The Fatty Acid Composition of Skeletal Muscle Membrane Phospholipid: Its Relationship With the Type of Feeding and Plasma Glucose Levels in Young Children

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Long-chain polyunsaturated fatty acids (LCPUFAs), including docosahexaenoic acid ([DHA] 22:6 n-3), are important components of cell membranes. Low levels of DHA and other LCPUFAs in skeletal muscle membrane phospholipid are associated with insulin resistance and obesity in adults. These findings may be influenced by both dietary and genetic factors. This study aimed to investigate the interrelationships between the type of infant feeding, skeletal muscle phospholipid fatty acid (FA) composition, and glucoregulation in young children. Skeletal muscle biopsies and fasting blood samples were obtained from 56 normally nourished young children (35 males and 21 females) aged less than 2 years (mean ± SE, 0.76 ± 0.06) undergoing elective surgery. The dietary history was taken, and muscle phospholipid FA composition was analyzed. Subgroups of totally breast-fed and age-matched formula-fed infants were compared. Breast-fed infants (n = 13; age, 0.54 ± 0.06 years) had a significantly higher percentage of DHA (3.63% ± 0.22% *v* 1.84% ± 0.11%, $P < .0001$) and total percentage of LCPUFAs (30.24 ± 0.87% *v* 25.17% ± 0.86, $P < .0001$) in muscle phospholipids compared with the formula-fed group (n = 12; age, 0.59 ± 0.08 years). The totally breast-fed group had lower plasma glucose levels than the formula-fed group (4.7 ± 0.2 *v* 5.4 ± 0.2 mmol/L, $P < .02$). Consistent with these findings, further analysis of a group of 39 children who had either never or not recently been breast-fed showed significant inverse correlations between fasting plasma glucose and the percentage of both DHA ($r = -.47$, $P < .003$) and total LCPUFAs ($r = -.38$, $P < .05$). The results of this study show that (1) breast-feeding increases LCPUFA levels in skeletal muscle membrane and (2) early development of relatively higher levels of LCPUFAs in the phospholipid of skeletal muscle, influenced both by type of feeding and by genetic predisposition, is associated with lower fasting plasma glucose. Early changes in skeletal muscle membrane phospholipid FA saturation may play a role in the subsequent development of diseases associated with insulin resistance.

LONG-CHAIN POLYUNSATURATED fatty acids (LCPUFAs), especially docosahexaenoic acid ([DHA] 22:6 n-3), are important components of cell membrane structural lipid. The retina and brain are rich in n-3 polyunsaturated fatty acids (PUFAs), particularly DHA,[1-3] and there have been a number of studies in infants of the relationships between diet and aspects of development of the nervous system.[4-9]

However, alterations in the fatty acid (FA) composition of membrane structural lipids are important throughout the body for a range of metabolic functions. Skeletal muscle is particularly important because it is the major site of insulin-mediated glucose uptake in the body.[10] Insulin resistance is associated with a cluster of prevalent diseases, including non–insulin-dependent diabetes mellitus, obesity, dyslipidemia, hypertension, and heart disease.[11-13] Studies from our laboratory and others have shown that the FA composition of skeletal muscle phospholipid is closely associated with insulin action in animals and humans.[14-18] In short, a higher proportion of LCPUFAs in muscle membrane phospholipid is associated with improved insulin action; conversely, a higher proportion of the more saturated FAs is associated with insulin resistance. The formation of LCPUFAs from most dietary fats is dependent on the activity of elongase and desaturase enzymes[19] (Fig 1). The activities of these particular enzymes have been crudely indexed in terms of product-precursor ratios,[20,21] and have also been shown to be closely associated with leanness and insulin sensitivity in adult humans.[17,18]

Although the relationships between skeletal muscle phospholipid FA composition and insulin action have been established in studies in adult humans, there have been no studies on the effect of early infant diet on skeletal muscle membrane FA composition. Furthermore, there are no reports in infants and young children on the relationship between skeletal muscle membrane FA composition and measures of carbohydrate metabolism. The aim of this study was to investigate (1) the effect of the type of infant feeding on skeletal muscle membrane FA composition and (2) the relationship between muscle membrane FA composition and simple measures of carbohydrate metabolism in young children.

SUBJECTS AND METHODS

Subjects

Fifty-six children (35 males and 21 females) aged less than 2 years who were undergoing elective surgery at The Royal Alexandra Hospital for Children (Camperdown, Sydney, Australia) were recruited for the study. These children had no history of poor weight gain, significant systemic disease, major congenital malformations, or previous surgery.

The types of elective surgery were correction of congenital talipes and several forms of cardiovascular, urological, or abdominal surgery. The muscle groups obtained were abductor hallucis (n = 30), rectus abdominis (n = 11), latissimus dorsi (n = 9), and external oblique (n = 6).

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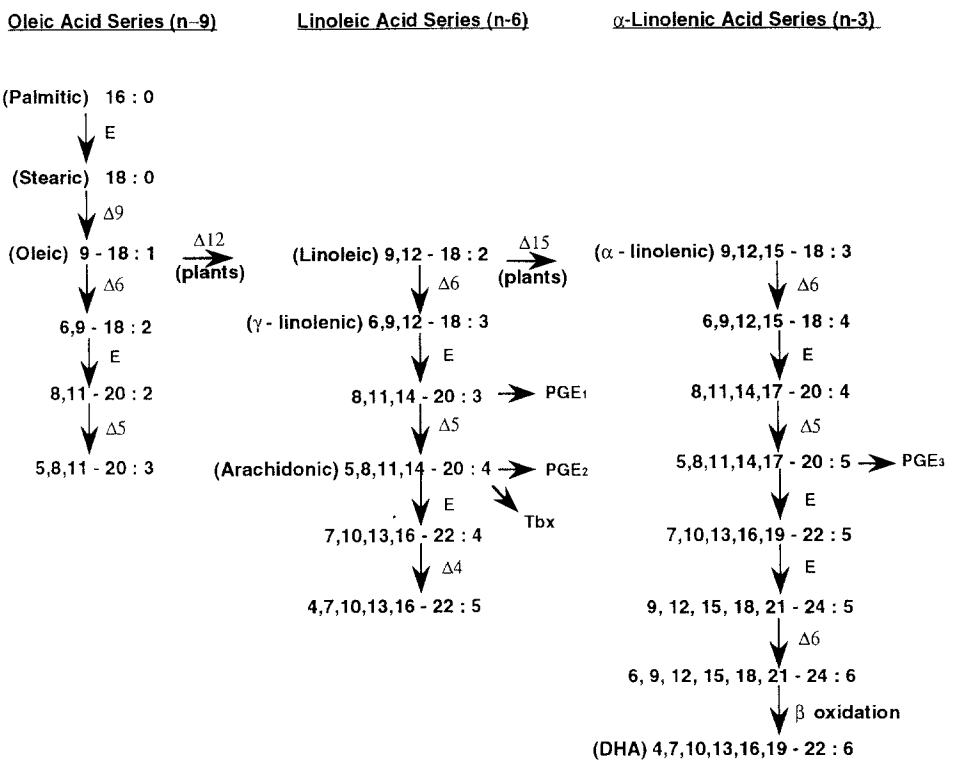

Fig 1. Biosynthesis of PUFAs, showing pathways of elongation and desaturation. E, elongase; Δx, fatty acyl-coenzyme A desaturases; PG, prostaglandin; Tbx, thromboxane.

The study protocol was approved by the Ethics Committee of The Royal Alexandra Hospital for Children.

Protocol

Patients were admitted to the hospital in the afternoon before the day of elective surgery. At this time, written informed consent was obtained from the parents. A detailed dietary history of the child was obtained focusing on the duration of breast- and/or formula-feeding, the time of introduction of solid foods, and the current diet, with particular reference to foods high in LCPUFAs. Length measurements (±1 cm) were made with an infantometer (Holtain, Dyfed, Wales, UK). Weight measurements (±0.01 kg) were made with electronic scales with the infants wearing minimal clothing.

A fasting blood sample (1.5 to 2.0 mL) was obtained at the time of insertion of an intravenous line directly after halothane induction of anesthesia and before intravenous fluids were given. This was analyzed for plasma glucose, plasma cholesterol, and serum insulin levels. Note that non–breast-fed infants underwent fasting for at least 6 hours for milk, solids, or formula and 4 hours for clear fluids. Breast-fed infants fasted for at least 4 hours.

A skeletal muscle biopsy (40 to 200 mg) was obtained in the operating room at the time of surgery and immediately freeze-clamped using aluminium tongs cooled in liquid nitrogen. The samples were stored at −70°C for later analysis of membrane phospholipid FA composition.

The study design was constrained by the ethical problems of obtaining muscle biopsies and fasting blood samples from normally nourished infants and young children. Due to these stringent considerations, it was not possible to construct a formal prospective study. The only ethical way to acquire muscle biopsies from this age group was to obtain consent from the parents of children undergoing specific types of elective surgery that involved routine incision of particular muscle groups. It was not ethically or practically feasible to control the scheduling of the operations, to set a fixed period for fasting preoperatively, or to prospectively control the child's diet.

Analytical Methods

Biochemical tests on blood samples were performed at the Departments of Endocrinology (insulin assays) and Pathology (cholesterol and glucose assays) of The Royal Prince Alfred Hospital (Sydney, Australia). Plasma glucose and cholesterol concentrations were measured with automated enzymatic techniques (Hitachi 747 multichannel analyzer; Boehringer Mannheim Australia, Sydney, Australia). Serum insulin concentrations were estimated with the Ultra Sensitive Human Insulin RIA Kit (Linco Research, St Charles, MO). All samples were pooled to minimize interassay variability.

The extraction and derivatization of the FA components of muscle phospholipids have been described elsewhere.[15] Phospholipid is almost exclusively associated with membranes, but our analysis does not differentiate between cellular membranes, eg, sarcoplasmic reticulum, plasma, and mitochondria. In brief, muscle tissue was homogenized in 2:1 (vol/vol) chloroform:methanol, and total lipid extracts were prepared according to the method of Folch et al.[22] Phospholipids were isolated from less polar lipids by solid-phase extraction on Sep-Pak silica cartridges (Waters, Milford, MA). The phospholipids were transmethylated, and methyl FAs were separated, identified, and quantified by gas chromatography. The content of individual FAs in skeletal muscle phospholipids was expressed as a percentage of total FAs. Identified minor FA peaks (<0.5% of the total) were excluded from the calculations.

Data Analysis

The content of individual FAs in skeletal muscle phospholipids was expressed as a percentage of the total FAs identified. Results for the different muscle groups were pooled because there were no major differences between them. Several FA indices were derived from the primary data: the total percentage of C20-22 LCPUFAs (sum of the percentage of the individual LCPUFAs 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-6, 22:5 n-3, and 22:6 n-3) and the mean degree of FA

unsaturation (the unsaturation index) calculated as the mean number of double bonds per FA residue multiplied by 100.

Length and weight measurements were compared with the National Center for Health Statistics reference population.[23] These measurements were normalized by expression as standard deviation (SD) scores.[24]

Dietary histories were analyzed using Australian food composition tables[25] extended to specifically index LCPUFA intake.

Statistical Analysis

All data are expressed as the mean ± SEM. Statistical analyses were performed using Statview II (Abacus Concepts, Berkeley, CA) and SPSS/PC version 6.0 (SPSS, Chicago, IL). The level of significance was set at P less than .05. Student's t test was used to compare anthropometric characteristics and fasting plasma glucose, serum insulin, and plasma cholesterol between subgroups of children. ANOVA was used to compare differences in muscle membrane phospholipid DHA levels among four different feeding subgroups. Differences in muscle membrane phospholipid FA composition between formula-fed and breast-fed subgroups of infants were analyzed by multivariate ANOVA. Relationships between fasting plasma glucose levels and muscle membrane FA composition were investigated by simple correlation and partial correlation (controlling for age and duration of breast-feeding).

RESULTS

Subject Characteristics

Table 1 shows the anthropometry and fasting blood results for the 56 children in the study. Results are expressed as the mean ± SE. The mean weight and length SD scores were not significantly different from zero, indicating that the children were comparable to the reference population.

The duration of breast-feeding for 56 children ranged from 0 to 0.90 years. In this study, breast-feeding duration was calculated as the actual duration of breast-feeding, whether sole (no food given other than breast milk) or partial (some solid foods and/or complementary formula feeding offered concurrently with breast-feeding). Further dietary analysis showed that food sources of LCPUFAs (eg, fish, egg yolk, lamb brains, and liver) were either absent or present in negligible amounts in the diet of children in this study.

The fasting plasma glucose levels (5.1 ± 0.1 mmol/L; range, 3.6 to 7.3) reported in this study are consistent with those from other studies of glucose levels in infants and young children after halothane induction of anesthesia.[26,27]

Relationship Between the Type of Feeding and Muscle Membrane FA Composition

For purposes of comparison of breast-feeding versus infant formula-feeding, the subjects were divided into two groups depending on the type of feeding. Only subjects aged less than 1 year were included, to allow age-matching between the groups.

Table 1. Anthropometry and Fasting Blood Results for 56 Children

Parameter	Mean ± SE	Range
Age (yr)	0.76 ± 0.06	0.23-1.96
Duration of breast feeding (yr)	0.23 ± 0.03	0-0.90
Weight SD score	0.00 ± 0.13	−1.80-2.23
Length SD score	−0.26 ± 0.13	−2.11-1.92
Insulin (pmol/L)	12 ± 1	5-25
Glucose (mmol/L)	5.1 ± 0.1	3.6-7.3
Cholesterol (mmol/L)	3.6 ± 0.1	2.0-5.8

Table 2. Comparison of Age, Fasting Blood Values, and Muscle Membrane FA Copmposition of Two Subgroups of Infants Aged Less Than 1 Year: Formula-Fed and Breast-Fed (mean ± SEM)

Variable	Formula-Fed (n = 12)	Breast-Fed (n = 13)
Age (yr)	0.59 ± 0.08	0.54 ± 0.06
Weight SD score	0.19 ± 0.23	−0.04 ± 0.27
Length SD score	0.09 ± 0.23	−0.38 ± 0.25
Glucose (mmol/L)	5.4 ± 0.2	4.7 ± 0.2†
Insulin (pmol/L)	11.8 ± 1.4	10.6 ± 1.4
Cholesterol (mmol/L)	3.2 ± 0.2	4.0 ± 0.2*
% of total FAs		
Saturated	30.25 ± 0.64	30.41 ± 0.44
14:0	0.32 ± 0.11	0.98 ± 0.27*
16:0	14.76 ± 0.49	14.39 ± 0.31
18:0	15.06 ± 0.24	14.83 ± 0.18
Monounsaturated	12.23 ± 0.74	12.64 ± 0.65
14:1	0.91 ± 0.33	0.91 ± 0.21
16:1	0.67 ± 0.10	1.02 ± 0.11*
18:1	11.86 ± 0.75	12.33 ± 0.60
n-6 polyunsaturated	49.77 ± 1.22	47.80 ± 0.92
18:2 n-6	28.69 ± 1.19	23.25 ± 1.33*
20:3 n-6	1.96 ± 0.12	2.32 ± 0.11*
20:4 n-6	16.71 ± 0.54	20.18 ± 0.60§
22:4 n-6	1.40 ± 0.15	1.21 ± 0.12
22:5 n-6	0.96 ± 0.09	0.81 ± 0.09
n-3 polyunsaturated	3.78 ± 0.37	5.25 ± 0.23‡
18:3 n-3	0.14 ± 0.03	0.05 ± 0.02*
20:5 n-3	0.38 ± 0.08	0.19 ± 0.05*
22:5 n-3	1.43 ± 0.22	1.37 ± 0.05
22:6 n-3	1.84 ± 0.11	3.63 ± 0.22§
Derived indices		
C20-22 PUFAs‖	25.17 ± 0.86	30.24 ± 0.87§
Unsaturation index¶	178.06 ± 2.76	190.07 ± 1.90‡

*P < .05.
†P < .02.
‡P < .002.
§P < .0001.
‖Calculated as the sum of the % of the PUFAs 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-6, 22:5 n-3, and 22:6 n-3.
¶Calculated as the mean no. of double bonds per FA residue multiplied by 100.

The formula-fed group (n = 12: eight talipes repair and four abdominal surgery) consisted of children who were breast-fed for less than 4 weeks, and the breast-fed group (n = 13: seven talipes repair and six abdominal surgery) consisted of children who were still being breast-fed at the time of surgery (Table 2). Compared with the formula-fed group, the breast-fed group had significantly greater proportions of the FAs 14:0, 16:1, and 20:4 n-6, DHA (22:6 n-3), sum of the n-3 PUFAs, C20-22 LCPUFAs, and the unsaturation index in muscle membrane phospholipid. However, the breast-fed group had significantly lower proportions of 18:2 n-6, 18:3 n-3, and 20:5 n-3 FAs compared with the formula-fed group. In addition, the breast-fed group had significantly (P < .02) lower fasting plasma glucose than the formula-fed group (4.7 ± 0.2 v 5.4 ± 0.2 mmol/L, respectively; Table 2).

Consistent with previous research,[28,29] the breast-fed group had significantly higher fasting plasma cholesterol than the formula-fed group (Table 2).

Relationship Between Muscle Membrane FAs and Blood Glucose Levels

When the total group was considered, significant inverse relationships were found between the fasting plasma glucose level and the percentage of DHA and C20-22 LCPUFAs in muscle membrane phospholipid ($r = -.28$, $P < .05$ and $r = -.32$, $P < .04$, respectively). However, these results may be influenced by the previous finding that breast-feeding has a profound effect on muscle membrane FA composition (Table 2).

We have previously shown[30] that the higher levels of DHA in muscle membrane phospholipid of breast-fed infants disappear relatively quickly after cessation of breast-feeding. In keeping with this finding, there was no difference in the current study between muscle membrane phospholipid DHA levels of children who had stopped breast-feeding at least 11 weeks before surgery (previously breast-fed) and an age-matched group of formula-fed children (formula-fed group). Both of these latter groups had significantly less DHA in the muscle membrane phospholipid than the currently breast-fed group (Table 3). Therefore, the formula-fed and previously breast-fed groups were combined to form a subgroup of 39 children to examine the relationship between the muscle membrane phospholipid DHA level and fasting plasma glucose level independent of the effect of breast-feeding (Table 4).

There was a significant ($P < .05$) positive correlation between the fasting plasma glucose and the percentage of 18:0 (a saturated FA) in muscle phospholipid. More interestingly, there were significant inverse correlations between fasting plasma glucose and the sum of the n-3 PUFAs, DHA, C20-22 LCPUFAs, and the ratio of 20:4 n-6 to 18:2 n-6 (Table 4). The latter ratio is the effective measure of throughput from the dietary intake to the longest, more unsaturated n-6 FAs. The significant ($r = -.47$, $P = .003$) inverse relationship between the percent DHA and the fasting plasma glucose is shown graphically in Fig 2. Note the wide range of both fasting plasma glucose and percent DHA in muscle membrane in this non–breast-fed group (3.6 to 6.7 mmol/L and 1.09% to 3.19%, respectively).

When this analysis was repeated after controlling for age and breast-feeding duration by partial correlation, the same inverse and positive correlations were found for muscle membrane FAs and fasting glucose levels, with one additional correlation: 18:2 n-6 ($r = .31$, $P = .02$).

There was a small but nonsignificant trend toward increased muscle membrane phospholipid DHA levels with age in this group of 39 children ($r = .26$, $P = .11$).

There were no significant correlations between the proportion of any of the muscle membrane LCPUFAs and fasting insulin levels.

Table 3. Comparison of Muscle Membrane Phospholipid DHA in Four Subgroups of Children

		Ceased BF		
	Currently BF	1-11 wk Presurgery	>11 wk Presurgery	Formula-Fed
Sex ratio (male:female)	9:5	2:1	12:7	12:8
DHA*	3.53 ± 0.22	2.02 ± 0.54	1.81 ± 0.10	1.90 ± 0.09

NOTE. Values are the mean ± SEM % of total FAs.
Abbreviation: BF, breast-feeding.
*$P = .0001$ (ANOVA).

Table 4. Profile of FAs in the Phospholipid Fraction of Skeletal Muscle and Simple Correlations With Fasting Plasma Glucose in 39 Children Who Either Had Never Been Breast-Fed or Had Not Been Breast-Fed for at Least 11 Weeks Presurgery (mean ± SEM)

Variable	% of Total FAs	Correlation With Fasting Plasma Glucose
Age (yr)	0.87 ± 0.08	−.22
FAs		
Saturated	30.96 ± 0.41	−.17
14:0	0.60 ± 0.14	−.26
16:0	15.17 ± 0.27	−.27
18:0	15.03 ± 0.18	.32*
Monounsaturated	11.78 ± 0.44	−.09
14:1	1.27 ± 0.18	.09
16:1	0.69 ± 0.06	−.04
18:1	11.46 ± 0.44	−.09
n-6 polyunsaturated	49.71 ± 0.50	.15
18:2 n-6	29.13 ± 0.52	.30
20:3 n-6	1.99 ± 0.09	.08
20:4 n-6	16.49 ± 0.32	−.27
22:4 n-6	1.23 ± 0.06	.02
22:5 n-6	0.84 ± 0.04	−.16
n-3 polyunsaturated	3.71 ± 0.18	−.37*
18:3 n-3	0.15 ± 0.02	−.15
20:5 n-3	0.34 ± 0.05	−.15
22:5 n-3	1.35 ± 0.09	−.24
22:6 n-3	1.86 ± 0.07	−.47‡
Derived indices		
C20-22 PUFAs§	24.60 ± 0.45	−.38†
Unsaturation index‖	175.75 ± 1.54	−.21
Ratio of 20:4 to 18:2	0.58 ± 0.02	−.34*

*$P < .05$.
†$P < .02$.
‡$P < .003$.
§Calculated as the sum of the % of the PUFAs 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-6, 22:5 n-3, and 22:6 n-3.
‖Calculated as the mean no. of double bonds per FA residue multiplied by 100.

DISCUSSION

This study demonstrates for the first time the significant effect of the type of infant feeding on the phospholipid FA composition of skeletal muscle, the major tissue of insulin-stimulated glucose uptake.[10] Infants who were breast-fed had a higher percentage of LCPUFAs in the muscle membrane structural lipids and a lower mean fasting plasma glucose level than infants who were formula-fed. To examine muscle phospholipid FA composition independently of the effect of breast-feeding, we investigated a subgroup of 39 children who either had never been breast-fed or had not been breast-fed for at least 11 weeks before surgery. For this group, significant inverse relationships were found between fasting glucose levels and LCPUFA levels, particularly the n-3 series, in muscle membrane phospholipid.

The results from the first part of this study are in keeping with the known differences in FA composition between breast milk and infant formula.[31-34] Infant formulas contain the precursor FAs linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3) but

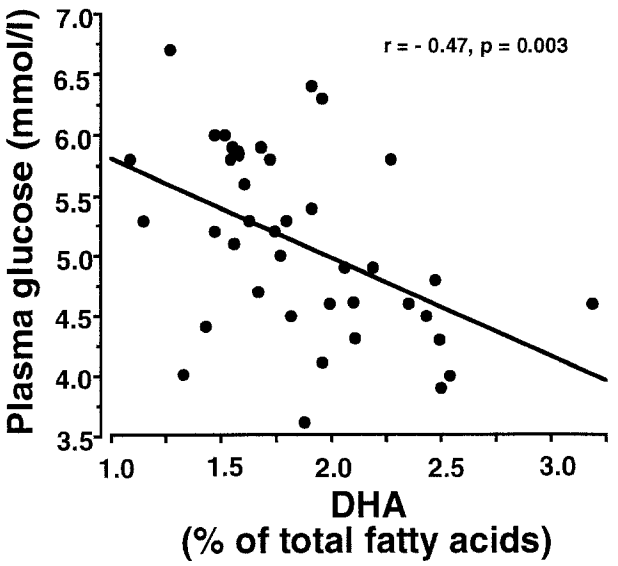

Fig 2. Fasting plasma glucose in relation to % DHA in the muscle membrane phospholipid of 39 children.

lack important LCPUFAs such as DHA (22:6 n-3) and arachidonic acid (20:4 n-6).[32] Likewise, the amount of weaning foods consumed by an infant supplies a negligible amount of LCPUFAs, especially DHA.[35] However, breast milk contains a range of LCPUFAs.[31] As a reflection of their diet, the formula-fed group of infants in this study had higher proportions of the precursor FAs in muscle membrane phospholipid and lower proportions of DHA and the other LCPUFAs compared with the breast-fed group.

In light of previous studies[16-18] on the relationship between skeletal muscle FA composition and insulin action, the results presented here are of interest. Breast-fed infants have a muscle phospholipid FA composition similar to that of insulin-sensitive adults, ie, a higher proportion of LCPUFAs, especially the n-3 series, and a correspondingly lower proportion of the n-6 series of FAs. In contrast, formula-fed infants have a muscle phospholipid FA composition similar to that of insulin-resistant adults, with the deficiency in DHA and other LCPUFAs of the n-3 and n-6 series substituted for by the insertion of linoleic acid, a major component of infant formula. In view of the relationship between insulin action and glucoregulation, it is of interest that the breast-fed group in this study had significantly lower fasting blood glucose than the formula-fed group ($P < .02$; Table 2).

A further important finding in this study is the significant inverse relationship between fasting plasma glucose and the percentage of LCPUFAs in skeletal muscle phospholipid of a subgroup of 39 children who had never or not recently been breast-fed. While halothane is thought to affect glucose regulation,[36] the blood sample was taken directly after induction of anesthesia and after a fasting period of at least 6 hours. However, it is likely that there was a significant degree of stress involved at the time of blood collection, which may explain why insulin levels were uniformly low. The higher plasma glucose level associated with a more saturated membrane lipid FA composition may then represent either a tendency to primary insulin resistance and/or a hyperreactivity to stress. Both have been proposed as elements of the "syndromes of insulin resistance" or, as termed by Björntorp,[37] "the civilization diseases." Although fasting insulin in the normoglycemic population is a marker of insulin action in adults nothing is known about this in infants and young children. It may be that fasting glucose is more closely related to insulin resistance than fasting insulin. Of course, in this population, it was not ethically possible to use a more invasive measure to directly assess insulin action.

Adult humans have the ability to elongate and desaturate the essential FAs provided the precursor FAs linoleic acid (n-6 series) and α-linolenic acid (n-3 series) are supplied in the diet.[19] Conversion of these precursors to the LCPUFAs is dependent on the activities of elongase and desaturase enzymes,[19] and indeed, the activities of elongase and Δ5-desaturase enzymes have been closely linked to leanness and insulin sensitivity in two separate adult populations.[17,18] Recent data demonstrate the ability of full-term neonates to biosynthesize DHA and arachidonic acid in vivo from the 18-carbon precursors, although the amounts produced are significantly lower than in breast-fed infants.[38,39]

None of the FA desaturase and elongase enzymes of the n-6 and n-3 PUFA pathways have been cloned in mammalian systems. Therefore, the only potential measures of desaturase and elongase activity in this study are the product-precursor ratios. Although these are far from ideal as indices of enzyme activity, it is nevertheless of interest that in this study the ratio of 20:4 to 18:2, the product-precursor ratio for the reactions catalyzed by Δ5-desaturase, elongase, and Δ6-desaturase enzymes, was found to be inversely related to fasting plasma glucose in the non–breast-fed group (n = 39).

LCPUFA levels in infant muscle phospholipid would appear to result from a complex interaction between the weaning diet, duration of breast-feeding, and LCPUFAs retained from the in utero environment and endogenous synthesis. In this study, there was no significant dietary intake of n-3 LCPUFAs in the children, and the effects of breast-feeding could be eliminated by focusing on the non–breast-fed group (n = 39). In this latter group, there was a broad range in muscle LCPUFAs. In utero storage would not appear to be a major factor affecting muscle PUFA levels, since DHA is not stored in white adipose tissue in infants[40] and thus could not be released from internal stores for incorporation into phospholipid. Additionally, if DHA in phospholipid were just residual and had been "hoarded" from the time in utero, then one should observe a decline in DHA over time in the children who were formula-fed. In contrast, the present results show a modest nonsignificant increase in DHA in muscle phospholipid with age in this group (n = 39). Therefore, the wide range of muscle membrane LCPUFAs observed in this study is consistent with a large variation in interindividual FA elongase and desaturase enzyme activities. This variation could be a primary metabolic difference between individuals, or it could be secondary to differences in insulin sensitivity and thus the overall pattern of daily glucose and insulin excursions.[41,42] Both possibilities raise important issues that require further investigation.

Assuming that the LCPUFA composition of muscle membrane phospholipid influences glucose metabolism, what might be the mechanism? It is possible that LCPUFAs may modulate the function of membrane proteins mediating insulin action,

such as insulin receptors and glucose transporters, by altering the fluidity of the surrounding lipid environment.[43] Another explanation is that LCPUFAs could influence insulin action by acting as precursors for generation of second messengers, such as eicosanoids or diacylglycerols[44] (Fig 1). Eicosanoids, which are physically active compounds known as prostaglandins, thromboxanes, and leukotrienes, are derived from C20 PUFAs.[45] The different actions of these biochemical mediators are dependent on the availability of precursor FAs in the phospholipid pool.

Animal data have shown that dietary manipulation can have lasting effects on neurodevelopment and health outcomes.[46] In addition, brief periods of dietary management in which human infants received either breast milk or PUFA-supplemented formula have been shown to have a positive impact on visual and neurodevelopmental function.[6-9] These findings are further supported by recent studies showing an increased proportion of DHA in the structural lipid of the cerebral cortex of breast-fed infants compared with those who were formula-fed.[4,5] Our study has found that once breast-feeding has ceased, there is a decrease in muscle phospholipid DHA to levels comparable to those of the formula-fed children over 3 months. For breast-feeding per se to have a long-term effect on the incidence of diabetes, one must postulate a critical period in the first year of life. However, more importantly, even in non–breast-fed children there is a threefold difference in DHA levels (Fig 2) reflecting several interrelated factors, one of which may be a wide range in desaturase and elongase activities in young children. An intrinsic predisposition to either high- or low-level activity of these enzymes would presumably have significant long-term consequences for membrane function and insulin action. It is possible that formula diets (ie, those lacking LCPUFAs) may act in concert with low levels of desaturase and elongase enzyme activity to result in a deleterious reduction in important muscle LCPUFAs in some individuals.

The results of this study show that (1) breast-feeding increases LCPUFAs in skeletal muscle phospholipid and (2) early development of a relatively lower percentage of LCPUFAs in skeletal muscle phospholipid, influenced by both type of feeding and genetic predisposition, is associated with higher fasting plasma glucose in young children. Such alterations in muscle membrane phospholipid FA saturation may influence the subsequent development of diseases associated with insulin resistance.

NOTE ADDED IN PROOF

Since submission of this report, Pettit et al[47] have published a study showing that Pima Indians who were exclusively breast-fed for the first 2 months of life have significantly lower rates of NIDDM in adulthood. Our current study provides a possible explanation for their findings.

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