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Effect of a maternal cafeteria diet on the fatty acid composition of milk and offspring red blood cells

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ABSTRACT

Previous studies have demonstrated that exposure to a maternal cafeteria diet during the lactation period alone produces detrimental effects to offspring metabolic health comparable to exposure during the entire perinatal period. The present study used a rodent model to assess the effect of a maternal cafeteria diet on the fat content and fatty acid composition of the dams' milk, and to determine the degree to which this was related to the fatty acid status of offspring on postnatal day 1 (PND1), weaning and 3 weeks post-weaning onto a standard rodent diet. As expected, omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) content of both the milk and pup red blood cells (RBCs) was lower in the cafeteria (CAF) group on PND1. At 2 weeks post-partum, milk produced by CAF dams had a higher total fat, saturated fat and n-6 PUFA content, however these differences were modest in comparison with the differences in maternal intake between groups. Offspring suckled by CAF dams had a lower n-3 LCPUFA and n-6 PUFA status at weaning and higher *trans* fatty acid levels at both weaning and 6 weeks of age. These findings indicate that the fat content and fatty acid composition of the dam's milk is altered by exposure to a cafeteria diet. While it appears that the dam has a significant capacity to buffer the transfer of most dietary lipids into the milk, the *trans* fatty acids in particular appear to be readily transferred, resulting in persistent increases in *trans* fatty acid status of the offspring after weaning. The potential physiological implications of this warrants further examination.

1. Introduction

Epidemiological, clinical and experimental animal studies have shown that maternal consumption of diets high in saturated fat, sugar or total calories during pregnancy and lactation is associated with an increased risk of obesity and metabolic disease in the offspring in postnatal life [1,2]. Cross-fostering studies in rodents have built on this evidence and shown that exposure to a maternal cafeteria diet during the suckling period alone is associated with adverse metabolic health outcomes in the offspring that are comparable to those resulting from exposure during the entire perinatal period [3,4]. Since maternal milk is the dominant source of nutrition for the offspring during suckling, these findings suggest that alterations to milk composition are likely to play an important role in the metabolic programming of the offspring.

The majority of studies that have focussed on the consequences of a maternal poor quality diet have provided a selection of palatable foods, which typically contain a higher proportion of total fat, sugar and carbohydrate and lower levels of protein as a percentage of total energy than the standard rodent diets fed to control dams [5–7]. In addition to the higher total fat content, the fatty acid composition of cafeteria diets used in these studies is also markedly different to the control diets, with higher proportions of saturated and trans fats and a lower omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA) content [5–7]. This is significant since the fat content and fatty acid composition of breast milk is considered to be closely related to maternal dietary intake [8]. In addition, previous studies have provided evidence that individual fatty acids have distinct roles in development. The n-3 LCPUFA are recognised as essential fatty acids for foetal and infant development, particularly the development of the brain and central nervous system [9], while perinatal exposure to excess levels of saturated or trans fats during has been linked to an increased

Abbreviations: ARA, arachidonic acid (20:4 n-6); ALA, α-linolenic acid (18:3 n-3); CAF, cafeteria diet-fed; EPA, eicosapentaenoic acid (20:5 n-3); DPA, docosapentaenioc acid (22:5 n-3); DHA, docosahexaenoic acid (22:6 n-3); FAME, fatty acid methyl esters; FID, flame ionisation detector; LA, linoleic acid (18:2 n-6); n-3 LCPUFA, omega-3 long chain polyunsaturated fatty acid; n-6 PUFA, omega-6 long chain polyunsaturated fatty acid; PND, postnatal day; RBC, red blood cells

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risk of obesity, insulin resistance and cardiovascular deficits [10,11]. Consequently, it is important to understand the impact of maternal diet on the balance of specific fatty acids in the milk. However, while previous studies have reported that the total fat content of milk from mothers fed a cafeteria diet is significantly increased relative to those fed on standard rodent diets [12], there are currently no studies which have determined how these diets affect the fatty acid composition of the milk or the fatty acid status of the offspring.

Thus, the primary aim of this study was to determine the impact of providing dams with a cafeteria diet during the lactation period on the fat and protein content and fatty acid composition of their milk, and the extent to which the fatty acid composition of the dams diet and milk related to the fatty acid status of the offspring during the suckling and post-weaning periods.

2. Materials and methods

2.1. Animals and feeding regime

This study was approved by the Animal Ethics Committee of the University of Adelaide. Twenty-six female Albino Wistar rats (200–250 g) and four male Albino Wistar rats (200–300 g) were used in this study. All rats were individually housed under a 12 hour (h) light/12 h dark cycle at a room temperature of 25 °C and allowed to acclimatise to the animal housing facility for at least one week before initiation of the experimental procedure. During this time rats were fed *ad libitum* on standard rodent feed (Specialty Feeds, Glen Forrest, Western Australia) with free access to water. All dams were weighed once per week throughout the experiment.

At the end of the acclimatisation period, the female rats were randomly assigned to either the Control ($n=14$) or a Cafeteria (CAF; $n=12$) group. Control rats were given free access to standard rodent feed while CAF rats were fed a cafeteria diet comprised of peanut butter, hazelnut spread, chocolate-flavoured biscuits, extruded savoury snacks, sweetened multi-grain breakfast cereal and an edible animal fat blend/rodent feed mix. Detailed macronutrient composition of the cafeteria diet and control diet has been published previously [13]. The fatty acid composition of the standard rat feed and each item in the cafeteria diet is shown in Table 1. All foods included in the cafeteria diet contained a higher amount of saturated fats (with the exception of peanut butter) and lower amounts of n-3 LCPUFA (with the exception of the hazelnut spread) compared to the standard rodent feed. The content of *trans* fatty acids (including *trans* 18:1 n-7, 18-1 n-6, 18:2 and 16:1 fatty acids) was also higher in the edible animal fat blend plus rat feed mix than in any other food type (Table 1).

2.2. Measurement of food intake

For both the Control and CAF dams, food intake was determined every two days and fresh food provided. For the Control dams, the weight of feed remaining at the end of the 2-day period was subtracted from the amount initially provided to determine the weight of feed consumed. For the CAF dams, the weight of each individual food type was subtracted from the amount of that food initially provided to determine the intake of each separate component of the cafeteria diet. The weight of each food consumed was multiplied by the energy, macronutrient and fatty acid content of the respective food type in order to calculate the intake of total energy, fat, protein, carbohydrate and each of the individual fatty acids for each experimental animal.

2.3. Mating and pregnancy

After 4–6 weeks on their respective diets, on the evening of diestrous/proestrous, two female rats were placed with a male rat for 24 h. The presence of sperm in vaginal smears on the following morning was taken as confirmation of successful mating and this was identified as gestation day 0. All dams were allowed to give birth naturally. The number of pups in each litter and the sex and birth weight of each pup were recorded and all litters culled to 8 pups, with 4 males and 4 females where possible, within 24 h of birth (culled pups were used for postnatal day 1 (PND1) samples). Pups were then cross-fostered to another dam which gave birth within the same 24 h period from either the same or different nutritional treatment group. Pups remained with their foster mother until weaning (3 weeks of age). After weaning, the offspring were housed in groups with their same-sex littermates (3–4 pups/cage) and were fed with standard rodent feed until the end of the experiment at 6 weeks of age. Pups were weighed every second day until weaning and once per week thereafter until the end of the experiment.

In the present study, the fatty acid status of offspring at birth was assessed in offspring born to both Control and CAF dams. The fatty acid status at weaning and 6 weeks of age was, however, only assessed in offspring born to Control dams and suckled by either a Control or CAF dam, such that the impact of exposure to the cafeteria diet exclusively during the suckling period could be determined.

2.4. Blood sample collection

Blood samples were collected from Control and CAF offspring within 24 h of birth (PND1). These pups were killed by decapitation and blood samples from all pups in a litter were pooled to provide sufficient volume for analysis. Blood samples were also collected from one male and one female pup from each litter of control pups at 3 weeks (weaning) and 6 weeks of age by cardiac puncture immediately following euthanasia with an overdose of CO_2. The blood was centrifuged at 3500*g* at 4 °C for 15 min. The plasma was removed and the red blood cells (RBCs) prepared for analysis of fatty acid composition as previously described [14].

2.5. Stomach contents and milk collection

Stomach contents were collected from pups of Control and CAF dams which were culled on PND1 and stored at −20 °C prior to analysis. Milk samples were collected from all dams during the second week of lactation. Dams were separated from their litters for 2–3 h and were given a single intraperitoneal injection of oxytocin (0.5 ml) 5 min prior to milking. The milk was expressed from the teats by gentle manual kneading with repetitive top to bottom stroking motions. Between 0.5 and 1.0 ml of milk was obtained from each dam and milk samples were frozen at −20 °C until further analysis. Protein concentration of the stomach contents and milk samples was determined by a validated Bradford method using bovine serum albumin as the standard [15].

2.6. Determination of total fat content and fatty acid composition

The total lipid content of standard feed and each individual component of the cafeteria diet and milk sample was determined gravimetrically following homogenisation and extraction in chloroform–methanol (2:1, v/v) [16]. Total lipids were also extracted from a sample of standard feed and each individual component of the cafeteria diet for the assessment of their fatty acid composition. For the RBCs, the phospholipids were separated from total lipid extracts by thin layer chromatography (TLC) on silica gel