Effects of linolenic and *trans* fatty acids on neonatal survival of C57BL/6 mice

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One of the four diets was fed to three generations of female C57Bl/6 mice. Diets contained one of two levels of linolenic acid (0.18 and 0.02 energy %) with or without trans *fatty acids (TFA; 0.0 and 2.2 energy %). All other components were identical in the four 10% fat diets. Females were mated and the survival of pups was monitored from birth to weaning. There were no significant differences among the diet groups in dam weight gain during pregnancy and lactation or in the average number of pups per litter. Survival was best for pups from dams fed the diet that contained the higher level of linolenic acid and no TFA. The poorest survival was observed for pups of dams fed the diet that contained TFA and the lower level of linolenic acid. Comparison over three generations revealed that pup survival on the TFA, low linolenic acid diet was progressively impaired. It was concluded that neonatal survival may be impaired by inadequate levels of dietary n-3 fatty acid and that an optimum level of linolenic acid should be considered in the context of other fatty acids present in the diet.*

Keywords: linolenic acid; *trans* fatty acids; mouse pup survival

Introduction

Many investigators have looked at the essentiality of linoleic and linolenic acids. The essential nature arises from the fact that animals cannot synthesize these fatty acids, which are precursors to the longer chain 20- and 22-carbon polyunsaturated fatty acid (PUFA) members of the n-6 and n-3 families. The 20- and 22-carbon, n-6 and n-3 PUFA are found in abundance in mammalian membranes and some serve as precursors to eicosanoids with important physiological functions.

Early studies[1–5] showed that infertility and other symptoms associated with essential fatty acid deficiency could be alleviated by diets containing linoleic acid. In these studies, feeding linolenic acid did not reverse fertility problems and had minimal effect on other symptoms of essential fatty acid deficiency, thus n-3 fatty acids were not considered essential.

Although n-3 fatty acids may not be necessary for conditions that specifically require n-6 fatty acids, they are essential during early development of the nervous system. Brain and peripheral nerve tissue contain high levels of 22-carbon n-3 PUFA[6] and several investigators[7–12] have stressed the essentiality of these n-3 PUFA during early brain development. The visual function of the retina in monkeys[13] and learning ability in rats[14–15] have been shown to be impaired by diets low in n-3 fatty acids. Additionally, in human studies[16–17] a nervous system dysfunction has been attributed to diets lacking n-3 fatty acids.

In preliminary studies with mice,[18] we found that tissue levels of n-3 PUFA were influenced by the level of *trans* fatty acids (TFA) and the level of n-3 PUFA in the diets, and that neonatal survival may have been reduced when dams were fed a diet that was both high in TFA and low in linolenic acid.[19] The current study was designed to test whether a difference in the dietary level of linolenic acid and the presence or absence of dietary TFA affects mouse pup survival when these diets are fed to dams during gestation and lactation.

Materials and methods

Diets

Two pairs of diets were used in this study. In pair A the linolenic acid was 0.18 energy %, while in pair B the linolenic

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Table 1 Dietary fatty acid composition[a]

Diet[b]	cis A	trans A	cis B	trans B
16:0	16.6 (1.1)	14.5 (0.5)	12.5 (0.5)	12.4 (0.5)
18:0	12.4 (0.7)	14.2 (0.5)	13.5 (1.2)	15.1 (0.4)
16:1 *cis* area	0.6 (0.1)	0.3 (0.02)	0.2 (0.02)	0.1 (0.01)
Σ18:1 *trans* area	*	10.9 (0.2)	*	10.7 (0.2)
Σ18:1 *cis* area	57.0 (1.7)	46.6 (1.4)	58.6 (0.4)	46.3 (1.2)
Σ18:2 *trans* area	—	0.3 (0.2)	—	0.3 (0.2)
18:2 (n-6)	11.0 (0.6)	10.5 (0.8)	12.1 (0.3)	12.0 (0.5)
18:3 (n-3)	0.9 (0.1)	0.9 (0.1)	0.1 (0.01)	0.1 (0.02)

[a]Values are means and (SD) of fatty acids as weight percent of total fatty acid methyl esters. $n = 6$ (—, < 0.1% or not detected).
[b]Cis A diet contained 0.18 energy % linolenic acid and 0.0 energy % TFA. Trans A diet contained 0.18 energy % linolenic acid and 2.2 energy % TFA. Cis B diet contained 0.02 energy % linolenic acid and 0.0 energy % TFA. Trans B diet contained 0.02 energy % linolenic acid and 2.2 energy % TFA.
*Small amounts (< 0.3 wt%) of a component with retention time similar to TFA was frequently observed.

acid was 0.02 energy %. For one of each pair, the octadecenoic acid was all *cis* (cis A or cis B); for the other diet of each pair, a part of the octadecenoic acid was replaced with TFA (trans A or trans B). Thus four diets were constructed so that the variables were the levels of linolenic acid and TFA *(Table 1)*. Total saturates, monounsaturates, and linoleic acid were kept essentially constant by varying the kinds and amounts of oils used in the 10% fat diets *(Table 2)*.

Every 2 weeks, mixtures of the oils containing 0.02% of the antioxidant tertiarybutylhydroxyquinone were prepared from fat components shown in *Table 2*. Prior to preparation of the diets, the fatty acid composition of the oil mixtures were assessed by gas-liquid chromatography.[20] Food pellets were dried overnight at room temperature and frozen at −20° C in small plastic freezer bags until used. All diets were changed on a daily basis and uneaten food was discarded.

Mice

Weanling C57Bl/6 mice were purchased from Taconic Farms, Germantown, NY, USA, and assigned to one of two diets, cis A or trans A. Mice were housed individually in plastic cages at 25° C and 55% humidity with a 12-hour light:dark cycle and maintained according to University of Maryland, College Park, animal care and use committee guidelines. Mating pairs were established and the offspring of the first litter were continued on the parents' diets until they were 8 weeks of age. At this point, four diet groups were established. Female litter mates on the cis A diet were assigned to either the cis A or cis B diet. Similarly, litter mates on the trans A diets were assigned to either the trans A or trans B diet. A smaller number of males were also allocated to the four diets in a similar manner. When the mice were 12 weeks of age, they were mated for the first generation study. Female offspring from the first surviving litters of the first generation study were used as dams in the second generation study. Females used in the second generation study were kept on the diets and mated at 6 weeks of age. Similarly, female offspring of the second generation study were saved for the third generation study. An effort was made to represent each family in each of the three generations. The number of dams mated per diet for the first, second, and third generations were 10, 15, and 11, respectively. A total of 144 dams were studied. For each dam, a maximum of three litters were examined, however the actual number of litters varied from 0–3 depending on the fertility of the dam. Males on the appropriate diet and generation were placed with females for 1 week and removed. If pregnancy was not evident after 2 weeks (based on weight gain) they were mated again. Attempts were continued until three litters were obtained.

Table 2 Fats and oils used in the diets[a]

Diets[b]	cis A	trans A	cis B	trans B
Cocoa butter[c]	27.3	19.8	34.7	24.2
Shortening[d]	0.0	33.4	0.0	33.4
Soybean oil[e]	4.5	9.8	0.0	0.5
Corn oil[e]	0.0	2.3	0.0	0.0
Olive oil[f]	68.2	34.7	0.0	0.0
Safflower oil[e]	0.0	0.0	7.3	10.2
High oleic sunflower oil[g]	0.0	0.0	58.0	31.7

[a]Values are g/kg of diet. All diets contained the same amounts of other components, listed here with their sources: sucrose 590g (Mazzeo and Sons, Hyattsville, MD, USA); casein 200g, alphacel 50g, AIN 76 mineral mix 40g, AIN 76 vitamin mix 15g, dl methionine 3g, choline bitartrate 2g (all from ICN Biochemicals, Cleveland OH); TBHQ .002g (Eastman Kodak, Rochester, NY, USA).
[b]Cis A diet contained 0.18 energy % linolenic acid and 0.0 energy % TFA. Trans A diet contained 0.18 energy % linolenic acid and 2.2 energy % TFA. Cis B diet contained 0.02 energy % linolenic acid and 0.0 energy % TFA. Trans B diet contained 0.02 energy % linolenic acid and 2.2 energy % TFA.
[c]Purchased from Wilbur Chocolate Company, Lititz, PA, USA.
[d]Purchased from Auth Bros., Washington, DC, USA.
[e]Purchased from local supermarkets.
[f]Pope Olive Oil, from Continental Smelkinson, Jessup, MD, USA.
[g]SVA Enterprises, Columbus, OH, USA.

Analyses

The amount of food consumed per day per pregnant dam or lactating dam and pups was determined from the weight of new pellets minus the weight of pellets remaining the next day. Pellets were checked for water loss overnight and the weight loss was insignificant. Food intake was examined during the last week of pregnancy and throughout lactation until weaning at 20 days postpartum. Over a 2 month period, a total of 45 dams became pregnant and were used to obtain food-intake and weight-gain data. This included dams from both the first and second generation studies. The dams and pups (entire litters weighed together) were weighed daily

except for the first 3 days after parturition when mortality due to handling was of concern. An estimate of feed efficiency for each dam and litter was obtained by dividing the weight of food eaten by the combined weight of dam and pups.

Data from the weight gain and feed efficiency study were analyzed by analysis of covariance[21] using the general linear models procedure (GLM) of the statistical analysis systems (SAS) (SAS, Institute, Cary, NC, USA).[22] In all three generation studies, survival was recorded daily from birth until weaning at 20 days. Statistical analysis of the survival data was done by analysis of variance of a split-split plot design with the GLM of SAS. The split-split plot design resulted from assigning generation to the whole plot, family to the subplot, and litter to the sub-subplot. Means were compared by least significant difference.

Results

Results of the feeding study during pregnancy are displayed in *Table 3*. Dam gain during pregnancy, food eaten, and number of pups per litter were not different among diets. During lactation, dam weight did not change significantly and the average weight of dams for the four diets ranged from 28.2 g–31.9 g (data not shown).

Although entire litters were weighed daily from day 3 to weaning at 20 days, some data were not available for some litters and the statistical analysis was done only on day 6 through day 18 *(Figure 1)*. The slope of the linear change in weight from day 6–18 is the regression estimate of average daily gain. Pups of dams fed the cis A diet had an average gain of 4.0 g/day, and when either the diet with low n-3 fatty acids (cis B) or the diet with TFA (trans A) was fed, the average daily weight gains were smaller (3.8 and 3.2 g/day, respectively). When these two components, TFA and low n-3 fatty acids, were combined in a single diet (trans B), one might expect a further decrease due to the presence of both in the diet. However, the average weight gain of 4.6 g/day on trans B diet was even greater than that observed on the reference diet (cis A). This interaction between diets containing low n-3 fatty acids and TFA was significant ($P < 0.02$).

Data relating to mortality are shown in *Figure 2*. Mortality was highest on days 1 and 2 postpartum,

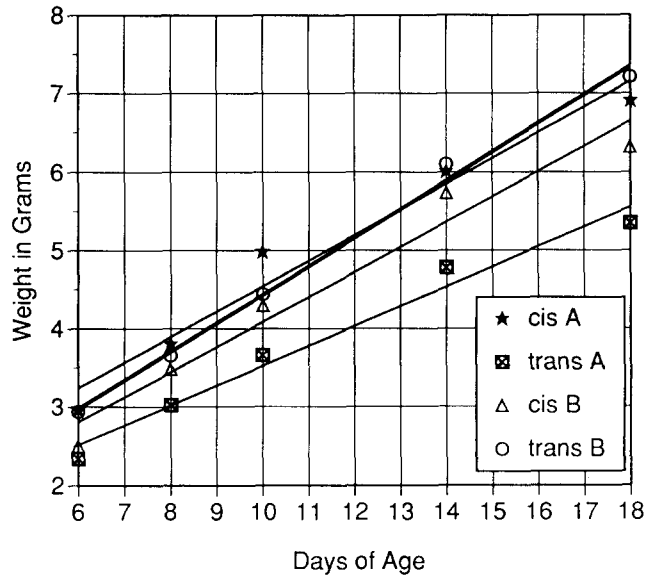

Figure 1 Average weight gain of pups during lactation. Cis A diet contained 0.18 energy % linolenic acid and 0.0 energy % TFA; trans A diet contained 0.18 energy % linolenic acid and 2.2 energy % TFA; cis B diet contained 0.02 energy % linolenic acid and 0.0 energy % TFA; and trans B diet contained 0.02 energy % linolenic acid and 2.2 energy % TFA. The following means were significantly different at $P < 0.05$: cis B versus trans B, trans A versus trans B, and cis A versus trans A.

when about two-thirds of all deaths on each diet occurred. The mortality pattern was similar throughout lactation and was virtually identical on days 1, 2, and 3. Mortality was highest for pups of dams fed the diet containing TFA and the lower level of n-3 fatty acids (trans B). In contrast, mortality was lowest for pups of dams fed the cis A diet, which lacked TFA and had the higher level of n-3 fatty acids.

When the total mortality data were examined by diet *(Table 4)*, there was no statistical interaction, therefore the following main effect comparisons were examined. The combined mortality for pups of dams fed the higher level of n-3 (cis A, trans A) was less than half that for pups of dams fed the lower level of n-3 (cis B, trans B) (21.3 versus 48.1; $P < 0.02$). Similarly, the combined mortality of pups from dams

Table 3 Food intake and weight gain of C57BL/6 dams during pregnancy[a]

Diets[b]	# of dams	Weight before pregnancy (g)	Avg. weight gain over entire pregnancy (g)	Average pups per liter	Average total intake (g) for last 6 days of pregnancy[c]
cis A	13	23.1 ± 1.3	13.8 ± 1.2	7.1 ± .6	26.7 ± 2.2
trans A	10	22.7 ± 1.3	13.0 ± 1.5	6.5 ± .9	25.5 ± 2.8
cis B	11	22.1 ± 1.3	13.6 ± 1.5	6.5 ± .8	26.0 ± 2.5
trans B	11	22.4 ± 1.2	13.7 ± 1.6	6.8 ± .8	26.2 ± 2.6

[a]Values for the last four columns are given as mean ± SEM and were not significantly different at $P \leq 0.05$.
[b]Cis A diet contained 0.18 energy % linolenic acid and 0.0 energy % TFA. Trans A diet contained 0.18 energy % linolenic acid and 2.2 energy % TFA. Cis B diet contained 0.02 energy % linolenic acid and 0.0 energy % TFA. Trans B diet contained 0.02 energy % linolenic acid and 2.2 energy % TFA.
[c]The dietary intake did not include the last day prior to parturition as food intake was often decreased at this time.

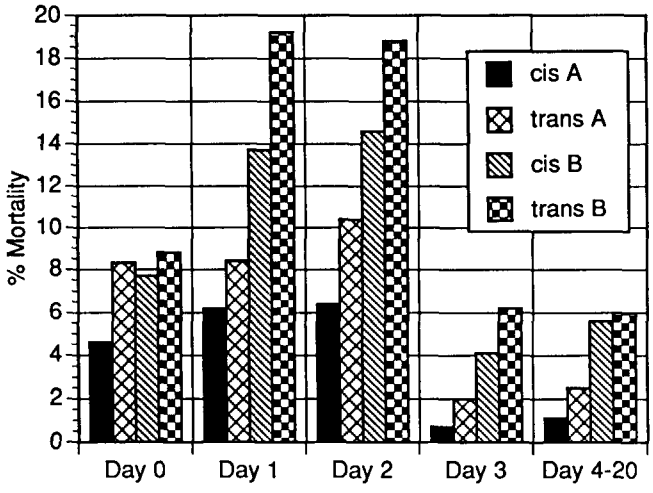

Figure 2 Mortality pattern during postpartum period. Cis A diet contained 0.18 energy % linolenic acid and 0.0 energy % TFA; trans A diet contained 0.18 energy % linolenic acid and 2.2 energy % TFA; cis B diet contained 0.02 energy % linolenic acid and 0.0 energy % TFA; and trans B diet contained 0.02 energy % linolenic acid and 2.2 energy % TFA.

fed the TFA diets (trans A, trans B) was significantly greater than that of pups from dams fed diets without TFA (cis A, cis B) (39.8 versus 29.7, $P < 0.04$). Although data for individual diet groups were not statistically different from each other, mortality of pups on the TFA diets were 10% greater regardless of the level of n-3 fatty acids. However, decreasing the level of linolenic acid resulted in a greater increase (27%) in mortality than did feeding TFA. The combined effect of feeding dams TFA and reducing the level of n-3 fatty acid in the diet resulted in an overall increase in percent mortality from 16% to 53%.

Data regarding mortality of pups from first, second, and third generation dams are given in *Table 5*. It should be noted that some data were excluded, as only families with offspring in all three generations were used in this statistical analysis. The diet effects were clearly manifested by the second generation and were even more evident by the third generation. Combined mortality on the lower n-3 diets (cis B and trans B) was significantly greater than the combined mortality on the higher n-3 diets (cis A and trans A) for either the second (52.5 versus 18.1, $P < 0.02$) or third generation (52.1 versus 20.0, $P < 0.003$). For the third

Table 4 Mortality data combined across generations and litters[a,b]

Diets[c]	Number of Dams[d]	Number of pups born	Number of litters born	Average pups born per litter	Percent mortality of pups
cis A	27 (31)	404 (438)	54 (61)	7.5 (7.2)	16.3 (19)
trans A	24 (35)	411 (556)	54 (76)	7.6 (7.3)	26.3 (32)
cis B	23 (30)	335 (444)	48 (65)	7.0 (6.8)	43.0 (46)
trans B	27 (35)	391 (512)	60 (77)	6.5 (6.6)	53.2 (57)

[a]Number in ( ) indicates total before some litters in generation 1 and 2 study were eliminated when there were no surviving offspring for generation 3 study.
[b]The following comparisons were significantly different: A diets versus B diets $P < 0.02$; cis diets versus trans diets $P < 0.04$.
[c]Cis A diet contained 0.18 energy % linolenic acid and 0.0 energy % TFA. Trans A diet contained 0.18 energy % linolenic acid and 2.2 energy % TFA. Cis B diet contained 0.02 energy % linolenic acid and 0.0 energy % TFA. Trans B diet contained 0.02 energy % linolenic acid and 2.2 energy % TFA.
[d]There were 36 dams on each diet, but some did not have pups.

Table 5 Mortality data from first, second, and third generation studies[a,b]

	Generation 1 study				Generation 2 study				Generation 3 study			
Diet[c]	# of Dams	# Pups born	# Pups died	% Mortality	# of Dams	# Pups born	# Pups died	% Mortality	# of Dams	# Pups born	# Pups died	% Mortality
cis A	5	80	27	33.8	13	164	13	7.9	9	160	23	14.4
trans A	5	97	25	25.8	8	103	29	28.2	11	211	54	25.6
cis B	4	52	19	36.5	8	100	49	49.0	11	183	76	42.0
trans B	4	60	12	20.0	12	170	95	56.0	11	161	101	62.7

[a]Includes only those families with offspring in all three generations.
[b]The following comparisons were significantly different: A versus B diets gen. 2 $P < 0.02$, gen. 3 $P < 0.001$. Trans B diet (gen. 3 only) versus cis A $P < 0.0001$ versus trans A $P < 0.003$ versus cis B $P < 0.02$.
[c]Cis A diet contained 0.18 energy % linolenic acid and 0.0 energy % TFA. Trans A diet contained 0.18 energy % linolenic acid and 2.2 energy % TFA. Cis B diet contained 0.02 energy % linolenic acid and 0.0 energy % TFA. Trans B diet contained 0.02 energy % linolenic acid and 2.2 energy % TFA.

generation, mortality was significantly greater on the TFA diet with lower n-3 fatty acid (trans B) than for any other diet (cis B, $P < 0.02$; trans A, $P < 0.003$; cis A, $P < 0.0001$).

A skin problem was observed that consisted of alopecia above the scapular region and was much worse on the low n-3 diets. Dams on the low n-3 diets with nonsurviving litters often did not exhibit maternal care of their newborn pups.

Discussion

The results of this study are consistent with an essential role of n-3 fatty acids and for an exacerbation of n-3 deficiency by TFA.* Although evidence for the essentiality of n-3 fatty acids in young animals has been reported previously,[13,14,23,24] to our knowledge this is the first study to suggest that TFA may exacerbate linolenate deficiency. Poor survival on diets containing TFA has been reported[3,25] but was ascribed to inadequate levels of linoleic acid.[26] It is unlikely that a deficiency in linoleic acid was responsible for the increased mortality in our study, as the level of linoleic acid was similar in all of our diets and was present in an amount considered to be adequate for rodents.

By the third generation, mortality was quite significantly increased on the diets containing TFA irrespective of levels of linolenic acid in the diet. Others have shown an increased depletion of n-3 fatty acids with successive generations[27–28] suggesting that increased mortality in subsequent generations in our study may be related to a generational depletion of n-3 in the dams that would ultimately be reflected in the levels available to the pups.

Surprisingly, the diet that had the highest mortality (trans B) resulted in the greatest increase in weight gain of surviving pups. The weight gain may have resulted from the smaller number of pups or a greater intake of milk on this diet. We have shown that feeding TFA resulted in a lower percentage of milkfat in lactating mice,[29] which, in turn, could have encouraged the pups to increase their milk intake to get enough calories. Nevertheless, weight gain due to an increase in body water content cannot be ruled out. Increased water consumption[1-2] has long been associated with deficiency of n-6 fatty acids and also has been reported when low n-3 fatty acid was fed.[30]

Increased mortality associated with the trans B diet, especially by the third generation, may have been due to insufficient tissue levels of 22-carbon n-3 PUFA in dams or pups. Low levels of linolenate would undoubtedly lead to low levels of 22-carbon n-3 PUFA, and TFA could exacerbate this situation by inhibiting the Δ^6 and Δ^5 desaturases,[31–33] which are important for providing the 20- and 22-carbon PUFA required for cell function. Mechanisms involving the induction of peroxisomes by TFA[34–39] may also be involved in altering tissue levels of PUFA. Increased β-oxidation due to increased numbers of peroxisomes could cause additional depletion of 22-carbon PUFA in cell membranes and exacerbate the problem of insufficient dietary n-3 fatty acid. This might be especially important in dams, because a study with partially hydrogenated marine oil has shown a three-fold increase in peroxisomes in females compared with males.[40]

In the absence of an optimum level of n-3 fatty acid(s), competition among potential desaturase substrates could result in inadequate levels of 22-carbon n-3 PUFA. Indeed, EFA deficiency symptoms have been reported[41] even though the dietary level of linoleic acid (2 energy percent) was presumably adequate. The EFA deficiency symptoms were attributed to competition for utilization of linoleic acid by the high level of oleic acid fed (69%, by weight of total fat, prior to gestation and 95% during gestation and lactation).

In our studies, the ratios of oleate:linoleate were similar in all diets, however, the ratios of oleate:linolenate were quite different and survival was lower on the diets with the higher ratios of oleate:linolenate. Similarly, there was a 10-fold difference in the ratio of linoleate:linolenate in our diets. Guesnet, Pascal, and Durand[24] found differences in rat pup mortality when oils with extremely different ratios of n-6:n-3 were fed to dams during pregnancy and lactation. Competition between oleate, linoleate, and linolenate for the Δ^6 desaturase could affect the level and type of PUFA available for use in membrane lipids. Thus in our studies, the level of 22-carbon n-3 PUFA expected in membrane lipids should be lower in the lower n-3 diets not only because they had lower levels of linolenic acid but also because they had higher ratios of linoleate:linolenate and of oleate:linolenate. Indeed, in related studies[42] we have observed lower levels of 22-carbon n-3 PUFA in brains and livers of males fed the cis B and trans B diets, compared with the A diets.

Several important membrane and cell functions, including enzyme activity, hormone receptor binding, and prostaglandin production,[43] could be affected by the 22-carbon PUFA levels in tissues. The neonatal survival problem in our study could possibly be due to cumulative effects of low 22-carbon n-3 PUFA in either maternal or fetal tissues. Most deaths occurred in the second and third days postpartum and may have been due to delay in lactation or failure of pups to suckle due to lack of opportunity presented by the dam or lack of ability to suckle on behalf of the pups. A crossfostering study might shed some light on these aspects. Improvement in survival when pups are transferred from dams on the low n-3 diet to dams on the higher n-3 diets would indicate tissue lipid composition of the dam rather than that of the pup was the most important factor.

*Partially hydrogenated fat was used as a source of *trans* fatty acids (TFA). In addition to TFA, partially hydrogenated fats also contain unusual *cis*-18:1 isomers, which were present in the trans A and trans B diets at ca. 9% of the total fatty acid methyl esters. Although the discussion in this paper is restricted to TFA, it is recognized that the results obtained herein could be due to the unusual *cis*-isomers or to a combination of the *cis*-isomers and the TFA present in the trans diets.

In conclusion, a problem with pup survival has been shown when dams were fed diets containing TFA or low n-3 fatty acid. The lower level of n-3 fatty acid had a greater effect on neonatal mortality than the presence of TFA, but the effects were cumulative and mortality was tripled when the diet contained both TFA and the lower level of n-3 fatty acid. We believe that n-3 fatty acids are required to provide 22-carbon PUFA, which may be important in neonatal mortality, and that n-3 fatty acid requirements should be considered in the context of other fatty acids present in the diet.

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References

1 Burr, G.O., Burr, M.M. (1930). On the nature and role of the fatty acids essential in nutrition. *J. Biol. Chem.* **86,** 587–621

2 Quackenbush, F.W., Kummerow, F.A., and Steenbock, H. (1942). The effectiveness of linoleic, arachidonic, and linolenic acids in reproduction and lactation. *J. Nutr.* **24,** 213–224

3 Kummerow, F.A., Pan, H.P., and Hickman, H. (1952). The effect of dietary fat on the reproductive performance and the mixed fatty acid composition of fat-deficient rats. *J. Nutr.* **46,** 489–498

4 Mackenzie, C.G., Mackenzie, J.B., and McCollum, E.V. (1939). Growth and reproduction on a low fat diet. *Biochem J.* **33,** 935–939

5 Tinoco, J., Williams, M.A., Hincenbergs, W.I., and Lyman, R.L. (1971). Evidence for nonessentiality of linolenic acid in the diet of the rat. *J. Nutr.* **101,** 937–946

6 Naughton, J.M. (1981). Supply of polyenoic fatty acids to the mammalian brain: The ease of conversion of the short-chain essential fatty acids to their longer chain polyunsaturated metabolites in liver, brain, placenta and blood. *Int. J. Biochem.* **13,** 56–62

7 Sun, G.Y. (1972). Effects of a fatty acid deficiency on lipids of whole brain, microsomes, and myelin in the rat. *J. Lipid Res.* **13,** 56–62

8 Mohrhauer, H. and Holman, R.T. (1963). Alteration of the fatty acid composition of brain lipids by varying levels of dietary essential fatty acids. *J. Neurochem.* **10,** 523–530

9 Samulski, M.A. and Walker, B.L. (1982). Maternal dietary fat and polyunsaturated fatty acids in the developing foetal rat brain. *J. Neurochem.* **39,** 1163–1168

10 Bourre, J.M., Pascal, G., Durand, G., Masson, M., Dumont, O., and Piciotti, M. (1984). Alterations in the fatty acid composition of rat brain cells (Neurons, astrocytes, and oligondendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. *J. Neurochem.* **43,** 342–348

11 Galli, C., Trzeciak, H.I., and Paoletti, R. (1972). Effects of essential fatty acid deficiency on myelin and various subcellular structures in rat brain. *J. Neurochem.* **19,** 1863–1867

12 Sanders, T.A.B., Mistry, M., and Naismith, D.J. (1984). The influence of a maternal diet rich in linoleic acid on brain and retinal docosahexaenoic acid in the rat. *Br. J. Nutr.* **51,** 57–66

13 Neuringer, M., Connor, W.E., Petten, C.V., and Barstad, L. (1984). Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. *J. Clin. Invest.* **73,** 272–276

14 Lamptey, M.S. and Walker, B.L. (1976). A possible essential role for dietary linolenic acid in the development of the young rat. *J. Nutr.* **106,** 86–93

15 Yamamoto, N., Saitoh, M., Moriuchi, A., Nomura, M., and Okuyama, H. (1987). Effect of dietary alpha-linolenate/linoleate balance on brain lipid composition and learning ability of rats. *J. Lipid Res.* **28,** 144–151

16 Holman, R.T., Johnson, S.B., and Hatch, T.F. (1982). A case of human linolenic acid deficiency involving neurological abnormalities. *Am. J. Clin. Nutr.* **35,** 617–623

17 Bjerve, K.S., Mostad, I.L., and Thoresen, L. (1987). Alpha-linolenic acid deficiency in patients on long-term gastric-tube feeding: estimation of linolenic acid and long-chain unsaturated n-3 fatty acid requirement in man. *Am. J. Clin. Nutr.* **45,** 66–77

18 Pax, J., Keeney, M., and Sampugna, J. (1989). Fatty acids in brain and liver of mice fed fish oil and partially hydrogenated fat. *FASEB* **3,** A948

19 Teter, B. (1989). *Alterations in milk fat percent and composition by isomeric fatty acids.* PhD Dissertation, University of Maryland, College Park, MD, USA

20 Sampugna, J., Pallansch, L.A., Enig, M.G., and Kenney, M. (1982). Rapid analysis of *trans* fatty acids on SP-2340 glass capillary column. *J. Chromatogr.* **249,** 245–255

21 Sokal, R. and Rohlf, F. (1987). *Introduction to Biostatistics,* 2d ed., W.H. Freeman and Co., New York, NY, USA, pp. 267–271

22 SAS Institute Inc. (1987). *SAS User's Guide: Statistics, Version 6.03,* SAS Institute, Inc., Cary, NC, USA, pp. 433–506

23 Budowski, P., Hawley, C., Crawford, M. (1980). L'effet protecteur de l'acide a-linolenique sur l'encephalomalacie chez le poulet. *Ann. Nutr. Alim.* **34,** 389–400

24 Guesnet, P., Pascal, B., and Durand, G. (1986). Dietary alpha-linolenic acid deficiency in the rat. 1. Effects on reproduction and postnatal growth. *Reprod. Nutr. Develop.* **26,** 969–985

25 Alfin-Slater, R.B., Morris, R.S., Hansen, H., and Proctor, J.F. (1965). Effects of non-essential fatty acids on essential fatty acid deficiency. *J. Nutr.* **87,** 168–172

26 Alfin-Slater, R.B., Aftergood, L., and Whitten, T. (1979). Nutritional role of hydrogenated fats (in rats). In *Geometrical and Positional Fatty Acid Isomers* (E.A. Emken and J. Dutton, eds.) American Oil Chemists' Society, Champaign, IL, pp. 53–74

27 Tinoco J., Babcock, R., Hincenbergs, I., Medwadowski, B., and Miljanich, P. (1978). Linolenic acid deficiency: changes in fatty acid patterns in female and male rats raised on a linolenic acid-deficient diet for two generation. *Lipids* **13,** 6–17

28 Bourre, J.M., Pascal, G., Durand, G., Masson, M., Dumont, O., and Piciotti, M. (1984). Alterations in the fatty acid composition of rat brain cells (Neurons, astrocytes, and oligondendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. *J. Neurochem.* **43,** 342–348

29 Teter, B.B., Sampugna, J., and Keeney, M. (1990). Milk fat depression in C57B1/6J mice consuming partially hydrogenated fat. *J. Nutr.* **120,** 818–824

30 Reisback, S., Neuringer, M., and Connor, W.E. (1990). Polydypsis in rhesus monkeys postnatally deficient in omega-3 fatty acids. *FASEB J.* **4,** A798

31 Mahfouz, M.M., Smith, T.L., and Kummerow, F.A. (1984). Effect of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat-liver microsomes. *Lipids* **19,** 214–222

32 Mahfouz, M., Johnson, S., and Holman, R.T. (1981). Inhibition of desaturation of palmitic, linoleic and eicosa-8,11, 14-trienoic acids in vitro by isomeric cis-octadecenoic acids. *Biochim. Biophys. Acta* **663,** 58–68

33 Mahfouz, M., Johnson, S., and Holman, R.T. (1980). The effect of isomeric *trans*-18:1 acids on the desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids by rat liver microsomes. *Lipids* **15,** 100–107

34 Thomassen, M.S., Christiansen, E.N., and Norum, K.R. (1982). Characterization of the stimulatory effect of high-fat diets on peroxisomal β-oxidation in rat liver. *Biochem. J.* **206,** 195–202

35 Wong, S.H., Nestel, P.J., Trimble, R.P., Storer, G.B., Illman, R.J. and Topping, D.L. (1987). The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver. *Biochim. Biophys. Acta* **792,** 103–109

36 Flatmark, T., Nilsson, A., Kvannes, J., Eikhom, T.S., Fukami, M.H., Kryvi, H., Christiansen, E.N. (1988). On the mechanism of induction of the enzyme systems for peroxisomal β-oxidation of fatty acids in rat liver by diets rich in partially hydrogenated fish oil. *Biochim. Biophys. Acta* **962,** 122–130

37 Hoy, C.E. and Holmer, G. (1988). Dietary linoleic acid and the fatty acid profiles in rats fed partially hydrogenated marine oils. *Lipids* **23,** 973–980

38 Gimenez, Y.B. (1984). *Effect of trans fatty acids on murine hepatic peroxisomes.* Master's thesis, University of Maryland, College Park, MD, USA

39 Panigrahi, K., Mohanty, S., and Sampugna, J. (1991). Effect of *trans* fatty acids on peroxisomes in 3T3-L1 Cells. *FASEB J.* **5,** A1158

40 Thomassen, M.S., Norseth, J., and Christiansen, E.N. (1985). Long-term effects of high-fat diets on peroxisomal β-oxidation in male and female rats. *Lipids* **20,** 668–674

41 Menon, N.K., Moore, C., and Dhopeshwarkar, G.A. (1981). Effect of essential fatty acid deficiency on maternal placental, and fetal rat tissues. *J. Nutr.* **111,** 1602–1610

42 Pax, J., Keeney, M., and Sampugna, J. (1991). Linolenic acid and *trans* fatty acids affect survival of mouse pups. *FASEB J.* **5,** A1446

43 Brenner, R.R. (1984). Effect of unsaturated acids on membrane structure and enzyme kinetics. *Prog. Lipid Res.* **23,** 69–96