its epidemic magnitude is a major global health issue, particularly in light of the increased morbidity and mortality associated with obesity-related metabolic disorders, such as type 2 diabetes, cardiovascular diseases, hyperlipidemia, and hypertension [1]. Obesity is characterized by an excess of white adipose tissue resulting from chronic positive energy imbalance [2]. As the disease develops, excess energy is stored in adipocytes as triacylglycerol, resulting in adipocyte hypertrophy [1]. When adipocyte storage capacity is exceeded, the pool of adipocytes increases through hyperplasia, with new adipocytes arising from differentiation of preadipocytes [3].

The fetal nutritional environment is key for the development of obesity in later life [4] due to developmental plasticity [5]. Evidence indicates that nutritional imbalances during pregnancy and lactation disrupt prenatal development, which can permanently affect the structure, function, and metabolism of several tissues and organs [6,7]. Obesity due to developmental plasticity appears to be

Abbreviations: CG, control group; EWAT, epididymal white adipose tissue; IF, interesterified fat; IG, interesterified group; IWAT, inguinal white adipose tissue; MWAT, mesenteric white adipose tissue; PG, palm group; PHF, partially hydrogenated fat; PO, palm oil; RPWAT, retroperitoneal white adipose tissue; TFA, *trans* fatty acids; TG, *trans* group.

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propagated through generations, further contributing to the already alarming obesity rates worldwide [8].

Changes in the composition of dietary fatty acids, which are involved in the regulation of several cellular activities, can lead to obesity [9] and other associated pathological conditions such as insulin resistance [10], hypertension, and cardiovascular disease [11,12]. Previous animal studies indicate that consumption of normolipidic diets rich in palm oil (PO) saturated fatty acid or in partially hydrogenated fat (PHF) *trans* isomers during lactation can favor fat retention in the offspring at young ages [13]. Given the deleterious health effects of consumption of *trans*-fatty acid (TFA), present in large amounts in PHF, reduced intakes during pregnancy have been recommended [14–17].

Several technologies have been developed to reduce or eliminate TFA from food products [18,19]. One such technology is interesterification, which rearranges fatty acids in triglycerides to yield customized melting features [20]. Alternatively, due to their high saturated fat content and higher melting point, tropical oils free of TFA, including palm, palm kernel, and coconut oil, have been used to replace partially hydrogenated fats in backed goods [21]. Whether consumption of these types of fat during pregnancy and lactation can affect body adiposity later in life remains controversial, particularly with regard to normolipidic diets containing different fatty acid compositions. The aim of this study was to investigate, in a murine model, whether the consumption of normolipidic diets containing PHF *trans* isomers or industrial lipid substitutes (interesterified fat, IF; or PO), during pregnancy and lactation, can predispose the offspring to altered adiposity in adulthood.

2. Material and methods

2.1. Animals, diets, and general procedures

C57BL/6 female mice were kept under controlled light and temperature conditions with free access to food and water.

Pregnant animals were housed in individual cages and divided into four groups. All groups were fed experimental isocaloric (4.1 kcal/g of dry weight) and normolipidic diets following the American Institute of Nutrition (AIN) [22] recommendations, but differing in fatty acid content (Table 1). The Control Group (CG) received a diet containing 7% soy oil, which contains the recommended amounts of essential fatty acids; the partially hydrogenated fat group (*trans* group, TG) received 6% partially hydrogenated vegetable oil plus 1% soy oil; the palm oil group (PG) received 5% palm oil plus 2% soy oil; and the interesterified group (IG) received 5% interesterified fat plus 2% soy oil. Soy oil was added to the diets to ensure the minimum requirement for essential fatty acids. Interesterified fat (IF) was prepared using different types of lipids such as palm kernel oil (2.5%), palm stearin (45%), soybean oil (45%), and fully hydrogenated fat (7.5%). IF was generously donated by Triângulo Alimentos, São Paulo, Brazil. The fatty acid composition of the diets is summarized in Table 2. The diets were maintained throughout gestation and lactation. On the day of delivery (day 0 of lactation), litters were adjusted to six pups per dam. At the end of lactation (day 21), male pups ($n = 6–8$ for each diet group) were separated from their dams.

From day 21 onwards, the offspring received control-growth diet (AIN-93G) containing 20% protein and 7% fat until 2 months of age. From 60 to 90 days of age, the offspring received the control-maintenance diet (AIN-93M) containing 14% protein and 4% fat from soybean oil, as recommended for adult animals [22]. The diets were prepared at the beginning of the study, kept in daily portions at −20 °C until use, and given to the animals every 24 h. Food consumption and body mass were measured daily and weekly, respectively. Offspring post-weaning relative body weight gain was calculated as ([Body weight at 21 days − Body weight at 90 days]/[Body weight at 21 days] %). At 90 days of life, animals were anaesthetized and sedated with intraperitoneal Ketamine (100 mg/kg of body mass) and Xylazine (16 g/kg body mass) and killed by cardiac puncture to collect trunk blood. Inguinal fat pads (IWAT), retroperitoneal fat pads (RPWAT), epididymal fat pads (EWAT), and mesenteric fat pads (MWAT) were collected and weighed. All procedures performed in this study were previously approved by the Experimental Research Committee of the Federal University of Rio

Table 1
Diet composition (g/kg).

Constituents (g/kg of diet)	Control diet (Soy oil)	Partially hydrogenated fat diet (trans)	Palm oil diet	Interesterified fat diet
Casein (vitamin free)	200.0 (140.0)	200.0	200.0	200.0
Cornstarch	397.4 (465.7)	397.4	397.4	397.4
Dextrinized Cornstarch	132.0 (155)	132.0	132.0	132.0
Sucrose	100.0	100.0	100.0	100.0
Cellulose	50.0	50.0	50.0	50.0
Salt mix G[a] (M)[b]	10.0	10.0	10.0	10.0
Vitamin mix[c]	35.0	35.0	35.0	35.0
β-Choline	2.5	2.5	2.5	2.5
Butylhydroquinone-BHT	0.014	0.01	0.01	0.01
Soy oil	70.0 (40.0)	10.0	20.0	20.0
Partially hydrogenated fat	–	60.0	–	–
Palm oil	–	–	50.0	–
Interesterified fat	–	–	–	50.0
Energy value (Kcal/Kg)	3950	3950	3950	3950

Amounts during maintenance periods (from 60 days of age onwards) are presented within parentheses (Reeves).

[a] Salt mix G (mg/kg diet) for growth, pregnancy and lactation periods: Calcium 5000.0, Phosphorus 1561.0, Potassium 3600.0, Sulfur 300.0, Sodium 1019.0, Chloride 1571.0, Magnesium 507.0, Iron 35.0, Zinc 30.0, Manganese 10.0, Copper 6.0, Iodine 0.2, Molubdenum 0.15, Selenium 0.15, Silicon 5.0, Chromium 1.0, Fluoride 1.0, Nickel 0.5, Boron 0.5, Lithium 0.1, Vanadium 0.1.

[b] Salt mix M (mg/kg diet) for maintenance periods: Calcium 5000.0, Phosphorus 1992.0, Potassium 3600.0, Sulfur 300.0, Sodium 1019.0, Chloride 1571.0, Magnesium 507.0, Iron 35.0, Zinc 30.0, Manganese 10.0, Copper 6.0, Iodine 0.2, Molubdenum 0.15, Selenium 0.15, Silicon 5.0, Chromium 1.0, Fluoride 1.0, Nickel 0.5, Boron 0.5, Lithium 0.1, Vanadium 0.1.

[c] Vitamin mix (mg/kg diet): retinyl palmitate 2.4; cholecalciferol 0.025; menadione sodium bisulfite 0.8; biotin 0.22; cyanocobalamin 0.01; riboflavin 6.6; thiamin hydrochloride 6.6; tocopherol acetate 100.

Table 2
Fatty acids profile (mg/g of diet) of total lipids of experimental diets consumed by mothers during pregnancy and lactation.

Fatty acids	CG	TG	PG	IG
Σ SFA	8.56 ± 0.38[a]	15.46 ± 3.39[ab]	25.23 ± 3.76[b]	16.62 ± 4.27[ab]
C16:0	5.83 ± 0.26[a]	8.24 ± 1.82[a]	20.40 ± 3.04[b]	13.35 ± 3.31[ab]
C18:0	1.93 ± 0.07[a]	6.47 ± 1.41[a]	3.53 ± 0.54[a]	2.41 ± 0.70[a]
Σ MUFA	13.90 ± 0.32[a]	21.93 ± 4.29[a]	22.96 ± 2.97[a]	10.62 ± 2.51[a]
C18:1 9c (Oleic)	11.89 ± 0.61[a]	8.26 ± 1.74[a]	21.64 ± 3.33[b]	9.44 ± 2.58[a]
Σ PUFA	22.53 ± 2.54[a]	4.33 ± 1.05[b]	13.00 ± 2.19[ab]	13.32 ± 4.26[ab]
Σ TFA	0.47 ± 0.07[a]	10.86 ± 3.03[b]	0.38 ± 0.04[a]	0.68 ± 0.18[a]
C18:2n-6 (AL)	20.98 ± 1.37[a]	3.91 ± 0.79[b]	11.92 ± 1.56[ab]	12.15 ± 3.55[ab]
C18:3n-3 (AAL)	2.07 ± 0.17[a]	0.34 ± 0.06[b]	1.00 ± 0.13[b]	0.86 ± 0.27[b]
Σ PUFA n-3	2.11 ± 0.17[a]	0.35 ± 0.06[b]	1.04 ± 0.13[b]	0.89 ± 0.29[b]
Σ PUFA n-6	21.17 ± 1.38[a]	4.13 ± 0.85[b]	12.21 ± 1.60[ab]	12.32 ± 3.60[ab]
n-6/n-3 ratio	10.03	11.80	11.74	13.84

Σ SFA: sum of saturated fatty acids; Σ MUFA: sum of monounsaturated fatty acids; Σ PUFA: sum of polyunsaturated fatty acids; Σ TFA: sum of *trans* fatty acids; LA: linoleic acid; AAL: alfa linolenic acid; Σ PUFA n-6: sum of n-6 polyunsaturated fatty acids; Σ AGPI n-3: sum of n-3 fatty acids. CG: control group; TG: trans fat group; PG: palm oil group; IG: interesterefied fat group. Values are expressed as mean ± standard error ($n = 6$ per group). Different letters at the same row mean statistical difference between experimental groups ($p < 0.05$).