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 explanation.

Key words: Maternal nutrition, fatty acids, milk, development

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.

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) contents [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 fetal 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 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 (200-250g) and four male Albino Wistar rats (200-300g) 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 two 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 to 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 3,500g at 4˚C for 15 minutes. 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 minutes prior to milking. The milk was expressed from the teats by gentle manual kneading with repetitive top to bottom stroking motions. Between 0.5 to 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 plates (Silica gel 60H; Merck, Darmstadt, Germany). A lipid class standard 18-5 (NU-CHEK Prep; Elysian, MN) was run on the plates for lipid

identification. The mobile phase for TLC was petroleum spirit/acetone (3:1, v/ v). The TLC plates were sprayed with fluorescein 5-isothiocyanate in methanol, and the lipid classes present were visualized under UV light. All solvents used for extraction and separation contained 0.005% (w/v) antioxidant, butylated hydroxyl toluene.

Total lipids from the milk samples, foods and the RBC phospholipid bands scraped from the TLC plates were transesterified with 1% H_2SO_4 in methanol at 70°C for 3 h. After the samples were cooled, the resulting fatty acid methyl esters (FAME) were extracted with *n*-heptane and transferred into vials containing a scoop of anhydrous sodium sulphate. FAMEs were separated and quantified by GC (Hewlett-Packard 6890; Palo Alto, CA) equipped with a capillary column (50 m x 0.32 mm id) coated with 0.25 μm film thickness silica (BPX-70; SGC Pty Ltd, Victoria, Australia), and a flame ionisation detector (FID). The injector temperature was set at 250 °C and the FID temperature at 300 °C. The oven temperature at injection was initially set at 140 °C and was programmed to increase to 220 °C at a rate of 5 °C per minute. Helium gas was utilized as a carrier at a flow rate of 35 cm per second in the column. The identification and quantification of FAMEs was achieved by comparing the retention times and peak area % values of unknown samples to those of commercial lipid standards (NU-CHEK Prep; Elysian, MN) using the Hewlett-Packard Chemstation data system. Total *trans* fatty acid content included the sum of *trans* 18:1 n-7, 18-1 n-6, 18:2 and 16:1 fatty acids. All solvents used in these experiments were of analytical grade and were purchased from Ajax Finechem Pty Ltd (Auckland, New Zealand) or Chem-Supply (South Australia, Australia). Other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

2.7 Statistical analysis

Data are presented as mean ± SEM. The effect of maternal diet on the dams' total energy, protein, carbohydrate and fat intake and the intake of each of the individual fatty acid classes during pregnancy and lactation was determined using a Student's unpaired t-test. Differences in the levels of protein, total fat and individual fatty acids of the stomach contents, milk samples and RBC phospholipids between the pups born to Control and CAF dams on PND1 were similarly determined. The impact of maternal diet during lactation and offspring sex on RBC fatty acid composition in offspring born to Control dams at 3 and 6 weeks of age was initially determined using a 2-way ANOVA with maternal diet during the lactation period (CAF or Control) and pup sex as factors. Since no sex differences or sex by treatment interactions were identified by the two way ANOVA, the data from male and female offspring were combined in the analysis. Correlations between maternal intake and fat composition of the milk with offspring fatty acid status were assessed using linear regression analysis. All statistical analyses were conducted using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The litter (dam) was used as the unit of analysis for all statistical tests. A probability of $P<0.05$ was considered statistically significant in all analyses.

3. RESULTS

3.1 Maternal nutritional intake during pregnancy and lactation

Mean daily energy intake was ~10% higher in CAF dams during pregnancy and ~16% lower during lactation compared to Control dams (Table 2). Dams fed the CAF diet consumed 4- to 5-fold more fat, and 40% and 54% less protein during pregnancy and lactation, respectively compared to Control dams (Table 2, $P<0.01$).

Dams in the CAF group consumed significantly more saturated fats (9-fold), monounsaturated fats (6-fold), *trans* fats (25-fold), linoleic acid (LA, 18:2n-6), total n-6 PUFA and α-linolenic acid (ALA)) as a percentage of their total daily energy intake compared to Control dams during both pregnancy and lactation (Table 2). The intake of arachidonic acid (ARA, 20:4n-6) and the n-3 LCPUFAs, eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), as a percentage of total energy intake was, however, significantly lower (by 4- to 30-fold) in CAF dams during pregnancy and lactation compared to Control dams (Table 2).

3.2 Effect of a maternal cafeteria diet during pregnancy on milk composition

Postnatal Day 1 (PND 1)

There was no difference in the protein or total fat content of the stomach contents collected from pups on PND 1 between the CAF and Control groups (Figure 1A and B). The stomach contents from the pups born to CAF dams, however, contained higher levels of monounsaturated and *trans* fatty acids (4.9-fold, $P<0.001$, Figure 2A and E), and lower levels of saturated fatty acids (2-fold, $P<0.01$) and n-3 LCPUFA (1.6-fold, $P<0.01$) compared to the pups of Control mothers (Figure 2). There were no differences in the level of n-6 PUFA in the stomach contents between groups (Figure 2C). The levels of monounsaturated fatty acids, *trans* fatty acids and n-3 LCPUFA, but not levels of saturated fat and n-6 PUFA, in the stomach contents were directly related to the levels of these respective fatty acids in the maternal diet (data not shown).

Mid-lactation

The milk from CAF dams had a significantly (0.3-fold) higher total fat content compared to the Controls (Figure 1A). The milk from dams consuming the cafeteria diet also contained higher proportions of saturated fat (0.3-fold, $P<0.01$), *trans* fats (12-fold, $P<0.01$) and n-6 PUFA (0.3-fold, $P<0.05$) as a percentage of total fatty acids compared to the milk from Control dams (Figure 2A-C,E). There were no differences in the proportions of n-3 LCPUFA in the milk between groups (Figure 2D). The total fat content and the amounts of saturated ($R=0.90$, $P<0.01$) and *trans* fats ($R=0.96$, $P<0.01$) in the milk were directly related to maternal dietary intake. There were no relationships, however, between maternal intake of n-6 PUFA or n-3 LCPUFA during the lactation period and the levels of these fatty acids in the milk at mid-lactation. The protein content of the milk was not different between the Control and CAF groups (Figure 1B).

3.3 Effect of maternal cafeteria diet consumption on offspring Fatty Acid Status

PND1

On PND1, pups born to CAF dams had lower LA, EPA, DHA (Table 3) and total n-3 LCPUFA (Figure 3D) levels in their RBC phospholipids compared to pups of Control dams. Conversely, the level of monounsaturated fats as a percentage of total fatty acids was higher in the CAF group (Figure 3B). There were no differences in the proportions of saturated fat, *trans* fats or n-6 PUFA in the RBC phospholipids between pups born to Control and CAF dams on PND1 (Figure 3).

Weaning (3 weeks of age)

At 3 weeks of age *trans* and monounsaturated fat levels were higher in pups suckled by CAF dams compared to those suckled by Controls (Figure 3B and E). Pups suckled by CAF dams also had lower levels of n-6 PUFA (Figure 3C) and n-3 LCPUFA (Figure 3D) in their RBC phospholipids compared to those suckled by Control dams (Table 3).

6 weeks

At 6 weeks of age, those pups that had been suckled by CAF dams had higher levels of monounsaturated and *trans* fatty acids compared to those suckled by Controls (Figure 3B and E). There were no differences in the levels of saturated fat, n-6 PUFA or n-3 LCPUFA between offspring suckled by Control or CAF dams at 6 weeks of age.

4. DISCUSSION

We have demonstrated that providing dams with a cafeteria diet during pregnancy and lactation was associated with significant alterations in the fat content and composition of the milk and that this had implications for the fatty acid status of the offspring. Interestingly, however, the magnitude of the effects of maternal cafeteria diet on milk composition were relatively subtle in comparison with the extent of differences in dietary fat intakes, and were dependent on the stage of lactation. A key finding was that n-3 LCPUFA status of the offspring of cafeteria dams was lower both on PND1 and at weaning, suggesting that a cafeteria diet has a negative impact on the supply of these essential fatty acids, which are known to have important roles in development [17, 18]. We also demonstrated that the higher *trans* fatty acid content of the cafeteria diet resulted in elevated *trans* fat content of the milk and increased *trans* fat status of the offspring, which persisted even after they had been maintained on a standard (*trans*-fat

free) rat chow for 3 weeks after weaning. These findings provide further support of the importance of maternal dietary fatty acid intake in determining milk composition, and highlight the importance of maternal nutritional intake during breast feeding, as well as during pregnancy, for the subsequent health of the offspring.

4.1 Maternal cafeteria feeding and milk composition

As expected, dams provided with the cafeteria diet had a markedly higher total fat intake, and higher intakes of saturates and *trans* fats and lower intakes of n-3 LCPUFA compared to dams consuming the standard rodent diet during both pregnancy and lactation. This pattern of dietary fat intake is comparable to poor quality western-style diets in humans [19]. A key finding of this study, however, was that the fatty acid content and composition of the milk and fatty acid status of the offspring was not entirely reflective of the maternal diet, and that the relationship between maternal dietary fat intakes and milk fat composition also appeared to vary with stage of lactation.

While it is well established that the fat content/composition of milk varies across lactation, to the best of our knowledge the current study is the first to report different impacts of cafeteria feeding on milk composition at two different time points in lactation. Thus, while further studies are required in order to confirm this finding, it raises the important possibility that the same pattern of maternal dietary intake at different stages of lactation could have distinct impacts on offspring development. In addition, while the level of total fat and saturated fatty acids were higher in the milk of dams consuming the cafeteria diet at mid-lactation, the magnitude of this effect was relatively modest in comparison with the differences in maternal

dietary intake, suggesting that the dam has a substantial capacity to buffer the milk supply from changes in dietary fat intake.

Despite the reduction in n-3 LCPUFA observed on PND 1 there were no differences in n-3 LCPUFA levels in milk collected at mid-lactation. This was unexpected, given that a number of human and animal studies have reported a direct relationship between maternal intake of n-3 LCPUFA and their concentrations in the breast milk [20-22]. However, these previous studies have largely focused on the impact of n-3 LCPUFA supplementation, rather than reductions in n-3 LCPUFA intake, and it is therefore possible that there are physiological mechanisms to maintain n-3 LCPUFA levels in mature milk when dietary intakes are low.

In contrast to fat, there were no differences in the protein content of either the early or mid-lactation milk between control and cafeteria dams, despite the lower protein intake of the cafeteria dams during both pregnancy and lactation. This is consistent with previous studies in both humans and animals [23-25] and suggests that lower protein intakes in the maternal diet, at least to the extent observed in the present study, does not translate into a reduced protein supply to the offspring.

4.2 Maternal cafeteria feeding and offspring fatty acid status

The fatty acid composition of the red blood cells in the first 24 hours after birth provides an indication of the direct effects of the maternal diet during pregnancy on *in utero* fatty acid supply. We found that the fatty acid composition in the pups, with the exception of a lower n-3 LCPUFA content, was remarkably similar between the control and cafeteria groups. This is consistent with the suggestion that placental transfer of fatty acids from the maternal to fetal

circulation is a highly regulated process [26]. Thus, the fetus appears to be largely protected against substantial shifts in maternal dietary fatty acid intake. Nevertheless, the reduced n-3 LCPUFA transfer to the fetuses of cafeteria-fed dams may still have significant implications for fetal development given the critical role of the n-3 LCPUFA in the neurodevelopment and immune function in the perinatal period [27, 28].

Interestingly, the differences we observed in fatty acid composition at weaning between offspring of control dams suckled by control or cafeteria fed dams were not consistent with maternal diet or milk composition. Offspring suckled by cafeteria-fed dams had a lower content of both n-3 and n-6 PUFA in RBC phospholipids at weaning, whilst n-6 PUFA levels were higher and n-3 LCPUFA levels not different in the milk of cafeteria dams at mid-lactation. It therefore appears that the n-3 LCPUFA supply during lactation was not sufficient to fully compensate for the lower n-3 LCPUFA supply in the immediate post-partum period. By 6 weeks of age, the n-3 LCPUFA status of offspring suckled by a cafeteria dam was no longer different to controls. Since the diet provided to the offspring post-weaning contained relatively low amounts of pre-formed n-3 LCPUFA, the majority of these fatty acids would have been derived from the short-chain n-3 PUFA, ALA. This suggests that consuming a diet with sufficient ALA levels for 3 weeks after weaning was sufficient for the offspring to restore n-3 LCPUFA status to control levels.

In contrast to the most other fatty acids, a significant finding of this study was that the increased *trans* fatty acid intake of dams consuming the cafeteria diet resulted in increased levels of *trans* fats in the milk in both early and mid-lactation and, perhaps more importantly, increased *trans* fatty acid status of the pups at both 3 and 6 weeks of age. This provides clear

evidence that *trans* fats in the maternal diet are transferred to the suckling offspring via the breast milk, and that this has persistent effects on the level of *trans* fats in the offspring. Whether this higher *trans* fat level has negative physiological consequences for the offspring remains to be determined, but is concerning given the well-described negative impacts of elevated *trans* fat intake on cardiovascular and metabolic health [29, 30]

4.3 Conclusion

Consuming a cafeteria diet, containing a high proportion of saturated and trans fats and low amounts of n-3 LCPUFA, during pregnancy and lactation was associated with significant changes in the fatty acid composition of the milk, however these changes were substantially smaller than the differences in fat intake between cafeteria fed and control dams, highlighting the significant capacity of the dam to buffer the transfer of dietary lipids into the milk. Nevertheless, offspring of cafeteria fed dams exhibited lower n-3 LCPUFA status both at birth and at weaning, and higher levels of *trans* fats which were still present at 3 weeks post-weaning. These findings have provided an important basis for interpretation of studies involving maternal dietary interventions during the lactation period and indicate a need for further studies investigating the long-term implications of exposure to milk supply with a high total fat, *trans* fat and n-6 PUFA content.

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Conflicts of Interest: The authors have no conflicts to declare.

Figure legends

Figure 1 The % total fat content (A) and protein content (B) of stomach contents collected Day 1 and milk collected Day 12 from Control (open bars) and Cafeteria diet exposed (closed bars) pups and dams. n= 14 Control, 12 CAF at each time point. Values are expressed as means ± SEM. * denotes significance at $P<0.05$.

Figure 2 The proportion of saturated fat (A), monounsaturated fat (B), N-6 PUFA (C), N-3 LCPUFA (D) and trans fatty acids (E) as a % of total fatty acids in stomach contents collected Day 1 and milk collected Day 12 from Control (open bars) and Cafeteria diet exposed (closed bars) pups and dams. n= 14 Control, 12 CAF at each time point. Values are expressed as means ± SEM. * denotes significance at $P<0.05$,** denotes significance at $P<0.01$ and *** denotes significance at $P<0.001$.

Figure 3 The proportion of saturated fat (A), monounsaturated fat (B), N-6 PUFA (C), N-3 LCPUFA (D) and trans fatty acids (E) as a % of total fatty acids in the RBC phospholipids of offspring suckled by Control (open bars) and Cafeteria diet exposed (closed bars) dams at Day 1, 3 weeks and 6 weeks of age. n= 9 Control, 6 CAF at each time point. Values are expressed as means ± SEM. * denotes significance at $P<0.05$,** denotes significance at $P<0.01$ and *** denotes significance at $P<0.001$.

Table 1 Fatty acid composition per 100g of the Control and cafeteria diet

	Control Diet	CAF DIET					
	Standard rat chow (5% fat)	Chocolate Biscuits (45% fat)	Savoury Snacks (35% fat)	Peanut butter (50% fat)	Hazelnut spread (36% fat)	Sweetened cereal (3% fat)	Animal fat+ chow (19% fat)
Total saturates (g)	6.95	18.2	16.9	6.41	8.51	13.8	16.1
Total monos (g)	13.3	12.6	14.9	27.8	19.8	12.8	14.7
Totals trans (g)	0.07	0.14	0.23	0.02	0.02	0.03	1.49
LA (g)	13.2	4.80	3.80	1.67	5.94	8.95	2.83
ARA (g)	0.02	0.00	0.00	0.00	0.01	0.01	0.01
N-6 PUFA (g)	13.3	4.82	3.80	1.69	5.98	8.99	2.91
ALA (g)	2.10	0.22	0.15	0.032	1.64	0.33	0.58
EPA (g)	0.10	0.00	0.00	0.00	0.00	0.00	0.01
DPA (g)	0.00	0.00	0.00	0.00	0.00	0.00	0.01
DHA (g)	0.20	0.00	0.00	0.00	0.00	0.00	0.04
N-3 PUFA (g)	2.60	0.23	0.15	0.032	1.64	0.33	0.69

Values are expressed as grams per 100g of feed; LA, Linoleic acid (18:2 n-6); ARA, Arachidonic acid (20:4 n-6); ALA, α-linolenic acid (18:3 n-3); EPA, Eicosapentaenoic acid (20:5 n-3); DPA, Docosapentaenioc acid (22:5 n-3); DHA, Docosahexaenoic acid (22:6 n-3).

Table 2 Maternal intake of fat (g/day), protein (g/day), total energy (kJ/day) and fatty acids as a proportion of daily energy intake (%) during pregnancy and lactation in control and cafeteria fed dams.

	Pregnancy		**Lactation**	
	Control Dams (n=14)	**CAF Dams (n=12)**	**Control Dams (n=14)**	**CAF Dams (n=12)**
Fat (g/day)	1.14 ± 0.03	6.68 ± 0.28 **	2.51 ± 0.06	10.77 ± 0.49 **
Protein (g/day)	4.86 ± 0.12	2.88 ± 0.1 **	10.70 ± 0.26	4.98 ± 0.24 **
Energy (g/day)	446.72 ± 10.76	500.46 ± 12.22 **	982.59 ± 23.54	821.29 ± 27.54 **
Total Saturates (%)	1.91 ± 0.05	17.68 ± 0.79 ***	1.85 ± 0.04	19.03 ± 0.71 ***
Total Monos (%)	3.66 ± 0.09	25.93 ± 1.24 ***	3.55 ± 0.08	26.95 ± 1.30 ***
Totals Trans (%)	0.02 ± 0.0005	0.49 ± 0.04 ***	0.02± 0.0004	0.66 ± 0.03 ***
LA (%)	3.62 ± 0.09	5.46 ± 0.24 ***	3.51 ± 0.08	5.30 ± 0.22***
ARA (%)	0.01 ± 0.0002	0.002 ± 0.0002 ***	0.01 ± 0.0001	0.003 ± 0.0002 ***
Total n-6 PUFA (%)	3.65 ± 0.09	5.50 ± 0.24 ***	3.53 ± 0.08	5.35 ± 0.23 ***
ALA (%)	0.57 ± 0.01	0.78 ± 0.05 ***	0.55 ± 0.01	0.71 ± 0.03***
EPA (%)	0.02 ± 0.0004	0.002 ± 0.0002 ***	0.01 ± 0.0003	0.0003 ± 0.0002 ***
DPA (%)	0.01 ± 0.0001	0.002 ± 0.0002***	0.01 ± 0.0001	0.0003 ± 0.0002 ***
DHA (%)	0.04 ± 0.001	0.01 ± 0.001 ***	0.04 ± 0.001	0.01 ± 0.001 ***
Total n-3 PUFA (%)	0.64 ± 0.02	0.81 ± 0.06 **	0.62 ± 0.01	0.75 ± 0.03***

*Values are expressed as means ± SEM. ** denotes significance at P<0.01 and *** denotes significance at P<0.001 LA, Linoleic acid (18:2 n-6); ARA, Arachidonic acid (20:4 n-6); ALA, α-linolenic acid (18:3 n-3); EPA, Eicosapentaenoic acid (20:5 n-3); DPA, Docosapentaenioc acid (22:5 n-3); DHA, Docosahexaenoic acid (22:6 n-3).*

Table 3 Red blood cell phospholipid fatty acid status of male and female offspring born to Control dams and suckled by either Control and CAF dams at 3 weeks and 6 weeks of age

3 Weeks	**Male**		**Female**	
	Control (n=9)	**CAF (n=6)**	**Control (n=9)**	**CAF (n=6)**
LA (%)	10.38 ± 0.29	8.27 ± 0.61**	9.74 ± 0.16	7.96 ± 0.51***
ARA (%)	18.36 ± 0.88	17.10 ± 2.69	18.28 ± 1.05	12.92 ± 1.88
ALA (%)	0.03 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
EPA (%)	0.29 ± 0.05	0.17 ± 0.03	0.30 ± 0.07	0.08 ± 0.04**
DPA (%)	2.28 ± 0.23	1.43 ± 0.43**	2.36 ± 0.23	0.98 ± 0.20***
DHA (%)	4.85 ± 0.59	3.93 ±1.08	4.46 ± 0.59	2.78 ± 0.70*
6 Weeks				
LA (%)	9.78 ± 0.21	9.63 ± 0.21	9.11 ± 0.33	8.83 ± 0.14
ARA (%)	19.79 ± 0.42	19.28 ± 0.56	21.28 ± 0.35	20.10 ± 0.42
ALA (%)	0.09 ± 0.01	0.10 ± 0.00	0.09 ± 0.01	0.08 ± 0.02
EPA (%)	0.32 ± 0.02	0.38 ± 0.025	0.36 ± 0.02	0.35 ± 0.02
DPA (%)	2.40 ± 0.05	2.20 ± 0.071***	2.13 ± 0.09	1.90 ± 0.09
DHA (%)	3.80 ± 0.06	3.95 ± 0.05	3.73 ± 0.13	3.90 ± 0.19

*Values are expressed as a percentage of total lipid in the phospholipids and are expressed as means ± SEM. ** denotes significance at P<0.01 and *** denotes significance at P<0.001; LA, Linoleic acid (18:2 n-6); ARA, Arachidonic acid (20:4 n-6); ALA, α-linolenic acid (18:3 n-3); EPA, Eicosapentaenoic acid (20:5 n-3); DPA, Docosapentaenioc acid (22:5 n-3); DHA, Docosahexaenoic acid (22:6 n-3).*

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