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Relationship between Milk Fatty Acid Composition of Dietary Fat during Lactation and Litter Growth in the Laboratory Mouse, Mus Musculus

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ABSTRACT

The relationship between milk fatty acid composition of dietary fat and litter growth was studied in the laboratory mice (MF1) fed during lactation macronutrient diets containing high fat (60%), medium fat (45%) and low fat (10%) diets between days 4 and 18 of lactation. Each diet contained 20% protein and low fat (LF) was used as the control diet. Food intake, maternal body mass and litter mass were recorded daily. To examine how dietary fat was transferred into milk for suckling litters, comparison of corresponding total lipid fatty acid profiles between the diets, milk and pup tissues was performed on all fatty acids that constituted more than or equal to 1% of all identified fatty acids. Mice fed on high fat (HF), medium fat (MF) and low fat (LF) diets reached asymptote in their daily food intake at 14.95 g day^{-1}, 16.30 g day^{-1} and 16.57 g day^{-1} respectively between days 12-17 of lactation. At weaning, litters from HF and MF fed mice were significantly heavier (109.33 g and 106.24 g) than litter from mice fed on LF diet (80.85 g). This was because the HF and MF-fed mice did not only consume more energy (306.52 kJ day^{-1} and 340.52 kJ day^{-1}) at peak lactation but also delivered more milk energy (203.20 kJ day^{-1} and 229.30 kJ day^{-1}) to their litters than the LF-fed mice (164.60 kJ day^{-1}). HF and MF diets had beneficial effects on lactation performance because they increased the capacity of mice to generate milk more efficiently and weaned heavier offspring than mice fed LF diet. The results show that majority of the fatty acids in the milk and pup/offspring tissues had dietary origin. This indicates that dietary fat elevated milk fat concentration in the lactating MF1 mice and that promoted faster growth in their litters. This provides support for the use of fatty acids as indicators of diet at higher trophic levels such as in the milk and offspring tissues of mice. Our data on the fatty acid composition of the laboratory mouse may provide an attractive novel animal model to be used to make guesses about dietary fat requirements and enable us to evaluate the importance of milk composition in lactation strategies.

Keywords: Lactation, dietary fat, milk fatty acid composition, laboratory mice, litter growth

INTRODUCTION

The defining characteristic of the class mammalian is the ability to produce milk, an externally secreted fluid designed specifically to nourish the young. The mammary gland is the sole organ for providing nutrients for nursing animals. Therefore, understanding the physiology of mammary gland and lactation is critical to understanding the biology of mammals (Hayssen, 1993). The evolution of lactation has incorporated many organizational changes. Biochemical changes involve carbohydrate, fat, and protein synthesis in the female and the catabolism in their young. Physiological changes include modification not only of hormonal control and metabolism, but also of behaviours of mothers and their young in order to implement and to integrate nursing and suckling (Hayssen, 1993). The first week of growth performance affects future growth performance (Loh *et al*., 1998) and the post-weaning growth performance mainly depends on pre-weaning growth (McConnell *et al*., 1987). The pre-weaning growth solely depends on the milk produced by dams and the composition of milk can affect the type of growth that occurs in the neonate. Milk fats are primarily utilized by the new born mammal for the deposition of body fat (Loh *et al*., 2002). Milk composition can be altered by diet to some degree. Lactose and milk protein concentrations generally are not subjected to major changes by modifying diet but milk fat can be altered by dietary fat level (Jackson *et al*., 1995). Therefore, there is the need to understand the issues about fat inclusion in the maternal diet as the nutritional value of

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milk produced during lactation may be important for improving pre-weaning growth and development of mammals.

During reproduction, the female mammal makes a large metabolic investment in the early development of the offspring. This investment continues until the offspring is metabolically independent from the mother. Lactation is a continuation of the metabolic investment of the mother and is the most energetically demanding part of reproduction due to milk production that increases greatly the nutrient needs of the mother (Król *et al*., 2007; Kagya-Agyemang *et al*., 2013a). The study of milk composition in a variety of species can provide useful information on the lactation strategies of mammals. The production of milk places high energetic demand on dams (Król *et al*., 2007; Kagya-Agyemang *et al*., 2013b) and hence, its components might be expected to evolve through natural selection. Most information about milk composition has been obtained from larger mammals or from traditional laboratory and domesticated mammals (Oftedal, 1984). As a result, the ability to evaluate the importance of milk composition in lactation strategies is hampered by our lack of knowledge about the milk of smaller species. Therefore, the MF1 laboratory mouse was chosen as a model for the present study.

The fatty acids of milk triacylglycerols are derived from *de novo* synthesis within the mammary gland from fat of dietary origin or fats mobilized from adipose tissues (Król *et al*., 2004). Variations in the fatty acid composition of the maternal diet during lactation alter the fatty acid composition of milk (Loh *et al*., 2002). Increment changes in the proportion of fat in the diets of rats during lactation have been shown to increase (Gregor and Warren, 1980) the milk fat concentration. Fats are the fatty acid esters of glycerol and are the primary energy depots of animals. Fats are used for long-term energy requirements during periods of extensive exercise or during periods of inadequate food and energy intake. The feeding of high fat diets to lactating rats resulted in diminished mammary gland lipogenesis and in reduced growth rates and increased mortality of the pups (Rolls *et al*., 1984). Other studies have shown that despite reduced mammary gland lipogenesis, pups of dams fed high fat diets grew better than did the controls (Gregor and Warren, 1980). These contradictory results might be due to differences in the experimental designs.

The present study sought to assess the relationship between milk fatty acid composition of dietary fat and the growth performance of litters from mice fed during lactation macronutrient diets with either a high, medium or low concentration of fat.

MATERIALS AND METHODS

Animals and Experimental Protocol

Sixty female mice (*Mus musculus* L.: out bred MF1) aged about nine weeks old (Harlan UK Limited, England) were used in this study. The animals were individually housed in cages (44 cm x 12 cm x 13 cm) with sawdust. Rat and mouse breeder and grower diet, (15.60 kJ/g gross energy, 18.80% crude protein, 60.30% carbohydrate, 3.40% crude oil, 3.7% crude fibre, 90% dry matter and 3.80% ash, Special Diets Services, BP Nutrition, Witham, UK) and water were supplied *ad libitum*. The environmental temperature was maintained at 21 ºC (range 20 to 22 ºC) on a 12 L: 12 D photoperiod with lights on at 07:00 h. Baseline measurement of body mass was used to allocate mice into three experimental groups with 20 animals per group. Each group had two replications with 10 animals per replicate. Completely randomized design was used for the study. Each female was paired with a male for 11 days after which the males were removed. Female body mass and food remaining in each hopper were weighed each morning between 08:00 h and 10:00 h, using a (Mettler Toledo, Switzerland) top-pan balance (± 0.01 g). Food intake was calculated from the difference between the amount of food provided and that left in the hopper. On the day of parturition, no measurements were made on the lactating mothers and their pups to avoid distress. From days 1-18 of lactation, maternal body mass, litter size, litter mass and pup mortality were monitored daily. On days 2-3 of lactation, mothers from each experimental group were presented with either high fat (HF), medium fat (MF) or low fat (LF) diet while still supplied with rat and mouse breeder and grower diet *ad libitum*. The diets used were HF diet (D12492) with 60% kcal from fat, MF diet (D12451) with 45% kcal from fat and LF diet (D12450B) with 10% kcal from fat (Research Diets, New Brunswick, NJ, U.S.A). The composition of experimental diets is shown in Table 1. From day 4 of lactation onwards, the dams were switched from the rat and mouse breeder and grower diet to HF, MF or LF diet exclusively. Low fat (LF) was used as the control diet. Maternal food intake was measured between days 5-18 of

lactation. Food intake was not monitored when mixed diets were presented on days 1-4. All animals were maintained in accordance with the United Kingdom Home Office animals (Scientific procedures) Act 1986.

Milk Collection and Analysis

On day 16 of lactation, twenty four (24) female mice made up of 8 animals from each dietary group and with litter size of 6-16, were separated from their pups for approximately 3 h. After this separation which was not long enough to affect milk production (Johnson *et al*., 2001; Król and Speakman, 2003a), milking was done manually from all teats after intra-peritoneal injection of oxytocin (1IU) under moderate isoflurane anaesthesia (Abbot Laboratories Ltd, Queensborough, England, UK). Oxytocin was used to stimulate milk let-down. Each mammary gland was palpated towards the nipple area and droplets of milk were collected in capillary tubes. Milk collection continued until no milk could be expressed. All the milk samples (0.4-0.6 ml from each female) were kept at -80 °C until further analysis for fatty acids. All analyses (Rowett Research Institute Analytical Services, Aberdeen, UK) were made on duplicate samples.

Table1. *Composition of Macronutrient Diets*

Diet code	D12492	D12451	D12450B
Diet	High Fat	Medium Fat	Low Fat (Control)
%	kcal	kcal	kcal
Fat	60	45	10
Carbohydrate	20	35	70
Protein	20	20	20
Total	100	100	100
Ingredients (g/kg diet)			
Casein	200	200	200
L-cystine	3	3	3
Corn starch	0	72.8	315
Maltodextrin	125	100	35
Sucrose	68.8	172.8	350
Cellulose	50	50	50
Soya oil	25	25	25
Lard	245	177.5	20
Mineral mix	10	10	10
Dicalcium phosphate	13	13	13
Calcium carbonate	5.5	5.5	5.5
Potassium citrate	16.5	16.5	16.5
Vitamin mix	10	10	10
Choline bitartrate	2	2	2
Gross energy (kJ g^{-1})	19.90	19.89	19.89

Source: *Research Diets, New Brunswick, NJ, U.S.A*

Milk Energy Output

Milk energy output was evaluated as the difference between metabolizable energy intake and daily energy expenditure (Król and Speakman, 2003b; Kagya-Agyemang *et al*., 2013b)).

Fatty Acid Analyses of Macronutrient Diets, Milk and Pup (Offspring) Tissues

Samples of high fat (HF), medium fat (MF) and low fat (LF) diets and total pups weaned on day 18 of lactation from dams fed on HF, MF and LF diets, respectively were thawed, individually weighed (±0.001 g, Ohaus Analytical Plus), and dried in a convection oven at 60 ºC for 14 days to constant mass. The total lipid fractions of macronutrient diets and pups tissues were extracted using a modification of the methods of Bligh and Dyer (1959). Each dry sample was ground and dissolved in 24 ml chloroform/methanol (2:1). After mixing the dissolved mouse tissues and each of the macronutrient diets with water (6 ml), the aqueous and chloroform/methanol phases were separated by centrifugation (3000 rpm) for 5 min. The aqueous layer was then discarded and the lipid layer of each sample was filtered through a Whatman IPS filter paper. The solvents were evaporated at 40 ºC under vacuum and the resulting lipid extracts (~ 50 mg) were saponified with 1 ml 0.5 mol l^{-1} potassium hydroxide in 95% ethanol for 90 min at 100 ºC. After adding diethyl ether (9 ml) and water

(3 ml), the aqueous phase (containing saponified material) and ether phase (containing non-saponified material) were separated by centrifugation (3000 rpm) for 5 min. The aqueous layer was then acidified with 5 mol l^{-1} sulphuric acid and free fatty acids extracted with 9 ml hexane/diethyl ether (19:1). The fatty acid extracts were stored at -20 ºC until further analysis.

Dry milk samples (~ 0.1 g) from lactating mice fed on all three diets, were defrosted at room temperature and thoroughly mixed on a votrex mixer. The lipid fraction was extracted from milk using a method modified from that of Bligh and Dyer (1959). Each milk sample was dissolved in chloroform, methanol and water at a ratio of 2:2:1.8. After this process, the aqueous and chloroform/methanol phases were separated by centrifugation (3000 rpm) for 5 min. The aqueous layer (top) was then discarded and the lipid layer of each sample was filtered through a Whatman IPS filter paper. The solvents were evaporated at 40 ºC under vacuum and the resulting lipid extracts (~ 50 mg) were saponified with 1 ml 0.5 potassium hydroxide in 95% ethanol for 90 min at 100 ºC. After cooling, the samples were acidified to pH 1.0 with 2 mol^{-1} hydrochloric acid (HCl). Saponified lipids were extracted into hexane, washed with distilled water and then dried over anhydrous sodium sulphate. All lipid samples were stored at -20 ºC under nitrogen in a glass vial secured with an aluminium-lined screw cap until further analysis.

Fatty Acid Composition

The lipid extracts of the three diets, milk samples, and pups tissues were trans-esterified to produce fatty acid methyl esters (FAMEs). FAMEs were prepared by reacting total lipids (10-20 mg) with dry methanol (0.5 ml) containing 2 mol l^{-1} HCl for 2 h at 100 ºC, and then dissolving the lipids in 1 ml hexane/diethyl ether (19:1). After mixing the dissolved FAMEs with water (0.5 ml), the aqueous and hexane/diethyl ether phases were separated by centrifugation (3000 rpm) for 5 min and the lipid layer dried by passing through anhydrous sodium sulphate. The solvents were evaporated at 35-40 ºC under a stream of nitrogen and the residue FAMEs were taken up in hexane containing 0.02% butylated hydroxytoluene (BHT). Analysis of the FAMEs was by capillary gas chromatography (GC) using a Hewlett Packard 5890A (Hewlett Packard, Sunnydale, CA, USA) fitted with a 50 m × 0.25 mm CP-SIL 88 column coated with a 0.25 µl film thickness (J & W Scientific, Folsum, CA, USA). The GC temperature was programmed from 160 ºC, held for 1 minute, increased by 10 ºC min^{-1} to 190 ºC, held for 3 min, again increased by 2 ºC min^{-1} to 230 ºC and finally held for 15 min. Samples (1 µm) run in duplicates were injected into a split injection system (1:15) and carried through the GC column with helium as the carrier gas. The GC was linked to a computerised integration system (Unicam 4880 software) to identify the peaks by comparison with absolute retention times (RTs) from a standard mixture (Supelco UK, Poole, Dorset, UK). The standard was run daily to determine accurate RTs. Individual fatty acids are designated in International Union of Pure and Applied Chemistry (IUPAC) shorthand nomenclature by carbon chain length**:**number of double bonds. A total of 10, 11, and 10 fatty acids were identified in the HF, MF, and LF diets. In the milk samples from lactating mice fed on HF, MF and LF diets, 18, 20, and 18 fatty acids, respectively were identified. Finally, 16, 15, and 14 fatty acids were identified in tissues of pups weaned from lactating mice fed on HF, MF, and LF diets, respectively. Each fatty acid from macronutrient diets, milk samples and tissues of pups was expressed as a percentage of the total fatty acids identified in each sample. All fatty acids identified in the macronutrient diets, milk samples and tissues of pups were used in the comparison of total fatty acid profile.

Statistical Analyses

The differences in baseline and pregnancy data between the three dietary groups for body mass and food intake were analysed using repeated measures analysis of variance (ANOVA). The significance of change in body mass and food intake over time was assessed by general linear modeling (GLM). Significant differences between days and diets were detected using the Tukey test. Asymptotic food intake across the three dietary treatment groups in late lactation was compared using ANOVA. The asymptotic food intake in late lactation was defined as the period during which no significant differences in food intake between days were detected. Direct comparison of the litter size and litter mass over the lactation period were made using ANOVA. Data are represented as means ± standard deviation (SD). All statistical analyses were performed using Minitab for Windows (version 14; Minitab Inc., State College, PA, USA**;** Ryan *et al*., 1985). All statistical significance were determined

at $P<0.05$. One-sample t test was used to test for differences in fatty acid composition of each macronutrient diet, milk from mice fed on macronutrient diets and tissues of their pups.

RESULTS

Maternal Body Mass

The body mass of HF (29.51±1.29 g), MF (29.52±1.33 g) and LF mice (29.52±1.42 g) was not significantly different (P>0.05) before mating. Body mass increased significantly during days 12-21 of pregnancy in HF (P<0.001), MF (P<0.001) and LF mice (P<0.001) reaching a peak of 53.96±4.18 g, 55.83±5.06 g and 56.28±5.24 g, for HF, MF and LF mice, respectively just before parturition. Mean body mass of mice in the three dietary groups was not significantly different during pregnancy (P>0.05) and averaged 44.74±7.04 g, 45.41±7.30 g, and 44.75±7.99 g for HF, MF and LF mice, respectively. Between days 1-4 of lactation when mice had a choice of standard rodent chow or their selected macronutrient diets, maternal body mass of mice in each group increased. Maternal body mass of HF, MF and LF mice did not show any significant difference (P>0.05) and averaged 41.39±3.82 g, 41.15±2.04 g, and 41.79±3.05 g on the day after parturition and increased to 42.67±3.18 g, 42.36±2.55 g, and 42.39±3.25 g on day 4 of lactation, respectively for HF, MF and LF mice. Female mice in all the three groups had a stable body mass between days 5-18 of lactation when the diet manipulation took place. The trend of change in body mass of HF, MF and HC mothers followed a similar pattern. There was a highly significant effect of day of lactation (P<0.001) on maternal body mass and also there was a highly significant effect of diet (P<0.05). However, there was no significant diet by day interaction (P>0.05). All Tukey pairwise comparisons among levels of diet showed that the LF-fed mice were significantly heavier (P<0.05) than HF and MF-fed mice between days 5-18 of lactation. The body mass of HF and MF-fed mice showed no significant difference (P>0.05).

Food Intake

The food intake of mice in the three dietary groups was not significantly different before mating (P>0.05). The animals consumed a mean of 5.28±0.51 g day $^{-1}$, 5.28±0.27 g day $^{-1}$ and 5.27±0.36 g day $^{-1}$ for HF, MF and LF mice, respectively. Food intake increased significantly during days 12-21 of pregnancy in HF (P<0.001), MF (P<0.001) and LF mice (P<0.001) reaching a maximum of 8.16±1.15 g, 8.04±1.13 g and 8.49±1.09 g before decreasing to 6.94±0.40 g, 6.92± 0.59g and 7.20± 0.57 g for HF, MF and LF mice, respectively on the day before parturition. The mean food intake of mice in the three groups (6.54±1.05 g day $^{-1}$, 6.29±0.94 g day $^{-1}$, and 6.57±1.24 g day $^{-1}$) was not significantly different throughout pregnancy (P>0.05). Diet manipulation started on day 4 of lactation, so there was no food intake data for days 1-4 of lactation when animals were fed on a mixture of rodent chow and the target diets. There was a significant increase in food intake between days 5-11 of lactation in mice fed HF (P<0.001), MF (P<0.001) and LF diets (P<0.001) from 10.45±1.86 g, 12.27±2.26 g and 12.43±2.68 g on the day after the animals were fed macronutrient diets exclusively to 13.20±2.60 g, 14.89±2.39 g and 16.33±1.88 g on day 11, respectively. Over the next 6 days (days 12-17), daily food intake remained constant (P>0.05) at an average of 14.95±1.14 g day $^{-1}$, 16.30±0.61 g day $^{-1}$ and 16.57±0.26 g day $^{-1}$ for mice fed HF, MF and LF diets, respectively. The food intake averaged over these 6 days was termed the asymptotic daily food intake. The corresponding asymptotic gross energy intakes were 345.40±26.41 kJ day $^{-1}$, 373.04±13.92 kJ day $^{-1}$ and 294.98±4.64 kJ day $^{-1}$ (equivalent to digestible energy intakes of 306.52 ±25.03 kJ, 340.52±13.49 kJ and 266.67±4.45 kJ) for mice fed HF, MF and LF diets, respectively. On day 18 of lactation, the daily food intake increased above the asymptotic level. This was because the pups started feeding directly on the food in the hoppers on this day. Over all, there was a highly significant effect of day of lactation (P<0.001) on maternal food intake and a highly significant effect of diet (P<0.001) on maternal food intake between days 5-18 of lactation. All Tukey pairwise comparisons among levels of diet showed that the mass of food intake of LF mice was significantly higher (P<0.05) than that of HF and MF mice between days 5-18 of lactation. The food intake of HF and MF-fed mice was not significantly different (P>0.05). There was no significant interaction effect (P>0.05) on maternal food intake during lactation.

Litter Size and Mass

The HF, MF and LF dams gave birth to an average of 10.5±2.86 (range 3-15), 10.7±2.31 (range 7-14) and 12.2±2.39 pups (range 8-17), respectively and weaned an average of 10.3±2.77 (range 3-14),

10.6±2.38 (range 7-14) and 11.2±2.78 pups (range 4-16), respectively. The litter masses of HF, MF and LF pups increased from 19.18±4.87 g, 19.69±4.25 g and 21.48±3.22 g at day 1 of lactation to 109.33±27.31 g, 106.24±20.02 g and 80.85±20.0 g, respectively at weaning. The mean mass of individual pups of HF, MF and LF mothers increased from 1.85±0.18 g, 1.84±0.13 g and 1.78±0.17 g at day 1 of lactation to 10.98±2.59 g, 10.26±2.10 g, and 7.68±2.56 g, respectively over the same period of time. The mean number of pups of HF, MF and LF-fed dams at day 1 of lactation were not significantly different (P>0.05) and they were also not significantly different (P>0.05) at weaning (day 18 of lactation). However, pups from dams fed HF and MF diets were significantly heavier than pup from dams fed LF diet (P<0.05) at weaning. Pups from dams fed HF and MF diets were not significantly (P>0.05) different from each other in terms of mass.

Milk Energy Output

The milk energy output (MEO) calculated on day 16 of lactation was significantly different (P<0.001) between lactating mice fed HF and MF diets and those fed LF diet and averaged 203.20±49.92 kJ day^{-1} (range 77.69-312.28 $kJday^{-1}$), 229.30±42.21 kJ day^{-1} (range 149.45-271.84 $kJday^{-1}$) and 164.60±30.59 kJ day (range 101.77-215.59 $kJday^{-1}$), respectively. Tukey pairwise comparisons among levels of diet indicate that MEO of both MF and HF mice were significantly higher (P<0.05) than that of LF mice. The MEO of MF mice was not significantly different (P>0.05) from that of HF mice. The relationship between growth of litters from HF, MF and LF mice and MEO showed a highly significant effect of MEO on litter growth (P<0.001) as well as a highly significant effect of the group (P<0.001).

Fatty Acid Composition

To examine how dietary fat was transferred into milk for suckling litters, comparison of corresponding total lipid fatty acid profiles between the diets, milk and pup tissues was performed on all fatty acids that constituted more than or equal to1% of all identified fatty acids (Tables 2, 3 and 4).

Chemical analysis (Table 2) showed that the most abundant fatty acid in HF diet was C18:1/oleic acid (50.41%), followed by C18:2/linoleic (24.24%), C16:0/palmitic (20.68%), C16:1/palmitoleic (2.09%), and C20:1 (1.15%) acids. Apart from these fatty acids, the rest were less than 1%. The most abundant fatty acid in milk when compared with the diet was oleic acid (30.78%), followed by palmitic (21.91%), linoleic (21.72%), and palmitoleic (2.13%) acids. The fatty acid profile of pup tissues was similar to that of the milk. The most abundant fatty acid in pup tissues was oleic acid (38.11%), followed by palmitic (20.74%), linoleic (17.50%), palmitoleic (2.32%), and C20:2/ (1.01%) acids (Table. 2).

Table2. *Fatty acid composition of total lipids extracted from high fat (HF) diet, milk and pup tissues*

Fatty acids	Mean percent of all fatty acids						
	Diet	t	*P*	Milk	t	*P*	Pup
C8:0	0.00	4.59	0.006*	1.43±0.76	4.08	0.010 *	0.16
C12:0	0.00	4.01	0.01*	4.21±2.57	1.91	0.115	2.21
C14:0	0.00	11.47	0.001*	6.01±1.28	-4.93	0.004*	8.59
C14:1	0.00	4.43	0.007*	0.05±0.02	4.43	0.007*	0.00
C15:0	0.07	0.82	0.448	0.09±0.04	-8.51	0.001*	0.24
C16:0	20.68	2.45	0.058	21.91±1.22	2.33	0.067	20.74
C16:1	2.09	1.02	0.355	2.13±0.09	-4.84	0.005*	2.32
C16:2	0.23	0.00	1.000	0.23±0.15	3.55	0.016*	0.00
C18:0	0.00	16.37	0.001*	5.06±0.75	-1.17	0.295	5.42
C18:1	50.41	-23.71	0.001*	30.78±2.02	37.29	0.001*	38.11
C18:2	24.24	-2.10	0.090	21.72±2.93	3.52	0.017*	17.50
C20:0	0.00	15.54	0.001*	0.10±0.01	-5.86	0.002*	0.14
C20:1	1.15	-1.21	0.282	0.92±0.47	3.37	0.002*	0.27
C20:2	0.70	1.53	0.188	2.51±2.91	1.26	0.262	1.01
C20:3	0.10	-3.36	0.020*	0.85±0.18	4.81	0.005*	0.48
C20:4	0.33	1.45	0.206	0.58±0.41	-7.39	0.001*	1.83
C22:0	0.00	1.51	0.191	0.21±0.34	0.14	0.891	0.19
C22:6	0.00	4.56	0.006*	0.21±0.11	-8.24	0.001*	0.58
C23:0	0.00	3.06	0.028*	0.73±0.58	3.06	0.028*	0.00
C24:0	0.00	3.03	0.029*	0.09±0.07	3.03	0.029*	0.00
Sd	12.70			8.87			9.76

Values for milk are means ± S.D

Significant differences at P value of 0.05 or less are represented by astericks (*).

Furthermore, chemical analysis (Table 3) showed that the most abundant fatty acid in MF diet was C18:1/oleic acid (50.22%), followed by C18:2/linoleic (25%), C16:0/palmitic (20.19%), and C16:1/palmitoleic (2.14%) acids. Apart from these fatty acids, the rest were less than 1%. For all MF-fed mice, the most abundant fatty acid in milk was oleic acid (32.59%), followed by palmitic (21.37%), linoleic (20.53%), and palmitoleic (2.52%) acids (Table 3). The fatty acid profile of pup tissues mirrored that of the milk. The most abundant fatty acid in pup tissues was oleic acid (34.68%), followed by palmitic (21.33%), linoleic (18.20%), and palmitoleic (2.69%) acids (Table 3).

Table3. *Fatty acid composition of total lipids extracted from medium fat (MF) diet, milk and pup tissues*

Fatty acids	Mean percent of all fatty acids						
	Diet	t	*P*	Milk	t	*P*	Pup
C8:0	0.06	3.57	0.038*	1.15±0.61	3.37	0.043*	0.12
C14:0	0.00	9.78	0.020*	7.16±1.46	-4.23	0.024*	10.27
C14:1	0.00	2.67	0.076	0.05±0.03	2.67	0.024*	0.00
C15:0	0.09	6.22	0.008*	5.51±1.74	5.99	0.009*	0.29
C16:0	20.19	3.84	0.031*	21.37±0.61	0.14	0.899	21.33
C16:1	2.14	2.15	0.120	2.52±0.35	-0.92	0.424	2.69
C18:0	0.00	14.92	0.001*	4.96±0.66	-1.77	0.176	5.55
C18:1	50.22	-13.52	0.001*	32.59±2.59	1.57	0.215	34.63
C18:2	25.00	-3.17	0.051	20.53±2.82	1.65	0.198	18.20
C20:0	0.20	-17.09	0.001*	0.09±0.01	-10.73	0.002*	0.16
C20:1	0.95	-0.26	0.808	0.86±0.69	1.85	0.161	0.21
C20:2	0.68	14.69	0.001*	1.66±0.13	9.76	0.002*	0.01
C20:3	0.28	5.74	0.010*	1.01±0.25	4.64	0.019*	0.42
C20:4	0.22	2.18	0.117	0.35±0.12	-22.84	0.001*	1.74
C22:0	0.00	1.57	0.215	0.03±0.03	-6.79	0.007*	0.16
C22:6	0.00	1.41	0.215	0.02±0.02	-44.55	0.001*	0.65
C23:0	0.00	1.71	0.187	0.08±0.09	1.71	0.187	0.00
C24:0	0.00	1.00	0.391	0.003±0.01	1.00	0.391	0.00
Sd	13.30			9.40			9.75

Values for milk are means ±SD

Significant differences at P value of 0.05 or less are represented by astericks (*).

Chemical analysis (Table 4) showed that the most abundant fatty acid in LF diet was linoleic acid (40.25%), followed by oleic (33.66%), palmitic (15.24%), stearic (7.36%), palmitoleic (1.20%), and C20:1 (1.01%) acids. Apart from these fatty acids, the rest were less than 1% For all LF-fed mice, the most abundant fatty acid in milk was oleic acid (24.64%), followed by palmitic (23.55%), linoleic (13.31%), stearic (3.27%), palmitoleic (2.48%), and C20:1 (1.03%) acids. The fatty acid profile of pup tissues was similar to that of the milk. The most abundant fatty acid in pup tissues was oleic acid (27.41%), followed by palmitic (23.86%), linoleic (12.54%), stearic (5.40%), palmitoleic (4.15%), and C20:3 (3.15%) acids (Table 4).

Table4. *Fatty acid composition of total lipids extracted from low fat (LF) diet, milk and pup tissues.*

Fatty acids	Mean percent of all fatty acids						
	Diet	t	*P*	Milk	t	*P*	Pup
C12:0	0.00	3.56	0.009*	7.08±5.62	2.02	0.083	3.06
C14:0	0.00	13.93	0.001*	18.36±3.72	1.66	0.141	16.17
C14:1	0.00	5.54	0.001*	0.18±0.01	5.54	0.001*	0.00
C15:0	0.00	3.55	0.009*	0.16±0.12	-9.76	0.001*	0.60
C16:0	15.24	4.53	0.003*	23.55±5.19	-0.17	0.870	23.86
C16:1	1.20	4.50	0.003*	2.48±0.80	-5.88	0.001*	4.15
C18:0	7.36	-26.17	0.001*	3.27±0.44	-13.61	0.001*	5.40
C18:1	33.66	-4.10	0.005*	24.64±6.21	-1.26	0.248	27.41
C18:2	40.25	-34.37	0.001*	13.31±2.21	0.98	0.361	12.54
C20:0	0.28	-9.60	0.001*	0.10±0.05	-5.81	0.001*	0.21
C20:1	1.01	0.10	0.921	1.03±0.51	4.98	0.002*	0.12
C20:2	0.36	2.55	0.038*	0.89±0.58	1.64	0.145	0.55
C20:3	0.38	4.03	0.005*	0.61±0.16	-44.74	0.001*	3.15
C20:4	0.00	5.43	0.001*	0.23±0.11	-5.67	0.001*	0.47

C22:0	0.26	-10.08	0.001*	0.12±0.03	-15.84	0.001*	0.34
C23:0	0.00	1.11	0.303	0.12±0.03	1.11	0.303	0.00
C24:0	0.00	2.89	0.023*	0.07±0.06	2.89	0.023*	0.00
C24:1	0.00	2.66	0.032*	0.07±0.07	2.66	0.320	0.00
Sd	12.09			8.51			8.65

Values for milk are means ± S.D

Significant differences at P value of 0.05 or less are represented by astericks (*).

DISCUSSION

Lactation is a continuation of the metabolic investment that a female mammal makes in the early development of its offspring during reproduction. As a result, lactation is the most energetically demanding part of reproduction due to milk production which increases greatly the nutrient needs of the mother (Król *et al*., 2007; Kagya-Agyemang *et al*., 2013b). In the present study, the HF and MF-fed lactating mice increased in body mass during lactation as a result of their high energy intake. However, the mice did not continue to increase their food intake until the end of lactation but instead reached asymptote from days 12-17, respectively.

The asymptotic daily food intakes (14.95 g day^{-1} and 16.30 g day^{-1}) corresponding gross energy intakes (345.40 kJ day^{-1} and 373.04 kJ day^{-1}) and net energy intakes (306.52 kJ day^{-1} and 340.52 kJ day^{-1}), litter mass at day 18 (109.33 g and 106.24 g) and milk production (203.20 kJ day^{-1} and 229.30 kJ day^{-1}) of HF and MF-fed mice were higher than that of the LF-fed mice (164.60 kJ day^{-1}). The feeding of fat (Table 1) to lactating mice impacted positively on reproductive performance of the HF and MF-fed mice because the specific dynamic action (SDA) for HF (4.5%) and MF (3.9%) diets were significantly lower than that of the LF (6.1%) diet (Kagya-Agyemang, 2009). As a result, the HF and MF-fed mice consumed more energy (306.52 kJ day^{-1} and 340.52 kJ day^{-1}) at peak lactation so the energy available for milk production was greatly increased. Therefore, pups from mothers fed HF and MF diets were heavier at weaning than those from LF-fed dams.

Averette *et al*. (1999) noted that dietary fat elevates milk fat concentration in sows and promotes faster growth in piglets throughout lactation. Increasing the fat content in the milk increases the energy supply to offspring thereby improving survival (Pettigrew, 1981) and pup growth rate. This indicates that the fat intake of lactating mice is correlated with the growth of their pups (Rolls *et al*., 1984; Loh *et al*., 2002). In the present study, the faster growth rate of the pups from HF and MF-fed mice was associated with the high energy exported as milk to the pups. The pre-weaning growth of offspring solely depends on the milk produced by dams and the composition of milk can affect the type of growth that occurs in the neonate. Milk fats are primarily utilized by the new born mammal for the deposition of body fat (Loh *et al*., 2002). This corroborates the findings of Del *et al*. (1997) and Averette *et al*. (1999) that increasing the fat content in milk increases the energy supply to offspring, thereby improving their survival and growth rate. In this study, positive effects occurred because the HF and MF-fed mice consumed much more food (14.95 g day^{-1} and16.30 g day^{-1}) and energy (306.52 kJ day^{-1} and 340.52 kJ day^{-1}) at peak lactation.

Fatty acids are the major form in which fat is made available as fuel for energy generation. Increased dietary fat intake and increased *de novo* lipogenesis are the main ways by which lactating mice are able to increase the fat content of their milk to support the faster growth of their pups (Kagya-agyemang *et al*., 2013a). At weaning, litters from HF and MF-fed mice were significantly heavier (109.33 g and 106.24 g) than pups on LF diet (80.85 g). This was evidenced by the fact that the HF and MF-fed mice not only consumed more energy (306.52 kJ day^{-1} and 340.52 kJ day^{-1}) at peak lactation but also delivered more milk energy (203.20 kJ day^{-1} and 229.30 kJ day^{-1}) to their pups than the LF-fed mice (164.60 kJ day^{-1}). Probably, the HF and MF-fed mice had the ability to directly transfer absorbed fatty acids into the milk to support the growth of their pups.

The LF diet contains 70% energy as carbohydrate (Table 1). It has been reported that conversion of carbohydrate to fat prior to oxidation is thermogenically costly and about 28% of the energy content of carbohydrate is lost as heat (Hellerstein *et al*., 1996; Hellerstein, 1999). This indicates that the LF-fed mice had to expend part of the energy consumed for *de novo* lipogenesis to produce enough milk to support the growth of their offspring. *De novo* lipogenesis is thermogenically costly and it is accompanied by energy expenditure of about 25% (Flatt, 1978). Throughout lactation, the growth of

offspring from HF and MF-fed mice was better when compared with that of LF-fed mice. This attests to the fact that the fatty acids present in the HF and MF diets promoted growth of pups. The positive effects of HF and MF diets on lactation performance were due to the ability of the HF and MF-fed mice to reduce the metabolic heat generated as a by-product of milk production (Kagya-Agyemang, 2009). At peak lactation, the HF and MF-fed mice assimilated more energy than the LF-fed mice and used the extra energy to generate more milk to promote the faster growth of their offspring.

CONCLUSIONS

The study has shown that fatty acids are the major forms in which fat is made available as fuel for energy generation and growth promotion in laboratory mice. Lactating mice can increase the fat content of their milk to support the faster growth of their litters through increased dietary fat intake. The information on the fatty acid composition of laboratory mice may be used to make guesses about dietary fat requirements and enable us to evaluate the importance of milk composition in lactation strategies.

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REFERENCES

Averette, L.A., Odle, J., Monaco, M.H. and Donovan, S.M. (1999). Dietary fat during pregnancy and lactation increases milk fat and insulin-like growth factor I concentrations and improves neonatal growth rates in swine. J. Nutr. 129:2123-2129**.**

Bligh, E. G. and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Del, P. M., Delgado, G. and Villalpando, S. (1997). Maternal lipid intake during pregnancy and lactation alters milk composition, production and litter growth in rats. J. Nutr. 127: 458-462.

Flat, J. P. (1978). The biochemistry of energy expenditure. Rec. Adv. Obesity Res. 2:211-217.

Grigor, M. R. and Warren, S. M. (1980). Dietary regulation of mammary lipogenesis in lactating rats. Biochem. J. 188: 61-65.

Hellerstein, M. K., Schwarz, J-M. and Neese, R.A. (1996). Regulation of hepatic de novo lipogenesis in humans. Ann. Rev. Nutr. 16:523-557.

Hellerstein, M. K. (1999). De novo lipogenesis in humans: metabolic and regulatory aspects. Eur. J. Clin. Nutr. 53: S53-S65.

Hayssen, V. (1993). Empirical and theoretical constraints on the evolution of lactation. J. Dairy Sci. 76: 3213-3233.

Jackson, J. R., Hurley, W. L. Easter, R. A., Jensen, A. H. and Odle, J. (1995). Effects of induced or delayed parturition and supplemental dietary fat on colostrum and milk composition in sows. J. Anim. Sci. 73: 1906-1913.

Johnson, M. S, Thomson S.C, and Speakman J. R. (2001). Limits to sustained energy intake I. Lactation in the laboratory mouse, *Mus musculus*. J.Exp. Biol. 204: 1925-1935.

Kagya-Agyemang, J. K. (2009). Limits to sustained energy intake: Effects of macronutrient intake during lactation in laboratory mice. PhD thesis submitted to the University of Aberdeen, Scotland, United Kingdom.

Kagya-Agyemang, J. K., Humbly, C., Sharon, M. E. and Speakman, J. R. (2013a). Relationship between hypothalamic neuropeptide Y and food intake in the lactating laboratory mouse, *Mus musculus*. ARPN J. Agric. Biol. Sci. 8:310-316.

Kagya-Agyemang, J. K., Król, E. and Speakman, J. R. (2013b). Influence of feeding fat during lactation on growth performance of pups and maternal organ morphology. Egerton J. Sci. & Technol. 13:163-185.

Król, E, and Speakman J. R. (2003a). Limits to sustained energy intake. VI. Energetics of lactation in laboratory mice at thermoneutrality. J. Exp.Biol. 206: 4255-4266.

Król, E, and Speakman J. R. (2003b). Limits to sustained energy intake. VII. Milk energy output in laboratory mice at thermoneutrality. J. Exp.Biol. 206: 4267-4281.

Król, E., Redman, P., Thomson, P. J., Williams, R., Mayer, C., Mercer, J. G. and Speakman, J. R. (2004). Effect of photoperiod on body mass, food intake and body composition in the field vole, *Microtus agrestis*. J. Exp.Biol. 208: 571-584.

Król, E, Murphy, M. and Speakman, J. R. (2007). Limits to sustained energy intake. X. Effects of fur removal on reproductive performance in laboratory mice. J. Exp.Biol. 210: 4233-4243.

Loh, T. C., Dodds, P. F. and Lean, I. J. (1998). Effects of weaning weight and first week growth performance post-weaning on subsequent performance. J. Vet. Mal. 10: 47-50.

Loh, T. C., Foo, H. L., Zurina, A. W. and Tan, B. K. (2002). Effect of feeding fat during pregnancy and lactation on growth performance, milk composition and very low density lipoprotein composition in rats. Mal. J. Nutr. 8(2): 125-135.

McConnell, J. C., Eargle, J. C. and Walsort, R. C. (1987). Effects of weaning weight co-mingling, group size and room temperature on pig performance. J. Anim. Sci. 65: 1201-1206.

Oftedal, O. T. (1984). Milk composition, milk yield and energy output at peak lactation: a comparative review. Symp.Zool.Soc. Lond. 51: 33-85.

Pettigrew, J. E. (1981). Supplemental dietary fat for peripartal sows: a review. J. Anim. Sci. 53: 107-117.

Rolls, J. B., Van Duijvenvoorde, P. M. and Rowe, E. A. (1984). Effects of diet and obesity on body weight regulation during pregnancy and lactation in the rat. Physiol. Behav. 32: 161-168.

Ryan, B. F., Joiner, B. L. and Ryan T. A., Jr. (1985). Minitab Handbook. 2nd edition. Boston, MA: PWs-Kent.