ANALYSIS OF FAT, FATTY ACIDS, AND ALPHA-TOCOPHEROL IN MATURE HUMAN MILK AND RELATIONSHIPS TO DONOR DIET

by

Judith Rose Britten

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ABSTRACT

A pilot investigation was undertaken to determine the macronutrient, energy, fatty acid composition, and α-tocopherol content of individual mature human milks being donated to the Health Sciences Centre. Relationships between ad libitum maternal nutrient and energy intake and donor milk composition were also examined. Eighteen donors participated in the study. Each supplied approximately 30 mL milk expressed at the first feed of the morning and a dietary record kept for three days prior to milk expression.

Milk analysis revealed considerable variation among donor milks for many nutrients. Mean protein content was 1.19 g/dL. Levels ranged from 0.85 to 1.82 g/dL. Lactose was found to vary the least compared to other macronutrients. Concentrations ranged from 5.88 to 7.38 g/dL, and averaged 6.87 g/dL. Total fat content was highly variable, ranging from 1.07 to 6.27 g/dL. Mean fat concentration was 3.24 g/dL. Energy content strongly reflected fat concentration ($r = 0.99$, $p = 0.0001$).

A total of 16 milk fatty acids were identified by gas liquid chromatography. Saturated fatty acids constituted 41.82% of the total fatty acids. The largest proportion of the saturates was in the form of palmitic acid (C16:0). Oleic acid (C18:1) accounted for 87.51% of the monounsaturates and 36.70% of the total fatty acids. Total polyunsaturated fatty acids (PUFA) contributed 14.89% to the total milk fatty acids. Linoleic acid (C18:2) was widely variable, ranging from 7.41 to

22.89%. Mean C18:2 level was 13.12%. Linolenic (C18:3) and arachidonic (C20:4) acids averaged 1.03% and 0.35%, respectively. Three trans-fatty acids were resolved in the donor milks. The marked variability in fatty acid content became even more pronounced when percent fatty acids were converted to absolute amounts.

Alpha-tocopherol content of the milks ranged from 85.00 to 735.50 μg/dL. Mean α-tocopherol was 317.24 μg/dL. A positive linear correlation ($r = 0.74$) between α-tocopherol and total milk lipid was highly significant ($p = 0.0004$). The α-tocopherol content (μmol/L) also varied closely with the C18:2 content (mmol/L) ($r = 0.74$, $p = 0.0004$). Ten of the 18 milks failed to meet current recommendations that low-birth-weight infants receive 0.5 mg α-tocopherol/ 100 kcal. Seven of the 18 milks failed to meet the recommendation that such infants receive at least 0.67 mg α-tocopherol/ g C18:2.

Correlation analysis between maternal diet and total milk lipid failed to indicate any association. Correlations were significant only when fatty acid values were expressed as weight percents of total methyl esters. Significant positive correlations were observed between dietary carbohydrate (g/day) and milk C12:0 ($r = 0.54$, $p = 0.02$) and C14:0 ($r = 0.54$, $p = 0.02$). Total milk saturated fatty acids (Wt.%) also correlated with carbohydrate intake ($r = 0.50$, $p = 0.03$). Dietary carbohydrate was negatively related to milk C18:2 ($r = -0.53$, $p = 0.02$) and total milk PUFA ($r = -0.53$, $p = 0.02$). Both dietary C18:2 and PUFA intake negatively correlated with milk C16:0. Amounts of C18:2 in the diet (g/day) were positively related to percent C18:2 in the milks ($r = 0.61$, $p = 0.007$). These results must be interpreted with caution,

however, as the database used for dietary analysis was limited in its fatty acid data. Also, removal of one outlier from correlation analysis resulted in a 65% decrease in the R^2 value and in a non-significant association. Correlation between dietary PUFA, which represented a much better estimate of dietary intake, and milk C18:2 was very close to a 5% level of significance ($p = 0.054$). Relationships between total dietary saturates and monounsaturates and milk composition were not observed. Nutrient intake was also not found to be related to milk trans-fatty acids or α-tocopherol.

The variability found in the donor milks supports the current practice of nutrient supplementation for low-birth-weight infants fed human milk. The composition of human milk can only partially be explained by maternal intake. The physiology of diet and lactation is complex and must not be over- simpified.

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This thesis is dedicated to the loving memory
of Mémé Theriault

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Chapter I

INTRODUCTION

There has been a dramatic increase in the survival rate of low birthweight (LBW) preterm infants. These infants are most often born with low stores of essential fatty acids and vitamin E and a limited capacity to absorb fat. As such, LBW preterm infants present special problems in nutritional management.

Knowledge and technology centering around management of LBW preterm infants continues to grow as the survival rate increases. Controversy seems to remain, however, as to the best source of nutrition for these infants. Often when their own mothers' milk is unavailabe, LBW infants are fed mature donor milk which offers immunological advantages not found in formula (Chandra, 1982) and easier digestibility. This practice has become controversial as such milk often contains lower amounts of nutrients than found in preterm milk or formula. In addition, immunological properties have been found to be altered by storage and processing used by many milk banks (Nutrition Committee, Can. Paed. Soc., 1981). The Breast Milk Programme at the Health Sciences Centre (HSC) is unique in its use of fresh donor milk. In addition, samples from different donors are not pooled.

Previous reports have presented analyses of unpooled human milk which have generally been expected to be representative of a lactating mother's 24 h milk production (Jensen et al, 1980; Anderson et al,

1891). Comparison of 'fore' (low-fat) and 'hind' (high-fat) milks and studies on the diurnal effect on composition have been reported (Hall, 1979; Neville et al, 1984). Alternately, there are publications on the composition of pooled donor milks (Anderson et al, 1983; Schandler and Oh, 1980). The above studies have supplied composition data based on pooled milks or mean results from any given number of individual milks. Individual milks, however, are fed to LBW preterm infants at HSC and comparative compositions of individual samples of donor milks have rarely been reported.

The lipid content of human milk has been found to be highly variable while the fatty acid composition shows predictable patterns (Fomon, 1974; Hall, 1979). Human milk generally contains 8 to 10% polyunsaturated fatty acids and recent studies suggest that amounts are increasing due to increased dietary intakes of PUFA (Guthrie et al, 1977; Vuori et al, 1982). Concern has been expressed that these increased polyunsaturates will be found in donor milks and an excess could lead to increased vitamin E requirements of LBW preterm infants (Jansson et al, 1978; Nutrition Committee, Can. Paed. Soc., 1981). It is thus important to monitor the levels of both tocopherol and polyunsaturated fatty acids found in breast milks being supplied to LBW preterm infants.

The present study was undertaken as an exploratory project. The purpose of this research was to examine the composition of the mature donor milk being fed to LBW preterm infants at the HSC and to relate this to the ad libitum dietary intakes of the donors. The objectives of this study were:

1. To examine the macronutrient and energy variability of individual donor milks.
2. To determine the fatty acid composition and tocopherol content of individual milks and examine their variability.
3. To examine the relationship between ad libitum maternal intake and donor milk composition.

Chapter II

REVIEW OF LITERATURE

2.1 COMPOSITION OF HUMAN MILK

The composition of human milk has been studied in detail by many investigators for several decades. It is beyond the scope of this investigation to review all nutritional components found in mature human milk. This section will focus primarily on the total fat, fatty acid, and tocopherol contents reported for mature breast milks. Tables of relevant findings will be presented in these subsections to serve as summaries. Protein and lactose concentrations in mature human milks will be outlined briefly.

2.1.1 PROTEIN AND LACTOSE CONTENT

The proteins found in milk provide the infant with nitrogen for growth, enzymes, hormones, and immunological components (Fomon, 1974). Levels of protein in human milks are most often calculated by multiplying total nitogen (TN) content, usually determined by Kjeldahl analysis, by a nitrogen conversion factor.

Variation in the content of TN has been reported. Swedish mothers were found to have milk TN levels of 161 mg/dL (1.01 g protein/dL) at 1.5 to 3.5 months lactation whereas milk from well-nourished Ethiopian mothers had a mean level of 197 mg TN/dL (1.23 g protein/dL) at the same

period (Hambraeus et al, 1978). A protein level of 1.43 g/dL was reported for young American mothers (Lipsman et al, 1985) and a higher level of 1.62 g/dL was found for milks from Canadian mothers who expressed milk during their first month of lactation (Lepage et al, 1984). Milks expressed up to 29 days lactation were noted previously for their higher levels of protein (Atkinson et al, 1978; Anderson et al, 1981).

Lactose is the dominant carbohydrate in human milk and provides approximately 40% of the energy (Hambraeus, 1977). Milk lactose levels have been found to remain constant during the course of a single feeding and throughout the day (Hall, 1979; Worthingon-Roberts, 1985). Anderson et al (1981) reported, however, that lactose concentrations increase significantly over the first 29 days of lactation. They suggested that the low levels found in samples obtained early in lactation were a consquence of higher nitrogen concentrations found at this time. Both nutrients are part of the water solube fraction and thus nitrogen may effectively decrease lactose levels of the milk.

Lactose levels in spot donor milk samples were found to range from 7.1 to 7.8 g/dL with a mean content of 7.4 g/dL (Anderson et al, 1983). A mean concentration of 6.76 g/dL was reported for 25 teenage mothers compared to 7.40 g/dL for 20 adult women (Lipsman et al, 1985). Lactose levels ranged from 7.05 g/dL at 1 month lactation to 7.62 g/dL at 6 months lactation for these 20 adult women, providing further evidence of the reported stability of milk lactose concentrations beyond the first month of lactation.

2.1.2 FAT CONTENT

Fat, which may contribute over 50% of the calories of human milk, provides essential fatty acids, the precursors of prostaglandins, fat soluble vitamins, sterols and phospholipids and probably other as yet unidentified compounds of nutritional importance (Ferris and Jensen, 1984). The fat component is also thought to act as a satiety factor (Jensen et al, 1978). Most (approximately 98%) of the lipid in human milk consists of triacylglycerols contained in a membrane-bound fat droplet known as the milk fat globule (Neville et al , 1983).

Fat is generally considered the most variable of all milk components, both in chemical composition and amount. The level found in human milk has been reported to range from 0.4 to 10.0 g/dL (Packard, 1982). Most values, however, fall between 2.1 g/dL (Fomon, 1974) and 4.5 g/dL (Hambraeus, 1977).

Milk fat content is usually reported as averages or based upon pooled milk from any given number of samples from one or more women. However, fat contents are variable not only between women but also within samples from an individual woman (Packard, 1982; Hytten, 1954a,b). Inherent differences between individuals may help to account for the wide range of values reported. Indeed, Prentice et al (1981) have shown that some mothers consistently produce milks of high fat concentrations while others continue to produce milk of lower fat concentrations. Direct comparison of fat values from one study to another is also made difficult by differences in sampling techniques. Much of the variability undoubtedly relates to the fact that samples usually represent single

spot collections obtained at different times during the day rather than complete emptying of one or both breasts during a 24-hour period. Hytten (1954a) and Gunther and Stainer (1949) and more recent researchers (Hall, 1979; Neville et al, 1984; Saner and Yuzbasiyan, 1984) have confirmed that milk fat concentration changes within a single feed, the foremilk containing less fat (1 to 2 g/dL) than the hindmilk (4 to 7 g/dL). Fat concentrations also changes during the day, with the total amount of lipid rising from early morning reaching a plateau about midday. The mean increase is usually about 2.5-fold.

Differences in fat levels also reflect length of gestation and stage of lactation. Anderson et al (1981) reported a 20 to 30% higher concentration of fat in preterm milk as compared to full term milk collected on similar days. Gross et al (1980) and Simonin et al (1984) obtained results in agreement with this while Lemons et al (1982) found no effect of prematurity on fat concentration. Colostrum, secreted during the first few days of parturition contains significantly less fat than mature milk (Macy et al, 1953; DHSS, 1981; Sann et al, 1981). Analysis of mature milk with progressing lactation has revealed significant increases in fat levels from 3.9 g/dL at 2 weeks postpartum to 5.2 g/dL at 16 weeks postpartum (Clark et al, 1982). These results have been confirmed by Lemons et al (1982), Hibberd et al (1982) and Harzer et al (1983). On the other hand, Underwood et al (1970), Lauber and Reinhardt (1979), Prentice et al (1981) and Butte et al (1984a) reported no significant differences in fat content. Some studies have reported constancy during later lactation and weaning after an initial increase (Guerrini et al, 1981; Garza et al, 1983; Bitman et al, 1983).

Also affecting fat concentration is method of milk expression. Use of mechanical pumps produces samples of greater volume and fat concentration than manual expression (Garza et al, 1982; Green et al, 1982). Participants in some studies expressed milk from one breast while others obtained samples from both breasts. Differences have been reported in lipid content of left and right breasts during the same feeding period (Hall, 1979; Prentice et al, 1981; Neville et al, 1984).

The observed variations in fat concentations within a feed, between breasts, throughout the day and on subsequent days underlines the importance of carefully selecting and describing the method of sample collection when making comparative studies. The limitations of human subjects expressing total 24-hour milk productions from an ethical and practical viewpoint must also be recognized. Thus, the exact approach taken depends on a number of factors, the most important being the objective of the study (Picciano, 1984).

The fat concentrations in mature milk have been examined around the world (Table 1). Approximate comparisons can be made between the various areas keeping in mind difficulties posed by variations with sampling and with analytical techniques.

Samples from New Hebridean milk representing 2 to 24 months postpartum showed a mean of 3.8 g/dL as reported by Peters (1953). In India, Belavady (1978) found little variation as lactation progressed to 12 months, the highest mean being 3.3 g/dL at 2 to 6 months. A slightly higher mean of 3.9 g/dL was reported for milk fat content of rural West African women (Prentice et al, 1981). These results were based on 12 h

TABLE 1

Fat Content of Mature Human Milk From Different Geographical Regions*

Country	Fat (g/dL)	No. Samples	Reference
New Hebrides	3.8	51	Peters (1953)
India	3.3	?	Belavady (1978)
West Africa	3.9	120	Prentice et al (1981)
Pakistan	2.8	134	Underwood (et al, 1970)
United States	4.1	10	Clark et al (1982)
Britain	4.8 3.2	612 129	Kon and Mawson (1950)
Britain	4.2	5	DHSS, 1977
United States	3.2	172	Finley et al (1985)
United States	4.8	13	Butte et al (1984a)

* All samples collected at least 30 days postpartum.

collections whereas Peters (1952) and Belavady (1978) collected 4 h samples.

Jenness (1979) has suggested that racial differences may be among possible correlates of compositional differences in human milk. At first glance the variation in fat concentrations shown in Table 1 between Pakistani (Underwood et al, 1970) and American (Clark et al, 1982) data might infer a racial difference. Milk in both studies was expressed at 6 weeks postpartum. Sampling techniques were fairly similar. Lipid contents of Pakistani milks were obtained by the Roese-Gottlieb method which uses extraction with ethyl ether-ethanol. Samples from American women were analyzed by a modified Folch extraction with 2:1 chloroform to methanol ratio. This method of extaction has been highly correlated with the Roese-Gottlieb technique, the procedure recognized by the Association of Official Analytical Chemists (AOAC) as "official" (Hundrieser et al, 1984). The Kon-Mawson study (1950) in England however, gave a similar difference in fat contents (4.8 and 3.9 g/dL) between two large groups sampled in that country. More recently, reports from Britain have shown a mean of 4.2 g/dL (DHSS, 1977). Data from different regions in the United States revealed more variation, ranging from 3.2 g/dL (Finley et al, 1985a) to 4.8 g/dL (Butte et al, 1984a).

Significant to human milk banking systems and thus to the infants for which they are designed is the variability shown to exist in the fat content of milk from individual donors. Anderson et al (1983) reported a range of 1.3 to 7.9 g/dL in single spot samples from nine donors. Watts (1985) found a larger range of 0.4 to 8.6 g/dL in samples from 30 donors.

2.1.3 FATTY ACID COMPOSITION

The principal lipids in human milk lipids are triacylglycerols. Like those of other species, human milk triacylglycerols have been found to contain a bewildering array of fatty acids including straight chain saturated acids from C6:0 to C24:0, various branched-chain acids, monoenoic, dieoic, polyenoic, keto, and hydroxy acids (Jenness, 1979). Jensen et al (1980) compiled a list of 183 fatty acids that have been found or tentatively identified in human milk. Most were present in trace amounts. Analyses usually report only the principal acids which are those readily detectable in a gas-liquid chromatogram (GLC) of the mixture of esters derived from a sample of unfractionated fat. Long-chain polyunsaturated derivatives of linoleic (C18:2ω6) and linolenic (C18:3ω3) acids are rarely reported because of their small concentrations in human milk and because of their long retention time on GLC (Gibson and Kneebone, 1980). However, concern has been expressed that few workers attempt to analyze fatty acids beyond C18:3 possibly because many equate C18:2 with total essential fatty acids (Gibson and Kneebone, 1981). In fact, the derivatives of linoleic acid have high biological activity and have been suggested to be essential because of their structural role in human brain and nervous tissue (Crawford et al, 1974; Gibson and Kneebone, 1981).

Compilations of fatty acid composition of human milk lipids show the characteristic prominence of palmitic acid (C16:0) among the saturated acids and oleic acid (C18:1) among the unsaturated (Jensen et al, 1978; Jenness, 1979; Clark et al, 1980; Gibson and Kneebone, 1981; Lammi-Keefe and Jensen, 1984). Contrary to this however, Crawford et al (1977)

reported data from five East African mothers whose infants were failing to thrive. Their milk revealed the principal fatty acids to be of 10 to 14 carbon chain length rather than the 16 and 18 carbon chain length. Total lipid content was approximately 1% or less. The authors suggested that the mothers were undernourished and the high shorter chain fatty acid content was indicative of inadequate lipid synthesis from carbohydrate.

Much of the early data on the fatty acid patterns in human milk was obtained in the late 1940's and 1950's. Results from this period and fatty acid data through to the early 1980's are included in Table 2.

Macy et al (1953) obtained results from pooled 24 h samples from 200 women whereas the data of Insull and Ahrens (1959) represent six analyses of 24 h collections from one breast and 5 random samples. Similar patterns were reported by both groups. Eight fatty acids constituted close to 90% of the total identified fatty acid methyl esters. Unlike other later researchers, Macy et al (1953) did not separate linolenic acid (C18:3) which can constitute up to 2% of the pattern according to the data of Guthrie et al (1977).

Compared to the earlier reports, the results of Guthrie et al (1977) showed a lower proportion of lauric (C12:0) and myristic (C14:0) acids, comparable amounts of palmitic (C16:0), slightly more palmitoleic (C16:1), stearic (C18:0), oleic (C18:1) acids, and almost twice as much linoleic acid (18:2), at 14.4%. In the same year, the Department of Health and Social Security (DHSS, 1977) complied milk data from 96 British women. The fatty acid patterns from this data were similar to

TABLE 2

Fatty Acid Composition Of Mature Human Milk Fat†

Reference	No. Samples	Fatty Acids‡ 12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Macy et al (1953)	200	5.5	8.5	23.2	3.0	6.9	36.5	7.8	---	---
Insull and Ahrens (1959)	11	6.8	8.5	20.9	2.4	7.3	35.8	7.1	0.9	0.6
Guthrie et al (1977)	110	3.8	5.2	22.5a	4.1a	8.7	39.5	14.4	2.0	---
DHSS (1977)	96	5.4	6.2	23.7	4.0	9.5	35.5	7.2	0.8	---
Jansson et al (1981)	24	4.4	6.3	23.0	3.8	8.4	38.0	12.9	1.4b	0.4
Gibson and Kneebone (1981)	61	4.1	5.6	22.4	3.8	9.2	35.0	10.8	0.6	0.4
Harzer et al (1983)c	14	5.8	7.5	23.3	3.9	8.6	36.0	10.0	0.7	0.4

† Fatty acids are expressed in mean Wt.% of methyl esters of total fatty acids.
‡ 12:0 = lauric acid; 14:0 = myristic acid; 16:0 = palmitic acid; 16:1 = palmitoleic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 20:4 = arachidonic acid.
a n=99
b 18:3 + 20:0
c day 22 lactation

that of Guthrie et al (1977), with the exception of C18:2 and C18:3, which were much lower. Although not shown in Table 2(because of incomplete reporting of the data) a later study in Britain on 22 specimens of pooled banked human milk (Smith et al, 1984) reported higher concentrations of C18:2 and C18:3 (11.2% and 1.2%, respectively) than those reported by DHSS (1977).

The data of Jansson et al (1981) was obtained from 24 Swedish mothers representing the 12th day to 5th month of lactation. As with other findings, the most abundant essential fatty acid in the milk was C18:2 acounting for, in this study, about 13% of the total fatty acids. In Australia, Gibson and Kneebone (1981) found almost identical fatty acid patterns in the milk of 61 women. Saturated fatty acids constituted 46% of the total fatty acids in the milk lipids, C16:0 accounting for 49% of the saturates and 22.4% of the total acids. Unsaturated fatty acids accounted for 54% of the total fatty acids. Most of these were monounsaturated which contributed 41% to the total fatty acids. The mean C18:2 content was about 11%. The authors noted the variability in the levels of linoleic acid from one mother to another. The range of values for C18:2 was 4 to 26%.

Harzer et al (1983) extensively examined milk from British and German mothers. Thirty eight fatty acids were identified and four unknown compounds were also detected. Selected portions of their data in Table 2 represents 14 pooled samples from day 22 of lactation. They included arachidonic acid (C20:4) in their compilation of polyunsaturated fatty acids along with C18:2 and C18:3. Other research groups listed in Table 2 also identified C20:4 (Insull and Ahrens, 1959; Jansson et al, 1981;

Gibson and Kneebone, 1981). Approximately 0.4% C20:4 has been found in breast milk lipids as reported by these researchers except for the early report of Insull and Ahrens (1959) who listed a value of 0.57%.

Harzer et al (1983) converted selected fatty acids from weight percents of total fatty acid methyl esters to mg fatty acids/dL milk. Such calculations allow for determination of absolute amounts of fatty acids and indicate more clearly the variation between individual milk samples as differences in total lipid content are incorporated into calculations. For example, these authors reported C18:2 to be 9.7% at day 1 of lactation for their subjects and 11.8% on day 36. Presented as mg, these data are converted to 184.5 mg/dL on day 1 and 423.4 mg/dL on day 36.

2.1.3.1 Physiological Factors Contributing to Fatty Acid Variability

Unlike the total lipid, the fatty acid composition presented in Table 2 shows remarkable similarity from one study to the next. Insull and Ahrens (1959) demonstrated that despite marked changes in lipid content during the feed, the fatty acid composition of the lipid did not differ in either breast or during the course of the feed. Emery et al (1978) found a high correlation ($r = 0.99$) for nine fatty acids with cumulative amounts of fat from three subjects and identical fatty acid composition of milk from left and right breast from two subjects in the 2nd to 4th month of lactation. They concluded that fatty acid analsyes of human milk would be representative of an entire nursing period if samples were taken from either breast at any time during the nursing. Such findings for mature milk were confirmed by Hall (1979). Gibson and Kneebone

(1980) found similar results for colostrum from 19 subjects (day 3 and 5 postpartum) and 30 fatty acids. Guthrie et al (1977) reported less within-day than between-day variation in the fatty acid patterns of samples collected for 5 days at 6 to 12 weeks lactation. Harzer et al (1983) failed to find any diurnal changes in fatty acid composition of total milk lipids in 24 individual samples.

The fatty acid composition of human milk has been observed to vary with stage of lactation. For example, capric acid (C10:0), C12:0, and C14:0 have been shown to be lower in colostrum than in transitional or mature milk (Read and Sarrif, 1965; Jansson et al, 1981; Gibson and Kneebone, 1981; Bitman et al, 1983). Long-chain polyunsaturates, on the other hand, have been found to be higher in colostrum than in later milk (Crawford et al, 1978; Jansson et al, 1981; Gibson and Kneebone, 1981; Bitman et al, 1983; Harzer et al, 1983).

Once mature milk is being secreted, however, the fatty acid composition remains faily consistent. Hall (1979) demonstrated this from the analyses of 39 milk samples from three women at different weeks lactation (8 weeks, 5 to 13 weeks and 13 to 24 weeks postpartum). Similar patterns for the major fatty acids in the three milks were found. Total lipid content ranged from 1.6 to 5.6 g/dL. The fatty acid pattern was practically identical for the shorter-chain acids up to C18:3. Variation between the milk of the three women occurred primarily in the proportions of the long-chain derivatives of C18:2 and C18:3.

Earlier, Underwood et al (1970) reported a relatively constant distribution of fatty acids in breast milk lipids over a 6 week to 24

month period. They noted that after 1 year a small decrease in the percentage of palmitic (C16:0) and an increase in C14:0 occurred. These changes were not statistically significant because of considerable variations in patterns between individuals. Linoleic acid (C18:2) concentration remained virtually constant throughout the study period.

Lauber and Reinhardt (1979) studied milk composition of 33 women from the Ivory Coast over a 23 month period. They also observed a rise in C14:0 along with a decrease in C18:1 during the first 11 months. More recently, Clark et al (1982) found no significant change in total fatty acid compostion with time. The total lipid content, however, increased significantly over the 2 to 16 weeks postpartum period. Thus, while the fatty acid pattern remained uniform, the actual concentration of individual fatty acids increased with total lipid concentration.

Data on the fatty acid composition from milk of mothers delivering at term (FT) or prematurely (PT) as reported by by Lemons et al (1982) revealed no significant differences for C16:0, C18:0, C18:1, and C18:2 acids. Chappell and Clandinin (1982) and Bitman et al (1983) reported higher levels of C12:0 and C14:0 acids in PT milk compared to FT milk. Higher levels of long-chain polyunsaturated fatty acids (C20 to C22) were also found in PT milk compared to FT milk (Bitman et al, 1983). They noted that as the milk for both PT and FT groups reached the mature stage (day 21 to 84 of lactation), the amounts of fatty acids were remarkably similar apart from levels of medium chain fatty acids and oleic acid. There were no differences in C18:2 between mature PT and FT milk.

2.1.4 TRANS FATTY ACIDS

Although the predominant geometrical isomer in dietary fat is the cis-isomer, some dietary fats, including human milk fat, contain varying proportions of trans-isomers. Jensen et al (1978) listed trans-isomer contents of 2 to 18% for milk fatty acids. Beare-Rogers et al (1979) determined that most of the polyenoic acids in human milk reacted with lipoxidase and thus contained the cis,cis-methylene interrupted series of double bonds. The trans-isomers were found primarily in the C18:1 (elaidic acid) fraction. In a bulk sample containing milk from 20 mothers, Gibson and Kneebone (1981) found only a trace of trans-C16:1 while trans-C18:1 represented approximately 10% of the total oleic acid present.

The vast majority of analyses of fatty acids, including resolution of trans-isomers have been done using gas liquid chromatography (GLC)(Lammi-Keefe and Jensen, 1984). The reliability of identifying and quantifying the trans-fatty acids by the GLC procedure was evaluated by Aitchison et al (1977). They made comparisons of GLC alone and in conjunction with argentation thin layer chromatography (TLC) using pooled milk samples for the comparisons. In the samples analyzed by argentation plus GLC the saturated fractions were found to contain peaks with relative retention times close to those of trans-isomers analyzed by GLC alone. For trans-C14:1 and trans-C16:1 the results were higher by GLC alone than by argentation plus GLC. Most of the trans-fatty acids in the samples were C18:1t whereas the greatest percentage of errors were in the relatively minor trans components. The conclusion was made that the errors in determinations of total trans- fatty acids

by GLC alone were small and that the procedure was suitable for study of trans-fatty acids.

A relatively wide range of levels of trans-fatty acids have been found to exist in human milk. Beare-Rogers and Nera (1979) reported concentrations of 6 to 18% in Canadian milk lipids. Aitchison et al (1977) found 3.2 to 5.4% total trans-fatty acids in early morning samples and 2.7 to 4.3% in pooled early morning and evening milk. Clark et al (1980) tentatively identified trans-isomers of C16:1 and C18:1. The quantities of trans-C18:1 ranged from 2.5 to 7.5% and averaged 4.5%. Piccano and Perkins (1977) observed a smaller range for trans-C18:1 of 2 to 4%. The Aitchison study (1977) found similar ranges.

Craig-Schmidt et al (1984) reported the milk C18:1t content of eight women on self-chosen diets to be 4.8% of total fatty acids. An earlier report from Hundrieser et al (1983) found a slightly lower mean value for C18:1t from 10 observations at 3.4%. Chappell et al (1985a) reported 0.5 to 4.5% eliadic acid concentration. They also observed very low levels of trans-isomers of C18:1ω7, C18:1ω5, and C18:2ω6. Trans-C14:1 and C16:1t were not found. Reports from several other groups have shown these isomers to be present in the 0.08 to 0.16% and 0.13 to 0.6% range, respectively (Aitchison et al, 1977; Clark et al, 1980; Harzer et al, 1983; Finley et al, 1985a).

2.1.4.1 Physiological Factors Contributing to Trans Fatty Acid Variability

The variability of trans-isomers has received considerable attention. Aitchison et al (1977) did not find differences in the percentages of trans-C18:1 and total-trans between early morning and evening samples. Chappell et al (1985a) examined the effects of lactational stage and gestational age on the trans-fatty acid content of Canadian samples. There were no significant differences in milk trans-fatty acids between days 7, 16, 22, 28, and 37 of lactation. Samples representing 24 h production expressed on days 16 and 37 postpartum by 14 mothers were also not significantly different. Eliadic acid concentration was significantly higher for colostrum (day 4 of lactation) than on all subsequent days studied. Levels of trans-C18:1 were found to be higher in milk of mothers delivering at term (38 to 41 weeks of gestation) than in preterm milk (38 weeks of gestation). Interestingly, rate of maternal postpartum weight loss was found to influence trans-fatty acid levels in the milk. Concentrations of trans-C18:1 were higher in the 4 to 7 kg weight loss group than in the low weight loss group (0 to 2 kg) throughout the study period. There were no apparent differences for expressed milk volume, energy intake or food selection between the two groups.

2.1.5 VITAMIN E CONTENT

The primary physiological function of vitamin E in humans is that of an antioxidant protecting cell membranes against lipid peroxidation (Tappel,1972; Lammi-Keefe and Jensen, 1984). In the premature infant,

born with low tocopherol stores and poor absorption, the effect of vitamin E is to prevent or correct the often reported hemolytic anemia (Hassan et al, 1966; Oski and Barness, 1967; Melhourn et al, 1971).

There is an increased requirement for dietary tocopherol as the unsaturation of the fat in the diet is increased (Horwitt, 1960; Harris and Embree, 1963). Hemolytic anemia has been documented in premature infants receiving increased amounts of polyunsaturated fatty acids (Hassan et al, 1966; Panos et al, 1968; Williams et al, 1975). There is concern that an excess of these fatty acids could produce peroxidation and increase the vitamin E requirements of infants fed breast milk (Committee on Nutrition, Am. Acad. Ped., 1977, 1985; Jansson et al, 1978; Nutrition Committee, Can. Paed. Soc., 1981). In 1979, Atkinson stated that it was not known whether human milk had an increased tocopherol concentration in proportion to the increased levels of polyunsaturated fatty acids.

Four chemically related compounds (α-, β-, γ-, δ-tocopherol) as well as four corresponding unsaturated derivatives (α-, β-, γ-, δ-tocotrienol) exert vitamin E activity. Of these, α-tocopherol is the most active form (Fomon, 1974; Packard, 1982). Kobayashi et al (1975) reported α-tocopherol to be 83% of the total tocopherol content in 18 mature milk samples. Jansson et al (1981) found γ-tocopherol to range from 5.0 to 32.4% of the total tocopherol in mature milk. Mean γ-tocopherol was 21.5%. β-tocopherol constituted 2.1% of the total tocopherol. The nutritional significance of β- and γ-tocopherols in human milk seems, however, of little importance, since the <u>in vivo</u> activity of γ-tocopherol is probably only 10 to 15% that of α- tocopherol (Bieri and Farrell, 1976).

The vitamin E or tocopherol content in human milk has been determined by many investigators. However, its concentration has been found to vary markedly. Fomon (1974) reported a mean of 180 μg α-tocopherol/dL in mature milk. Levels of vitamin E ranged from 130 to 330 μg d-α-tocopherol /dL according to Packard (1982). The cause of this variation might be partly due to the determination procedure for tocopherol. Methods of analysis have included colorimetric, fluorometric, GLC, and most recently, high pressure liquid chromotography (HPLC). The majority of researchers in the 1940's through to the late 1960's used a colorimetric assay which posed problems of reproducibility, specificity of individual tocopherols, and interference from other compounds (Kobayashi et al, 1975; McMurray and Blanchflower, 1979). The HPLC has been proven to be sensitive and reproducible and is the method currently recommended (Lammi-Keefe and Jensen, 1984). Jansson et al (1981) and Vaisman et al (1985) found the HPLC to be equally effective on fresh and frozen milk samples.

As shown in Table 3, there is a wide range of values for the vitamin E content of mature human as reported by different laboratories. The table includes investigators who have assessed vitamin E content as either total tocopherol and/or α-tocopherol. Presented on a per gram milk lipid basis, the tocopherol variability is also noteworthy. Concentrations were found to range from a low of 37 μg total tocopherol/g lipid as reported in the early study of Quaife (1947) to a very high level of 324 μg α-tocopherol/g lipid found by Hashim and Asfour (1968).

TABLE 3

Tocopherol Content of Mature Human Milk

Reference	No. Samples	µg/dL milk	µg/g milk fat
Quaife (1947)	4 pools	110-150a	37-58
Harris et al (1952)	10	238(100-480)b	72(25-107)
	1 pool	141	49
Woodruff et al (1964)	20	175	--
		98c	--
Hashim and Asfour (1968)	5	1200d	324e
Herting and Drury (1969)	8	99(52-166)f	38
Kobayshi et al (1975)	3	180(84-310)g	74(47-126)g
		148(83-259)h	62(34-108)h
Lauber and Reinhardt (1979)	33	360	117i
Jagadeesan and Prema (1981)	17	450	--
Jansson et al (1981)	24	405j	123k
		310l	94m
Vaisman et al (1985)	7	106n	--

a) Values are total tocopherol unless otherwise indicated. b) Values in parentheses indicate range of tocopherol content. c) α-tocopherol, calculated from 56.2% of total tocopherol. d) α-tocopherol. e) Based on 3.7 g fat/dL. f) α-tocopherol. g) Contents of α-,ß-,γ-,δ-tocopherol summed. h) α-tocopherol, based on 2.4 g fat/dL. i) Based on 3.07g fat/dL. j) Calculated from µmol/L. k) Calculated from µmol/g. l) α-tocopherol,calculated from µmol/L. m) α-tocopherol,calculated from mg α-tocopherol equivalent. n) FT milk, at 5 weeks postpartum.

The earlier investigations reveal much more variability than those of the late 1970's and early 1980's. Quaife (1947) used a colorimetric assay on four pools of milk from 14 mothers one to eight months postpartum. Total tocopherol ranged from 110 to 150 μg/dL. Using the same procedure, Harris et al (1952) found an average of 238 μg total tocopherol/dL in 10 samples obtained from mothers 3 to 37 weeks lactation. Tocopherol values ranged from 100 to 480 μg/dL. A mean of 141 μg/dL in another composite sample obtained from a milk bank was also reported in this study. The authors speculated that this lower concentration may have been due to loss during a nine month storage period. Total tocopherol levels in the Woodruff et al (1964) study averaged 175 μg/dL, while α-tocopherol constituted about 98 μg/dL. Little information regarding assay methodology was provided by Hashim and Asfour (1968) who reported a very high level of α-tocopherol (1200 μg/dL or 324 μg/g lipid) in pooled milk from five mothers. In eight frozen fresh milk samples Herting and Drury (1969) found a considerably lower concentration of α-tocopherol at 99 μg/dL using thin-layer chromatography and colorimetric measurements. In 1975, Kobayashi et al analyzed milk samples from Japanese women at various stages of lactation. Thin-layer and gas-liquid chromatography as well as mass spectrometry were used to separately determine individual tocopherols and tocotrienols. Mean total tocopherol content expressed as the sum of each tocopherol was calculated at 180 μg/dL for three mature milks collected at 30 to 39 days postpartum. Levels of α-tocopherol ranged from 34 to 108 μg/g lipid. Average content was 62 μg/ g lipid or 148 μg/dL milk. Milk from Ivory Coast women had a mean vitamin E content of 360 μg/dL as reported by Lauber and Reinhardt (1979). Jagadeesan and Prema (1980) observed a

tocopherol concentration of 450 μg/dL in the milk of undernourished women from India who expressed milk after 3 months lactation. Using HPLC with fluorescence detection, Jansson et al (1981) determined the various tocopherols in milk from Swedish mothers. Mature milk had a mean total tocopherol level of 405 μg/dL, fairly similar to that for the Indian mothers' milk. The α-tocopherol content of the samples was 310 μg/dL. Vaisman et al (1985) also utilized an HPLC method. However, they found a much lower tocopherol concentration of 106 μg/dL in term milk expressed at 5 weeks lactation.

2.1.5.1 Physiological Factors Contributing to Vitamin E Variability

Several physiological factors have been found to affect vitamin E levels in human milk including length of gestation and stage of lactation. In two of three recent studies, preterm (PT) milk was shown to have a considerable higher tocopherol content than term (FT) milk. Gross and Gabriel (1985) determined the vitamin E content as α-tocopherol in 10 pooled milk samples from mothers 3 to 12 months lactation who had delivered at term and in milk from mothers who delivered infants less than 35 weeks gestation. The pooled FT milk contained 230 μg α-tocopherol/dL whereas the PT milk had higher quantities ranging from 850 μg/dL during the first postpartum week to 370 μg/dL during the sixth week. Initial α-tocopherol content of PT milk (1100 μg/dL) was not significantly different than FT milk α-tocopherol content (1500 μg/dL) in a recent Canadian study (Chappell et al, 1985b). However, on all subsquent study days (7 to 37) the α-tocopherol level was found to be significantly higher in PT milk. This observation of high

α-tocopherol concentration in PT milk parallels the reported higher total fat content of PT milk compared to that of FT milk (Anderson et al, 1981). Vaisman et al (1985) did not find significant differences in tocopherol content as a function of gestation. Tocopherol concentation was higher in FT than in PT milk one week after delivery but thereafter the rate of decline of tocopherol was greater in FT milk than in PT milk.

Correlations between stage of lactation and tocopherol content of breast milk have been noted. Jansson et al (1981) observed very high tocopherol levels in colostrum (26.1 μmol/L) as compared to either transitional (13.5 μmol/L) or mature milk (9.4 μmol/L). The difference between these milks was due to levels of α-tocopherol. Levels of ß- and γ-tocopherols were similar at all three stages. Earlier, Kobayashi et al (1975) found similar patterns in milk expressed 2 to 39 days postpartum from Japanese women. Lammi-Keefe et al (1985) attributed significant decreases in α-tocopherol in milk samples as lactation progressed from 2 to 16 weeks to both time postpartum and lipid content. Significant correlations between tocopherol concentration and week of lactation ($p < 0.05$) were also reported in the Vaisman et al (1985) study.

2.2 EFFECT OF DIET ON MILK COMPOSITION

Many factors are known to affect the composition of human milk. In previous sections, the diurnal effects as well as the effects of stage of lactation and gestation on composition were noted. Maternal nutrition has also been found to exert some effect on breast milk composi-

tion, although the regulatory factors that partition dietary nutrients into body stores or milk production are poorly understood (Motil et al, 1986).

This section will be limited to a discussion of previous findings regarding maternal diet effects on the fat and fatty acid composition of human milk. Within the subsection dealing with the effects of diet on fatty acid composition there will be a discussion of the effects of both manipulated and ad libitum diets.

2.2.1 DIETARY EFFECTS ON LIPID CONTENT

While total fat has been shown to be the most variable component of milk between women and within women (Packard, 1982), Atkinson (1979) stated that it does not seem to reflect the total amount of fat in the maternal diet. Data do exist which suggest that the milk fat content is decreased in poorly nourished mothers, although malnutrition probably has a greater effect on milk volume than composition (Whitehead, 1979). In 1979, Jenness suggested that racial differences may be among possible correlates of compositional differences in human milk. However, Jelliffe and Jelliffe (1978) complied data on fat contents of mature human milk from well- and poorly- nourished communities, suggesting that dietary practices and not racial differences per se may reflect differences in reported fat values. In general, lower fat concentrations were noted in more poorly nourished mothers. For example, Crawford et al (1974) found levels as low as 2.0 g/dL in milk from Tanzanian mothers whose children were at risk to marasmus and kwashiorkor. Diets in parts of Tanzania are predominately carbohydrate and fat may comprise only 7% of the calorie intake (Casey and Hambidge, 1983).

Extreme variation in the milk fat has been found to exist in both adequately and inadequately nourished regions. Ferris and Jensen (1984) have noted lower amounts of fat in milk of donors from socioeconomically deprived areas. Contrary to this, Marin et al (1984) found fat values of 3.7 g/dL in well nourished Brazilan women and 4.4 g/dL in malnourished women from the same region. Even gross malnutrition such as that seen in Karachi, Pakistan may be associated with milk fat levels greater than 3.5 g/dL (Lindblad and Rahintoola, 1974). The reported wide range of fat values and an overall mean of 3.9 g/dL in underpriviledged African women was similar to that found in Western societies (Prentice et al, 1981). Lauber and Reinhardt (1979) found milk fat levels of 3.1 g/dL from Ivory Coast women who consumed diets poor in protein, fat and calories to be within range of other published values for developing countries, but about 25% lower than those for Western countries.

Early studies have focused on diet manipulation to determine effects on milk fat levels. Deem, in 1931, found that increasing the fat content but not the energy of the diet increased fat levels in the milk of five well-nourished New Zealand mothers. High protein diets resulted in decreased milk fat concentrations. In a 1932 study by Shukers et al, one woman who consumed slightly less dietary fat and much more carbohydrate than the two other subjects produced a lower fat milk. Insull et al (1959) stated that daily fat production did not appear to be influenced by intakes of 0%, 40% or 70% fat in one mother's diet. Deb and Cama (1962) reported that protein supplementation of Indian women taking 1850 calories/day increased the fat content of their milk but not the milk yield or total protein content. Also in India, Karmarkar et al

(1963) studied the effects of supplementing diets of very poor women with extra protein and fat. Initial average intakes consisted of 1300 calories, 18 g fat and 21 g of protein. The authors found that milk fat concentrations increased from 3.8 g/dL up to a maximum of 4.7 g/dL which was obtained on an intake of 50 to 55 g fat.

Hall (1979) postulated that diurnal changes in the composition of mothers' milk follow the requirements of the infant and do not depend on the mothers' diet. The work of Harzer et al (1983) did not confirm this hypothesis. They found different diurnal patterns of milk lipids in the samples from German and English mothers and suggested this was likely due to differences in meal patterns of both groups. In 1984, Harzer and his coworkers reported on the short-term effects of diet on milk lipids. They used a cross-over design in which three subjects consumed a low fat - high carbohydrate diet (diet A) and a high fat - low carbohydrate diet (diet B). Both diets contained 2500 calories. In Diet A, protein contributed 20% of the energy, fat 15%, and carbohydrate 65% whereas diet B was comprised of 15% protein, 50% fat and 35% carbohydrate. Each mother remained on one diet regimen for 7 days and then changed to the alternate diet. Early morning milk samples were obtained one day prior to the study and on days 1, 3, 7, 8, 10 and 14. Total lipids were analyzed enzymatically as triglycerides (TG). Marked alterations in TG contents of the milk samples were observed for the different diet regimens. When the mothers were on the low fat diet (A), TG values were 4.1 g/dL but when on the high fat diet (B), TG levels averaged 2.6 g/dL.

Comparing milk composition of 25 American teenagers and 20 adult women, Lipsman et al (1985) did not find any significant relationship

between milk nutrient levels and maternal intake. Nutrient intakes for both groups were above recommended dietary allowances. Finley et al (1985a) also failed to find significant correlations between percent milk fat and any dietary variable in a study of well-nourished vegetarian and non-vegetarian mothers. Packard (1982) noted that two women on the very same diet may show wide variations in milk composition. She stated that in such circumstances, genetic influences would seem significant. Also, frequency of milk output, stress and lack of mammary gland stimulation prior to feeding are possible factors causing lower levels of fat or of milk yield.

In summary, early research on the effects of diet on milk lipid content revealed inconsistent results. Fat and protein supplementation of poorly nourished mothers did lead to increased milk fat levels in a few studies. However, both ad libitum intakes and diet manipulation of well-nourished mothers have generally failed to result in significant correlations with milk fat.

2.2.2 DIETARY EFFECTS ON FATTY ACID CONTENT

2.2.2.1 Effects of Manipulated Diets

The fatty acid composition of milk lipids has been shown to be very susceptible to dietary manipulation. Thiemich (1899, as cited by Insull et al, 1959), the first researcher believed to have altered human milk fat by dietary means reported changes in the iodine number of milk fat after feeding unsaturated fat to mothers. Fatty acids can be altered by changes in the type and amount of dietary fat, dietary carbohydrate or total energy intake (Casey and Hambidge, 1983). The widely quoted work

of Insull et al (1959) demonstrated this when they attempted to estimate how much of the milk fat was derived from dietary as opposed to endogenous sources. Four isocaloric (including one ad libitum), one hypocaloric and two hypercaloric diets were given to one subject, each diet consumed for at least four days duration. On the isocaloric diets, the fatty acids of the milk reflected those in the diet. For example, when lard constituted 40% of total dietary calories, C16:0 and C18:1 predominated in the milk at levels of 23.8% and 42.6%, respectively. When corn oil replaced lard in this diet, both C16:0 and C18:1 decreased considerably (to 12.7% and 28.7%) whereas C18:2 + C18:3 became the dominate fatty acids totalling 42.0%. The milk that resulted from the mother consuming the hypocaloric and essentially fat-free diet (1.4 g/day) had an increased proportion of C12:0 reflecting increased synthesis within the mammary gland. The two hypercaloric diets were strikingly different in that one contained 0% calories from fat whereas 70% of the calories of the other were derived from corn oil. When the excess calorie, fat-free diet was consumed, the percents of C12:0 and C14:0 doubled at the expense of C18:0, C18:1, and C18:2 + C18:3 indicating promotion of endogenous synthesis of fatty acids from carbohydrate precursors. On the other hand, the hypercaloric diet containing corn oil resulted in a milk containing almost five times the amount of C18:2 + C18:3, mainly at the expense of the saturated fatty acids compared to levels that resulted from the fat-free regimen.

The studies of Potter and Nestel (1976) and Mellies et al (1978, 1979) evaluated the effects of varying maternal dietary polyunsaturated and saturated fats on milk fatty acids. Both studies included a high

saturated fat diet (meat, cheese, milk, butter, eggs) and a high polyunsaturated fat diet (fats derived mainly from vegetable oils). The Mellies et al (1978, 1979) study also included an ad libitum dietary period which was found to be quite similar to the saturate-rich regimen. In both studies, it was reported that the linoleate content of the breast milk increased rapidly in response to increased levels in the diet. Also, on the polyunsaturate-rich diets, the milk content of C18:0, C16:0 and C14:0 were reduced. In the Mellies et al (1978) study, alterations in the fatty acid levels on the polyunsaturate-rich diet were more pronounced than in the study of Potter and Nestel (1976). Myristic acid decreased to 4.6% from 6.7%, C16:0 fell to 18.4% from 24.5% and C18:2 increased to 25.9% from 10.0% on this diet.

The milk of one woman ingesting an extremely high level of polyunsaturated fatty acids (300 mg of C18:2 and 45 mg of γ-18:3 per day in capsule form) for the treatment of multiple sclerosis was studied by Hall in 1979. Effects of dietary changes were also assessed. Milk samples were obtained prior to and three and six weeks after reducing intakes of saturated fatty acids and significantly increasing polyunsaturates by consuming 3 kg of green leafy vegetables and 600 g of fish per week. When compared to the milk composition of 39 samples obtained from three other women, C18:2 was twice as high and levels of C16:0 and C18:0 were considerably lower. The amount of arachidonic acid (C20:4ω6) was not significantly different from that found in the milk of the other women.

In the Harzer et al (1984) study reported earlier, mothers were advised to use vegetable oils and high linoleic acid margarines when

preparing the high-fat dietary regimen (Diet B). On diet B, the C18:2 content of the total lipids increased by more than 30%. On diet A (low-fat), levels of medium chain fatty acids were higher (C12:0 = 5.01%, C14:0 = 7.61%) than on diet B (C12:0 = 3.90%, C14:0 = 6.10%).

In 1984, Harris et al posed the question "Will dietary ω-3 fatty acids change the composition of human milk?". They found their answer by studying the milk of eight women who were given supplements of a fish oil concentrate rich in ω-3 fatty acids, including docosahexaenoic acid (DHA,C22:6ω3) in concentrations ranging from 5 g to 47 g per day for various lengths of time. The milk levels of long-chain ω-3 fatty acids rose from 0.2% to 1.1% after 4 weeks of consuming 5 g of fish oil per day and to 1.7% after comsumption of 10 g fish oil per day for 2 weeks. The milk of one mother who consumed 47 g of fish oil per day had levels of 4.8% of these fatty acids.

In general, the above studies have shown that milk fatty acid composition can change drastically when diet composition is altered. Fats containing large quantities of C18:2 such as the corn oil in the Insull et al (1959) study, produced C18:2-rich milks and suppressed saturated fatty acid levels. Diets rich in saturated fats tended to produce saturate-rich breast milks.

2.2.2.2 Effects of Ad Libitum Diets

The above studies have focused on some form of dietary manipulation to show the effects of dietary regimens on the fatty acid composition of human milk. However, Crawford et al (1976) and Hall (1979) have

stressed that such drastic altering of both quantity and quality of dietary fat and carbohydrate present an erroneous picture of normal variations in the fatty acid patterns of breast milk. The data shown in Table 4 displays variation of the major fatty acids in breast milk of women from various countries. Women were eating ad libitum.

The 11 American women in the Insull and Ahrens (1959) study were divided into two groups. Six women had been lactating for 12 days and were on a hospital diet consisting of 2200 to 3000 kcalories, 40% of which was derived from fat. The other five women, lactating for 35 to 180 days, were at home eating ad libitum. Precise food consumptions for either groups were not determined. Fatty acid composition was quite similar for all milk samples. Excluding the unusual occasion of a nursing mother gaining weight or on a very low fat diet, the authors of this study felt it was reasonable to assume that the major determinant of milk fatty acid composition is the nature and quantitiy of the maternal dietary fat intake. To observe such effects, however, there must be marked qualitative differences in maternal dietary fats from one meal to the next. In this study, where differences between milks of the 11 women consuming different meals were minute, the authors believed that the effect of such dietary variation would only be manifested in the breast milk within days, not within hours and as such, much of the expected irregularities in fatty acid composition patterns would be evened out by the time dietary fatty acids would be transported into the milk.

The data of Read et al (1965a) revealed just how different milk fatty acid patterns could be among women. As seen in Table 4, four different

TABLE 4

Fatty Acid Patterns Of Mature Milk Of Mothers Eating Ad Libitum, Percentages Of Total Fatty Acid (Wt.%)

		Fatty Acid†								
Reference	N	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Insull and Ahrens (1959)	6a	7.0	8.8	21.0	2.2	7.2	35.7	6.4	0.8	0.7
	5b	6.6	8.2	20.6	2.7	7.4	36.0	8.0	0.9	0.5
Read et al (1965a)	17c	16.5	12.7	17.5	1.4	6.0	39.6	1.0	---	---
	9d	5.0	8.5	26.7	4.5	7.9	35.4	6.3	---	---
	31e	7.2	9.7	22.2	3.0	4.3	33.5	15.2	---	---
	19f	5.8	8.5	24.0	2.0	7.1	39.6	11.3	---	---
Underwood et al (1970)	60	4.9	10.1	39.2	2.5	5.8	31.4	7.0	---	---
Sanders et al (1978)	4g	3.3	8.0	27.6	3.6	10.8	35.3	6.9	0.8	0.5
	4h	3.9	6.8	16.6	1.2	5.2	31.3	31.7	1.5	0.7
Lauber and Reinhardt (1979)	33	9.3	15.2	27.9	3.2	6.9	25.4	5.7	0.3	0.3
Vuori et al (1982)	33	4.2	7.3	25.3	3.9	10.3	29.6	11.4	---	---
Finley et al (1985)	77i	5.1	7.7	24.0	3.2	8.7	33.0	14.7	1.5	0.3
	79j	6.0	8.2	22.5	2.8	7.4	30.7	18.4	1.6	0.3
Kneebone et al (1985)	26k	8.9	10.0	26.9	4.2	4.1	30.8	8.8	0.3	0.5
	15l	5.3	6.5	22.0	3.0	5.2	33.8	17.0	0.4	0.6
	10m	8.4	8.9	25.8	3.4	5.0	30.7	10.7	0.3	0.6

† Wt.% of Methyl Esters

a) Inpatient subjects
b) Outpatient subjects
c) Tanganyikan subjects
d) Bedouin subjects
e) Jordanian subjects
f) Lebanese subjects
g) Omnivore subjects
h) Vegan subjects
i) Non-vegetarian subjects
j) Vegetarian subjects
k) Malay subjects
l) Chinese subjects
m) Indian subjects

ethnic groups of women were studied. Each group represented women eating ad libitum. Although the diet information obtained was only approximate, major differences were apparent between each group. Such differences were reflected in fatty acid content of their milks. The Tanganyikans averaged a daily intake of 2800 kcalories, of which 75% was derived from carbohydrates, mainly starches. Intakes of fat were very low, averaging 7%. Milk fatty acids reflected this with a C18:2 level of only 1.0% and relatively high levels of C12:0 and C14:0 at 16.5% and 12.7%, respectively. Such results were similar to those reported by Insull et al (1959) when they fed low fat, high carbohydrate diets to lactating women. Interviews with the Bedouin subjects in the study of Read et al (1965a) revealed a daily intake of approximately 1500 to 2000 kcalories, of which 40% was fat, predominated by animal products. These women produced a milk with a high C16:0 content (26.7%) and fairly low levels of C18:2 (6.3%). A similar pattern was observed for the milk from the Lebanese women who were well-nourished, consuming an average of 2350 kcalories, 51% of which was fat. Much of their fat intake (70%) was derived from olive oil. Aminal fat contributed 10% of their fat intake. The Jordanian women consumed a similar number of kcalories to that of the Bedouins but 30 to 40% of these kcalories were obtained from vegetable oils. Variation in dietary intakes of C18:2 between these two groups were reflected in their breast milks with levels of 15.2% for the Jordanian subjects and 6.3% for the Bedouins. It was concluded that on a relatively low intake of carbohydrate, the C18:2 content of the dietary fat was reflected in the fatty acid content of the milk. However, when dietary carbohydrate was increased and the level of fat lowered, as was the case for the Tanganyikan subjects, the nature of of the fat ingested had little effect.

Data from 60 Pakistani women were reported by Underwood et al (1970). The women resided in two areas within a 10 - mile radius of each other and were representative of poor to low - middle income groups. The information shown in Table 4 was obtained at 6 months lactation. Dietary evaluation revealed intakes of 1600 to 1800 kcalories, 70% of which were from carbohydrates. The 10 to 15% fat kcalories were derived mainly from ghee, a clarified butter fat which contains about 50% palmitic acid. In contrast to the Tanganyikan subjects in the Read et al (1965a) study, who consumed a similar percentage of calories from carbohydrate, high levels of C12:0 and C14:0 were not observed in the milk from these women. The Pakistani women consumed approximately 1,000 kcalories per day less than the Tanganiykan women which may have exerted some effect. The higher C16:0 (39.2%) and lower C18:1 (5.8%) levels compared to most other studies likely reflect the composition of the primary dietary fat, ghee. Linoleic acid levels of 7.0% were quite similar to those found in the milk of Bedouin women in the Read et al (1965a) study whose diets were largely of animal origin.

Table 4 depicts the effects of vegetarianism on the fatty acid composition of breast milk as studied by two groups of researchers. In general, both studies found milk composition to be a reflection of major dietary differences, with vegetarians consuming less animal fat and ominvore or non-vegetarians using lower levels of vegetable fat. The milk of non-vegetarian mothers in the studies of Sanders et al (1978) and Finley et al (1985a) contained larger amounts of C16:0, C16:1, C18:0 and C18:1 than that of vegetarians. All these fatty acids are known to be derived either directly or indirectly from maternal intake (Read et

al, 1965b). Vegetarian mothers produced milks with higher C18:2 and C18:3 levels than did non-vegetarian mothers, reflecting their higher vegetable fat intakes. Multivariate analysis in the study of Finley et al (1985a) revealed a significant positive correlation between milk C18:2 and vegetable fat intake and a negative correlation with total fat intake. Linolenic acid (C18:3) also increased as the intake of vegetable fat increased. Multivariate analysis also indicated that C18:0 was the only fatty acid that correlated significantly with dietary carbohydrate ($r = -0.22$, $p \leq 0.05$). This finding extends the results of Read et al (1965b) demonstrating that C18:0 decreases after consumption of a high carbohydrate meal. The Finley et al (1985a) study also found that C14:0 increased as dietary carbohydrate increased ($r = +0.15$) as shown by simple regression analysis.

The diets of the 33 Ivory Coast women in the Lauber and Reinhardt (1979) study consisted mainly of yam, plantain and cassava. Fat and protein accounted for only 10% and 15% of total kcalorie intake, respectively. Energy intake was slightly below recommendations for lactating women. Analysis of the milk fatty acids showed similar trends to that of milk produced by other women consuming large quantities of carbohydrates, with high levels of C12:0 and C14:0. Other striking differences were in the levels of C18:2, C18:3 and C20:4, their percentages being considerably lower than European or American data.

Vuori et al (1982) also studied the relationship between maternal diet and fatty acid composition of 33 women. These Finnish subjects were studied over two survey periods, the first at weeks 6 to 8 postpartum, the second at 17 to 22 weeks postpartum. Samples were expressed

manually before and after each feeding. Protein accounted for 16% of the total dietary energy while fat and carbohydrate contributed 39% and 45% to dietary energy, respectively. Linear correlation coefficients were calculated between parameters of maternal diet and fatty acid composition of the breast milk. Carbohydrate intake revealed a significant, although negative correlation ($n = 33$, $r = -0.38$, $p < 0.05$) with arachidic acid (C20:0) of the breast milk while positive correlations were observed for both protein ($r = 0.36$, $p < 0.05$) and fat intake ($r = 0.38$, $p < 0.05$) with breast milk C16:0. Total levels of saturated and polyunsaturated fatty acids in the maternal diet and breast milk were also positively correlated, as were the levels of C18:2 in the diet and breast milk.

Within the final study listed in Table 4 (Kneebone et al, 1985) the diets and breast milks of three distinctly different cultures were compared. Intakes of the mothers were based on food frequency and recall methods conducted on four mothers from each group. The Islamic Malay mothers excluded pork from their diets and mainly used a palm oil for cooking. The Chinese mothers consumed the largest amount of energy. Their diet was quite diverse as they included all types of meat as well as fish. Vegetable oil was their cooking fat. The Indian mothers were vegetarians, although some ate small quantities of fish. They commonly used a highly saturated cocount oil. Milk analysis showed Chinese milks to have less saturates than the other two groups, with lower amounts of C12:0, C14:0 and C16:0. The largest difference in composition between the three groups was in the level of C18:2. The Chinese mothers' milk had an average level of 17.0% while that from the Malay mothers was 8.8%

and from the Indian mothers was slightly higher at 10.7%. These differences were believed to have been caused from the distinct differences in diet.

Most evident in Table 4 is the trend for milk C18:2 levels reported in the 1980's to be considerably higher than those observed by authors of earlier studies. Guthrie et al (1977) made note of the changing food consumption patterns in the United States with dramatic shifts from animal to vegetable fats. They also recognized that while milk fat represents a variety of fatty acids provided by diet, depot fat and that synthesized in the mammary gland, changes in diet may be responsible for changes in fatty acid patterns in breast milk. Canadian food consumption patterns have also been changing and since 1950 there has been a marked shift to lower amounts of fats from animals and higher amounts from vegetable oils (Anderson et al, 1977; Watts, 1977; Danielson and Robbins, 1984). This represents shifts in the fatty acid composition of Canadian diets. The main vegetable oil source now in Canada is canola (Vaisey-Genser, 1983) and this may influence the fatty acid composition of Canadian mothers' breast milk.

In summary, the research discussed in this subsection revealed considerable variation in the fatty acid composition of breast milks from mothers eating ad libitum. Milk in these studies reflected distinct eating practices of mothers from many areas around the world. For groups of women consuming diets not drastically different from each other, as in the Insull and Ahrens (1959) investigation, milk fatty acid patterns were found to be quite similar. Diets high in carbohyrate and low in total fat generally revealed higher levels of milk saturates,

particularly C12:0 and C14:0, compared to diets containing higher levels of fat. Studies comparing vegetarians and non-vegetarians, whose intakes of C18:2 significantly differed, showed corresponding differences in milk C18:2 concentrations.

2.2.3 DIETARY EFFECTS ON TRANS FATTY ACID CONTENT

In the above section it has been shown that total amounts of fatty acids can be influenced by caloric intake and relative amounts of fat and carbohydrate in the diet. The effects of diet on trans-fatty acids have also been studied. In a 1983 publication, Hundrieser et al noted that trans fatty acids may comprise up to 7 to 10% of total calorie intake in some American diets. Beare-Rogers and Nera (1976) stated that the production of such acids during hydrogenation and their presence in the diet are associated with their appearance in breast milk.

An extensive study to examine the effects of ad libitum diets on milk trans-fatty acids was undertaken by Aitchison et al (1977). A total of 11 women, 4 to 8 months lactation, participated for a one week period recording dietary intakes and expressing milk samples either once in the morning or both in the morning and evening. For three out of the seven days duplicate meals were collected for laboratory analysis. Over the one week period nutrient intakes were found to vary considerably, not only between the mothers but also within individuals. The only trans-fatty acid to appear in all 11 diets was C18:1t, with levels ranging from 1.00 to 8.27 g/day. Based on visual observation by graphically plotting the results, the authors noted a relationship between diet and the milk for nine out of the 11 subjects. Correlation coefficients

between percentages of C18:1t and total trans-fatty acids in diet and milk were calculated. A significant correlation ($p < 0.01$) between diet and milk samples of the same evening was found. Correlations between grams of trans-fatty acids in the diet and percentages in milk were not significant, however.

Data presented by Harzer et al (1984) on the fatty acid composition of total milk lipids from one mother revealed little change in trans-fatty acids when she was on a low fat diet followed by a high fat diet. Trans-C16:1 and trans-C18:2 were evident in her milk the day prior to beginning the study but did not appear during the following two week experimental period.

In another cross-over study, Craig-Schmidt et al (1984) had eight women, 2 months postpartum, consume two 5-day diets, each identical except for sources of visible fats. One diet contained nonhydrogenated fats such as butter, corn oil and lard whereas the hydrogenated diet contained margarine, hydrogenated oils and shortenings. Analysis of these diets revealed a level of 9.72 g trans-C18:1 for the hydrogenated diet and 0.86 g trans-C18:1 for the nonhydrogenated diet. Early morning milk samples were obtained by manual expression. Results of the milk analysis showed a significant correlation ($r = 0.91$) with diet for trans-C18:1. A 12-fold higher level of C18:1t was found when the women were on the hydrogenated diet. Of all the fatty acids analyzed, C18:1t was the only one to be significantly different ($p < 0.01$) between dietary periods on every day. Small amounts of C16:1t were observed during the two dietary periods. Slightly higher amounts of C18:2tt and C18:2ct were found in milk when mothers were on the hydogenated diet.

Three types of margarines, each of different trans-fatty acid concentrations were fed to Canadian womem in a recent study by Chappell et al (1985a). Food intakes were recorded by the mothers for the 24 h period prior to expressing milk samples. Once again, it was demonstrated that the milk composition responded to the margarine cross-over. Milk C18:1 trans was altered significantly ($p < 0.001$) following consumption of low, medium or high C18:1 trans margarines.

2.2.4 DIETARY EFFECTS ON VITAMIN E CONTENT

In the report of Kon and Mawson (1950), it was revealed that amounts of many vitamins in human milk are influenced by proportions in the maternal diet. However, little information is available concerning dietary effects on the fat soluble vitamins in breast milks. Only one study was found that examined the effects of maternal diet on the vitmain E content of breast milk. Chappell et al (1985b) obtained milk samples during the first 6 weeks of lactation. Diet intake was recorded the day prior to milk collection day. Apparent relationships between dietary variables and levels of vitamin E in the milk were not found. Further to this report, Ali et al (1986) stated that their findings did not suggest dependence of milk vitamin E levels on maternal diet. Dietary intakes of vitamin E were not recorded and direct comparison of diet and milk variables were not made, however.

Chapter III

MATERIAL AND METHODS

Procedures involving milk sample, dietary and clinical information collection were approved by the Ethics Review Committee of the Faculty of Human Ecology and the Committee on the Use of Human Subjects in Research, Faculty of Medicine, University of Manitoba.

3.1 SUBJECTS

All subjects were donors to the Fresh Breast Milk Programme at the Health Sciences Centre (HSC),Winnipeg, Manitoba. According to programme protocol, each donor was screened by HSC for general level of health, health of family members, use of medications, age of their infant (preferably over six weeks), hepatitis B surface antigen, and cytomegalovirus.

Initial contact with the donors was made with the assistance of the coordinator of the Breast Milk Programme. A letter explaining the proposed research (Appendix A) and a consent form (Appendix B) were mailed to all donors by the coordinator. Signed consent forms were returned to HSC and subsquently forwarded to the investigator who then contacted each volunteer by telephone to arrange two interviews.

A total of 22 donors were interviewed. Four volunteers were unable to supply milk samples; one because of illness and three others because

of decreased milk supply on the day of collection. Thus, a total of 18 subjects were included in the study.

3.2 NUTRIENT INTAKE

Three day estimated food records were used to assess usual individual intakes. To help maximize reliability of the food records, subjects were shown how to measure food items during the initial interview using standard household measuring utensils and a 30 cm ruler. An example of a one day food record was left with each subject as was a set of general instructions (Appendix C) on how to complete diet forms. The importance of the donors not altering their normal dietary habits was emphasized.

The day immediately following completion of the three day food record, diet forms were reviewed in a second interview. Using food models (mounds, spoons, plates) and the ruler, portion sizes of all food items were reviewed and any revisions noted. Particular attention was given to the amounts and types of fats eaten. When possible, ingredient lists were received for homemade and combination foods.

When interview appointments were being set up, 12 of the 18 (66.7%) subjects stated that it would not be convenient to be interviewed during weekends. Thus, dietary information was collected on three consective weekdays only.

3.3 CLINICAL INFORMATION

Clinical information related to length of lactation, age, parity, use of vitamin/mineral supplements and changes in diet since their pregnancy was collected during the second interview (Appendix D).

3.4 MILK SAMPLING AND STORAGE

During the first interview each donor received a 50 mL plastic vial which was labelled with their subject code and milk expression date. Because milk samples were to be representative of that being donated to HSC, each donor was instructed to follow their 'normal' procedure used for expressing milk. Thus, both manual expression and breast pumps were used and milk was expressed from either one or both breasts. Only mature milk was received. All milk samples were expressed the day immediately following completion of the dietary record. The samples represented a mixture of 'fore'(low fat) and 'hind'(high fat) milk from the first morning feed. Approximately 30 mL milk samples were collected from each donor.

Subjects were instructed to refrigerate the samples after their milk was expressed for HSC, mixed and the appropriate portion poured into the vial provided. Samples were collected by the invesigator at the end of the second interview and transported on ice to the nutrition laboratory at the University of Manitoba for analysis. Immediately upon arrival at the laboratory, the samples were thoroughly mixed and proportional amounts divided into 15 mL vials with screw top lids, flushed with nitrogen, frozen and stored at below -20^{0}C until analysis.

3.5 MILK ANALYSIS

3.5.1 PROTEIN DETERMINATION

Total nitrogen content of frozen milk samples was determined by the micro-Kjeldahl method of the Association of Official Analytical Chemists(1980) with slight modications. To 30 mL digestion flasks containing 1 g titanium dioxide catalyst, 0.2 mL thawed milk, boiling beads and 2 mL concentrated sulfuric acid were added. After the samples became clear they were digested for 30 minutes.

The flasks were cooled and 3 to 5 mL water was added to dissolve the solids. The sample plus boiling beads were then transferred to the distilling flask which was rinsed three times with a minimum volume of water. Two drops methyl red-methylene blue indicator and 5 mL saturated boric acid solution were added to a 50 mL beaker which was placed under the condenser top with the top extending below the surface of the solution. Eight mL 50% NaOH was added to the still. Approximately 15 mL of the distillate was collected and titrated with 0.01N HCl until the endpoint.

Protein content of duplicate samples was calculated by the following formula:

Protein(g/dL)=Total Nitrogen(TN)(mg/dL) x 6.25/1000.

where TN(mg/dL)=(mL HCl x Normality x 14 x 100)/0.2 mL sample

3.5.2 LACTOSE DETERMINATION

Lactose content of duplicate frozen milk samples was determined using the phenol-sulfuric acid method for total carbohydrates (Barnett and Towab, 1957). To a 100 mL volumetric flask, 1 mL milk was added and diluted to 100 mL with distilled water. A 0.1 mL portion of this diluted milk was placed in a test tube and a 1/5000 dilution was prepared by adding 4.9 mL water. A 2 mL sample of this diluted milk was transferred to a 20 mL screw cap tube and 1 mL of 5% phenol was added.

Then, using a rapid-delivery pipet, 5 mL sulfuric acid was added. After allowing the capped tubes to stand for ten minutes at room temperature, they were quickly mixed on a Vortex mixer and allowed to stand a further thirty minutes. The absorbance was measured on a Bausch and Lomb Spectronic 20 spectrophotometer (Fisher Scientific, Winnipeg, Manitoba) at 490 nm against a water blank. The mean of two absorbance readings was used to estimate the lactose content of the sample from a standard curve.

3.5.3 FAT DETERMINATION

Total fat content of the milk samples was determined using a modified version of the Roese-Gottlieb extraction procedure (Dorea et al, 1981). A premeasured 1 mL frozen milk sample was added to a 20 mL screw cap tube. Then, 0.125 mL ammonium hydroxide, 1.0 mL ethanol, 2.5 mL ether and 2.5 mL petroleum ether were added, the tube being mixed on a Vortex mixer for one minute after addition of each reagent. The solution was allowed to stand until the upper layer cleared. This layer was then

transferred to a 20 mL weighed and tared vial. The extraction procedure was repeated a second and third time using 3.0 mL ether:petroleum ether (1:1 vol/vol). The upper layers were again given time to clear and then were added to the vial set on a warm hotplate. The solvents were allowed to evaporate completely.

The vials containing the extracted fat were dried in a vacuum oven at 80°C for 45 minutes, then cooled in a desiccator. The total lipid content of the sample was then determined gravimetically. Duplicate analyses were performed and the mean of the two measurements was used to calculate the fat content of the sample.

3.5.4 CALCULATION OF ENERGY CONTENT

Energy content of the milk samples was calculated using values representing the energy for combustion for milk products (Report of the Joint FAO/WHO Ad Hoc Expert Committee, 1973). The caloric values used were: 5.65 kcal/g for protein, 9.25 kcal/g for fat, and 3.95 kcal/g for lactose.

3.5.5 FATTY ACID DETERMINATION

The fatty acid composition of milk samples was determined using gas liquid chromatography (GLC). The lipids were extracted from duplicate premeasured 1 mL frozen samples according to the method of Folch and Lees (1957). Fatty acid methyl esters of the lipid extract were prepared using boron trifluoride as a catalyst, following the procedure of Metcalfe et al (1966). The methyl esters were evaporated to dryness

under nitrogen and 150 to 200 uL hexane were added. The fatty acid composition was analyzed on a 1700 Varian aerograph gas liquid chromatograph equipped with dual flame ionization detectors and a Hewlett-Packard Model 3380-S recording integrator. The fatty acids were resolved on a 8 ft. by 1/8 in. (2.44 m by 0.318 cm) stainless steel column packed with GP 3% 2310/2% SP-2300 on 100/120 Chromosorb W.AW. Using a one minute delay,the column temperature was programmed from 185°C to 220°C at 2°C/minute.

Trans-isomers were determined using a 20 ft. by 1/8 in. (6.10 m by 0.318 cm) stainless steel column packed with 15% OV-275 on 100/120 Chromosorb P(AW-DMCS). This column was run isothermally at 220°C. An eight minute delay program was used.

For both analyses, carrier gas flow rate was 30 mL/minute, the injector port temperature 230°C, detector port temperature 250°C, air and hydrogen flow rates were 250 mL and 25 mL/minute, respectively. Results were expressed as relative percent of each fatty acid.

3.5.6 CALCULATION OF GRAMS FATTY ACIDS PER DECILITER MILK

The fatty acid composition determined by GLC was expressed as weight percent of total methyl esters. To determine actual amounts of fatty acids in the milk, values were converted to grams fatty acid/dL milk. The following equation, adopted from Posati et al(1975) was used:

Wt.% FAME x F x (g lipid/mL milk) = g FA/dL milk

where FAME =fatty acid methyl esters

F is defined as the conversion factor determined using the equation:

F = (g FA/g FAME) x 1.005

The constant, 1.005, is the ratio of three times the average molecular weight of the component methyl esters to the average molecular weight of the milk triacylglycerols

FA = fatty acid

Since milk fat has a relatively large amount of low-molecular-weight fatty acids, a different conversion factor was applied to each fatty acid as listed in Appendix E.

3.5.7 VITAMIN E DETERMINATION

The vitamin E content of duplicate frozen milk samples was determined following an adapted method of McMurray and Blanchflower (1979). One mL of thawed and thoroughly mixed milk was placed into a 15 mL screw cap tube and 2 mL 2% ascorbic acid in ethanol was added. After heating at 70°C for three minutes, 0.5 mL 60% potassium hydroxide was added, the mixture heated for five minutes. The tubes were then cooled on ice and 2 mL deionized water along with 3 mL hexane were added, the tubes capped and placed on a Vortex mixer for 30 seconds. After standing for five minutes, the mixture was centrifuged for five minutes in an IEC International Centrifuge Model CS (International Equipment Company,Boston,Mass.), the top layer (hexane) removed to a 5 mL vial. The water/ethanol layer was then re-extracted with an additional 3 mL of

hexane and centifuged as before. The hexane layer in the 5 mL vial was evaporated in a 30°C water bath using nitrogen and when near dryness the second hexane extraction was added and evaporated to dryness. The vial walls were washed down with 200 uL ethyl ether followed by 200 uL methanol.

Using a 100 uL syringe, either 20 or 50 uL of the extract was injected into a Beckman 110A high-pressure liquid chromatograph (HPLC) equipped with a Beckman Model 160 UV detector set at 280 nm and a Hewlett Packard 3390A recording integrator set at 0.2 in./minute. A LiChrosorb RP-18 10 um 250 mm by 4.0 mm column and a Whatman Co:Pell ODS precolumn was used. The mobile phase used in the HPLC was methanol:water(97:3). The flow rate was 2 mL/minute.

A d-α- tocopherol standard (Fisher 6340, Winnipeg, Manitoba) was run in conjunction with the samples. The mean of two peaks associated with the elution of d-α-tocopherol was used to estimate sample content from a standard curve.

3.6 NUTRITIONAL ANALYSIS

Three day food records were coded for computer analysis. All quantities of both solid and liquid food items were converted into grams for analysis. The percent edible portion and weight per volume or size of the foods were determined from the U.S. Handbook #456 (Adams, 1975). Mean macronutrient intakes were assessed using the Nutrient Analysis Program (NAP) of the Department of Foods and Nutrition at the University of Manitoba. Data for the program was based on that of the 1983 Canadian Nutrient File of Health and Welfare Canada.

In an attempt to analyze the donors' diets for individual fatty acids, including linoleic acid, several computer based nutrient analysis programs were used. Firstly, food items were coded for the 1978 Nutrient Analysis Program (NAP) of the Department of Foods and Nutrition. Insufficent data for fatty acid analysis led to the development of three additional programs based on databases from Health and Welfare Canada (1980, 1981, 1983). Using the 1983 database, a total of 345 food items were coded for analysis. Linoleic acid values were available for only 85 (24.64%) of these foods. Values for other individual fatty acids were available for fewer food items.

To determine where the major deficiencies were in the database with regard to available fatty acid values, all foods were then placed into one of seven food groups. The food groupings found in the 1978 version of NAP served as a base for those used in this study. The number (and percent) of available values in the various food groups for C18:2, total saturated, monounsaturated, and polyunsaturated fatty acids and total tocopherol is outlined in Appendix F (Table 26). The only two groups that provided sufficient estimates of individual fatty acid intake were the milk and milk products groups and the fats and oils group. The bread and cereal group, a known source of fatty acids (Paul et al, 1980) did not contain any C18:2 values. The percentage of food items having total saturated, monounsaturated, and polyunsaturated fatty acid values, was, however, sufficient enough in all food groups to allow for making reasonable estimates of dietary intake.

The percent contribution of energy from fat, carbohydrate and protein was assessed. The percent contribution of energy, fat, carbohydrate,

protein, total saturated, monounsaturated, and polyunsaturated fatty acids from the seven food groups was also calculated. These food groups included: milk and milk products; meat, fish, poultry and eggs; breads, cereals, and nuts; fruits and vegetables; food fats and oils (butter, margarines, vegetable oils, salad dressings); snack and dessert items (candy, cakes, cookies, alcohol, carbonated beverages, fruit drinks); and a miscellaneous group which included condiments, spices, sugars, and commerical soups prepared with water.

3.7 STATISTICAL ANALYSIS

Means, standard errors of means, and coefficients of variabilty were calculated for milk and diet nutrient composition using the computer based programs in the Statistical Analysis System (SAS) (SAS Institute Inc., 1982-1986). Individual and group data was calculated for both milk and dietary variables. Pearson's correlation coefficents were determined for the association between donor variables and milk composition. Correlation coefficients were also calculated for the association between nutrient intake and milk composition. Regression analysis was used to further substantiate correlation analysis. Scatter diagrams were plotted to indicate non-significance between diet and milk variables. Student's t-test was calculated to determine differences in both milk and diet content collected at different periods. Correlations and t-tests were reported as significant if $p < 0.05$.

Chapter IV

RESULTS AND DISCUSSION

4.1 SUBJECTS

All subjects in this pilot investigation were donating breast milk to the Fresh Breast Milk Programme at HSC. Of the 22 mothers who had volunteered, 18 were included in the study. Ten subjects were interviewed during July, 1982 and eight donors were interviewed during a three month period from November, 1982 to January, 1983.

Clinical data on the mothers is summarized in Table 5. Subjects ranged in age from 21 to 34 years with a mean age of 26.7 years. All donors had breastfed their infants since birth. Length of lactation for the group was 31.7 weeks (approximately 7 months) at the time of milk collection. Subject 15 had been breastfeeding for the shortest period of time at 11 weeks, whereas subject 12 had been lactating for 71 weeks, the longest period in the sample. All but four of the donors (77.8%) were primiparous; 3 (16.7%) had two children while only one mother (5.5%) had three children.

For donation to HSC, 11 out of the 18 (61.1%) subjects expressed milk once a day, usually at the first feeding of the morning. Six out of the 18 (33.3%) usually expressed milk twice per day, in the morning before feeding their own infants and again in the evening. One subject (5.5%) consistently expressed milk four times per day for HSC. Most donors

TABLE 5

Summary of Clinical Information on 18 Donors

	MEAN	RANGE
Maternal Age (years)	26.7	21 - 34
Weeks Lactation	31.7	11 - 71
Parity	----	1 - 3

expressed milk by hand pumping rather than using a mechanical or electrical pump. The methods used by the subjects to collect milk samples for the present investigation were very much representative of those used for milk being donated to HSC. Additional information collected on the individual donors is presented in Appendix G (Tables 27 to 30).

A student's t-test was used to determine if subjects interviewed during the two time periods significantly differed with respect to clinical variables. No significant differences were found between donors contacted in the summer or winter months regarding age, length of lactation or parity.

4.2 NUTRIENT COMPOSITION OF MATURE DONOR MILK

The macronutrient and energy content of the 18 individual donor milks is presented in Table 6. Mean values, standard errors of the means and coefficients of variability were calculated for each nutrient. Results of the investigation revealed considerable variation among donors for many nutrients.

4.2.1 PROTEIN CONTENT

The protein content of the 18 individual milk samples is presented in Table 6. These protein levels, calculated from total nitrogen (TN) ranged from 0.85 g/dL to 1.82 g/dL. The mean protein concentration was 1.19 g/dL (190.5 mg TN/dL). This is slightly higher than the range of 1.0 g/dL to 1.2 g/dL reported by Lonnerdal et al (1976a) for mature breast milk. A mean protein level of 1.01 g/dL (161 mg TN/dL) in milk from 12 mothers 1.5 to 3.5 months postpartum was observed in that study.

Mean TN values for milk from well - nourished Ethiopian mothers at 1.5 to 3.5 months lactation was 197 mg/dL which corresponds to a protein content of 1.23 g/dL (Hambraeus et al, 1978). Lauber and Reinhardt (1979) found a protein concentration of 0.96 g/dL in milk obtained from Ivory Coast women. They suggested that this low level was due to differences in analytical methodology between comparison studies. Protein concentration was assayed by a Biuret method which did not measure non-protein nitrogen. Hibberd et al (1982) reported a range of 1.13 to 2.07 g protein/dL for mature milk from 10 mothers collected from day 8 to day 36 lactation. They used the Lowry colorimetric technique

TABLE 6

Macronutrient And Energy Content of Mature Donor Milk

Donor	Protein (g/dL)	Lactose (g/dL)	Fat (g/dL)	Energy (kcal/dL)
1	1.82	6.40	5.17	83.36
2	1.12	6.25	2.35	52.74
3	1.27	6.69	3.97	70.30
4	1.18	6.36	1.63	46.88
5	1.17	6.75	1.81	50.04
6	1.27	5.88	6.27	88.36
7	1.12	6.55	4.08	70.48
8	1.46	6.70	2.59	58.66
9	1.33	7.38	1.49	50.42
10	1.32	6.92	1.70	50.52
11	1.11	7.31	2.42	57.49
12	1.14	7.13	5.55	85.95
13	1.02	7.36	2.86	61.28
14	0.96	7.04	4.43	74.20
15	1.16	7.20	3.11	63.78
16	1.04	7.07	3.55	66.61
17	0.85	7.28	4.22	72.58
18	1.00	7.34	1.07	44.53
Mean	1.19	6.87	3.24	63.79
SEM	0.05	0.10	0.36	3.21
C.V.	18.24	6.47	46.66	21.35

as did Patton and Huston (1984) and Lipsman et al(1985) who reported mean values of 1.33 g/dL and 1.43 g/dL, respectively. Determination of true protein content by amino acid analysis gave a range of 0.8 g/dL to 0.9 g/dL (Lonnerdal et al, 1976b). Lepage et al (1984), using a modified Kjeldahl procedure, reported a higher nitrogen content of 259 mg/dL in milk from Canadian women who expressed milk during the first month of lactation. This value corresponds to a protein level of 1.62 g/dL, considerably higher than the mean concentration observed in the present study. This high level was not unexpected as milk protein has been shown to be higher during the first 29 days of lactation (Anderson et al, 1981). Anderson et al (1983) found the protein content to range from 0.6 g/dL to 1.2 g/dL for spot donor milks. Mean TN content, 190 mg/dL, was similar to that in this study and slightly lower than the 199 mg TN/dL found in donated pooled milk from mothers one week to eight months postpartum in the Schanler and Oh (1980) study. The high variability among individual samples reported by Anderson et al (1983), Schanler and Oh (1980) and Gross et al (1981) was also evident in the present investigation. The protein content of the milks was representative of previous findings for mature donor milks, both in their range of values and mean concentration.

Differences in mean protein content of samples collected during the summer ($n = 10$) and winter ($n = 8$) months were determined by a Student's t-test at $p = 0.05$. Protein level for summer milks averaged 1.31 g/dL while that for the winter milks averaged 1.04 g/dL. The t-test found these means to be significantly different ($t = 3.339$, $p = 0.004$).

4.2.2 LACTOSE CONTENT

The lactose content of the donor milk samples is shown in Table 6. The group mean concentration was 6.87 g/dL. As expected, the lactose content varied little from one donor to another. Concentrations ranged from 5.88 g/dL to 7.38 g/dL. This, however, is a wider range that that of 7.1 g/dL to 7.8 g/gL reported by Anderson et al (1983) for nine American spot donor samples. These samples had a mean lactose content of 7.4 g/dL, somewhat higher than the value observed in this study. Hibberd et al (1982) determined lactose content enzymatically in 10 samples beginning at day 8 of lactation. Values ranged from 4.33 g/dL to 6.44 g/dL over a 28 day period. The mean value was reported at 5.34 g/dL on day 36 of lactation. This wide range and lower mean level was probably due to the time period in which samples were obtained. Anderson et al (1981) noted increasing lactose concentrations over the first 29 days lactation. Lipsman et al (1985) also used enzymatic analyses to determine lactose levels of milk from American teenagers and adult women. They observed a mean of 6.76 g/dL over a six month period for milk from the teenage mothers and a mean of 7.40 g/dL for milk from adult mothers. Values of 7.0 g/dL to 7.6 g/dL were reported by Lonnerdal et al (1976a) for Swedish mothers 0.5 to 6.5 months postpartum. A similar lactose concentration to that found in the present study and to the milk from teenage mothers in the Lipsman et al (1985) study was observed in Ivory Coast milk samples collected after one month postpartum by Lauber and Reinhardt (1979). They reported a concentration of 6.74 g/dL using a modification of the phenol - sulfuric method similar to that utilized in the present study.

In summary, the range of lactose content for the donor milks in the present study was slightly wider than several previous reports for mature milks. Mean concentration was somewhat lower than in American and Swedish samples but similar to averages reported for milks from American teenagers and Ivory Coast women.

The t-test procedure used to determine differences in protein content of summer and winter milks was also used to test for differences in lactose content. A "t" value of -4.617, $p = 0.0007$ was found, verifying that the means of 6.58 g/dL and 7.22 g/dL for summer and winter milks, respectively, were significantly different. Pearson's correlation coefficient analysis did not find a significant relationship between protein and lactose although a negative trend seemed possible ($r = -0.443$, $p = 0.065$). An inverse relationship between protein and lactose concentrations has been noted (Anderson et al, 1981; Smith, 1985).

4.2.3 FAT CONTENT

Individual milk fat contents along with the group mean, standard error of the means (SEM) and coefficients of variability (C.V.) are presented in Table 6. A student's t-test was performed to test for differences in fat content between milks collected during the summer and winter months. No significant differences were evident between samples collected at the different time periods ($t = -0.402$, $p = 0.693$).

The mean fat content of the donor milks was 3.24 g/dL. This level is similar to the 3.25 g/dL found for 172 vegetarian and non-vegetarian mothers in the Finley et al (1985a) study. These mothers expressed milk

from one breast at the second nursing period of the day. The donors in the present study expressed samples at the first feeding of the day. A level of 3.2 g/dL was also reported by Belavady (1978) for Indian mothers, seven to 12 months lactation, who obtained samples nearly 4 h after the first feeding. Higher average fat levels of 3.8%, 4.5% and 3.9 g/dL were reported by Macy et al (1953), Hambraeus (1977) and Harzer et al (1983), respectively for mature milk. Samples in these studies represented 24 h collections.

Evident in Table 6 was the considerable variation in fat content among mothers. Values ranged from 1.07 g/dL for milk from donor 18 to 6.27 g/dL for milk from donor 6. Hundrieser et al (1984) reported a wider range of 0.5 g/dL to 8.0 g/dL for mothers at least four weeks postpartum who expressed milk with the aid of an electric breast pump. A smaller range of 2.74 g/dL to 5.57 g/dL for triglycerides was found for milk expressed on days 8, 15, 22, 29 and 36 by 10 mothers in the study of Hibberd et al (1982). Anderson et al (1983) collected nine spot donor milk samples at different times during the day to represent milk routinely donated to a milk bank. They found fat levels ranging from 1.3 g/dL to 7.9 g/dL with a mean value of 4.7 g/dL. This range was greater than that in the present study, which also used procedures to represent milk being donated to a milk bank. However, unlike the study of Anderson et al (1983), all samples for the present investigation were expressed at a specified time, that being the first feeding in the morning. Many researchers including Hytten (1954b), Hall (1979) and Spencer and Hull (1981) have noted a diurnal effect on fat content of human milk. Significantly less fat has been found in milk expressed

early in the morning (6 a.m.) compared to that during the rest of the day. For this study, donors expressed milk samples between 5 a.m. (donor 15) and 11 a.m. (donor 11) with the mean time of expression being 8:30 a.m. and thus could be expected to result in lower fat levels than would have been found had samples been taken later in the day, or for a complete 24 h expression.

The reported variability of human milk fat was confirmed in the present study. For the 18 milk samples in this study, the coefficient of variability (C.V.) for fat was 46.6%. This value was higher than that observed by Butte et al (1984a) who reported a C.V. of 28% for 21 subjects who expressed single, morning samples by breast pump or by Neville et al (1984) who found a C.V. of 27.9% for six subjects who either hand-expressed or obtained samples using a breast pump. A considerably lower C.V. (14.5%) than that in the present study was reported by Clark et al (1982) for samples obtained from 10 mothers. On the day of collection, their subjects were instructed to express milk from one breast using an electric breast pump. Samples were obtained during a morning and an afternoon feeding. Prior to analysis, equal amounts of these samples were pooled. A mean fat level of 3.9 g/dL at week 2 of lactation and 5.2 g/dL at week 16 was found. It was felt that the repeated measurements and the rigid sampling procedure were factors that led to the decreased lipid variation.

Diurnal variation is only one factor among many that have been correlated with milk fat concentrations. Pearson's correlation coefficients (r) were calculated for the association between milk fat levels and other factors previously reported to have an effect. These are shown in Table 7.

TABLE 7

Pearson's Correlation Coefficients (r) Between Milk Fat and Subject Variables

	r	Probabilty
Fat x Weeks Lactation	0.536	0.022
Fat x Parity	-0.035	0.891
Fat x Method of Expression†	-0.147	0.559

† Expressed by hand or breast pump.

There was a significant correlation coefficient (r = 0.536, p = 0.022) between milk fat and length of lactation. Other researchers have also observed higher milk fat concentrations as length of lactation increased (Clark et al, 1892; Hibberd et al, 1982; Harzer et al, 1983). However, Guerrini et al (1981) and Bitman et al (1983) observed fairly stable levels of milk fat during later lactation. The milk from the mothers in the present investigation was not studied over time, however, the correlation between fat level and weeks lactation would support the observations of Clark et al (1982), Hibberd et al (1982), and Harzer et al (1983). A correlation coefficient (r) of 0.536, p = 0.022 (Table 7) between milk fat and lactation length was found.

In 1981, Prentice et al reported parity of the mother to be a major influence on the level of breast milk fat concentration. Primiparous mothers had significantly higher levels than multiparous women, even after correcting for stage of lactation. Finley et al (1985a) observed

a negative correlation between milk fat and parity for vegetarian and non-vegetarian mothers (n = 177, r = -0.23). As shown in Table 7, the correlation between milk fat and parity in the present study was negative although not significant.

The association between method of milk expression (by hand or with the aid of a breast pump) and milk fat is also presented in Table 7. A negative, non-significant correlation was found. Garza et al (1982) observed a 25% higher concentration of milk fat obtained by electric pump than that of hand expressed milk. This difference, however, was not statistically significant. A similar finding was also reported by Green et al (1982) who compared fat levels (creamatocrit) obtained when mothers used manual expression, two brands of mechanical pumps and an electric pump.

4.2.4 ENERGY CONTENT

The gross energy content of the donor milks in this investigation was calculated from the measured levels of protein (6.25 x N), lactose, and fat using the formula:

$$\text{Kcal/dL} = \text{protein (g/dL)} \times 5.65 + \text{lactose (g/dL)} \times 3.95 + \text{fat (g/dL)} \times 9.25.$$

These factors are based on the heats of combustion for the individual nutrients. In 1981, Anderson et al found energy levels calculated using these factors averaged 97% of those determined by bomb calorimetry.

As shown in Table 6, the mean energy content of the donor milks was calculated to be 63.79 kcal/dL with values ranging from 44.53 kcal/dL for donor 18 to 88.36 kcal/dL for donor 6. The energy values for spot donor milk in the study of Anderson et al (1983) were highly variable as well. Concentrations ranged from 45 kcal/dL to 101 kcal/dL. The mean level was 73.1 kcal/dL, a value higher than that found in the present study. They determined the energy content by bomb calorimetry and found no significant difference when total energy was calculated from fractional analysis of protein, fat, and carbohydrate. The mean energy content for 10 mothers at day 36 of lactation in the study of Hibberd et al (1982) was closer to the mean content of the milks in the present study, at 61 kcal/dL. Bomb calorimetry was used to determine energy concentrations. Butte et al (1984a), also used the bomb calorimeter and reported an average energy level of 67.7 kcal/dL for 13 mothers from two to 12 weeks postpartum. For the 16 mothers at four months lactation in the Dewey and Lonnerdal (1983) investigation, the range in energy content was very large. Values were reported to range from 46 kcal/dL to 113 kcal/dL with a mean level calulated at 78.7 kcal/dL. The present study confirms the high variability of previously reported energy values for mature human milk.

It was reported by Lucas et al (1978) and confirmed by Lemons et al (1980) that the energy content of human milk is directly related to its lipid content. Garza et al (1983) also observed a significant correlation between fat concentration and total calories ($r = 0.83$). The regression equation, fat (g/dL) = 0.07 kcal/dL - 1.99, $r = 0.79$, $n = 126$, was calculated by Lemons et al (1980). Presented in Figure 1 is

the relationship found between total milk lipid and energy content of the donor milks. When plotted out in a similar manner as Lemons et al (1980), a correlation of $r = 0.99$ ($p = 0.0001$) was obtained. This was based on the regression equation fat (g/dL) = 0.11 kcal/dL - 3.79. Plotted with energy content being dependent on the fat level of the milk (Figure 2), a regression equation of kcal/dL = 34.78 + 8.96 x g fat/dL was found. Analysis of the relationship between protein and lactose and total milk calories revealed very low and non-significant correlations ($r = 0.164$, $p = 0.513$ and $r = 0.269$, $p = 0.280$, respectively).

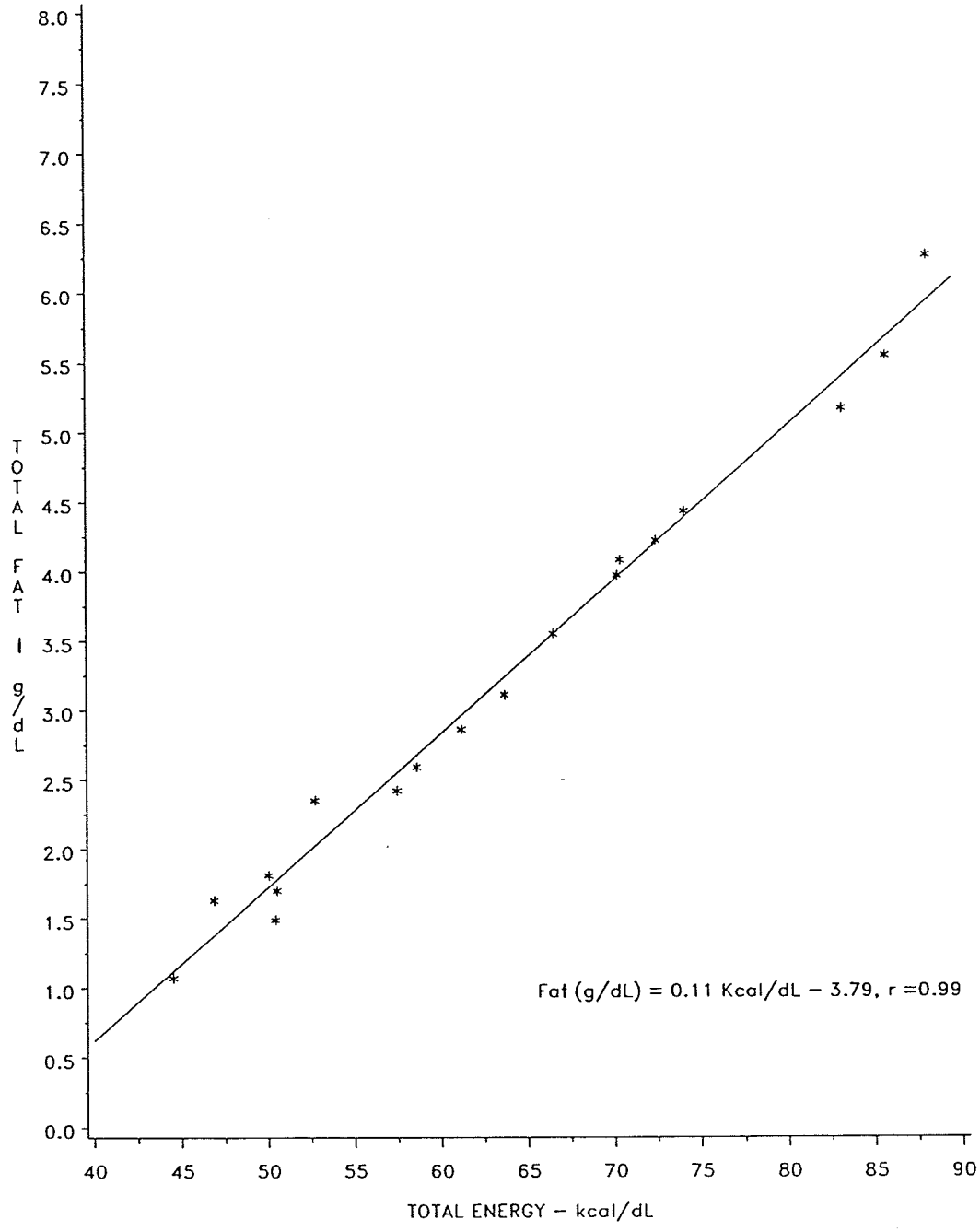

Figure 1: Relationship Between Fat (g/dL) and Energy Content (kcal/dL)of Donor Milk Plotted Similarly to Lemons et al (1980)

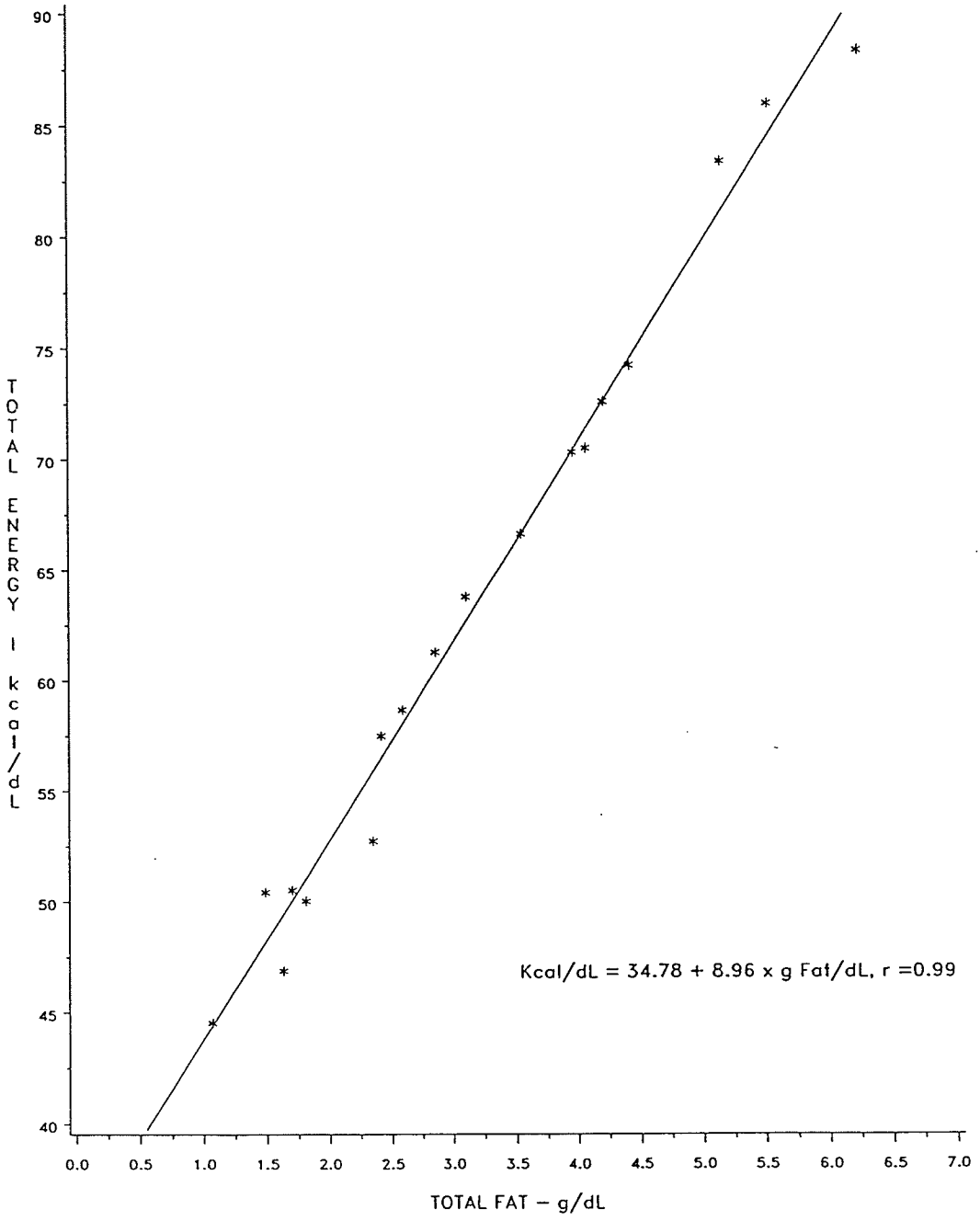

Figure 2: Relationship Between Fat (g/dL) and Caloric Content (kcal/dL) of Donor Milks with Caloric Level Dependent on Fat Concentration

4.2.5 FATTY ACID COMPOSITION

Results of fatty acid analysis for the 18 donor milks expressed as weight % of methyl esters are presented in three tables (Tables 8, 9, and 10). Gas liquid chromatography of the methyl esters showed 16 identifable fatty acids, which accounted for 98.65% of the total lipid content. In addition, there were up to six unidentified fatty acids in the donor milks. These contributed a maximun of 2.41% to the total fatty acids for any one donor. Over 93% of the lipid was present in eight major fatty acids from C12:0 to C18:3. The fatty acids found to vary the most from one donor to another, as indicated by coefficient of variability were: C8:0, C14:1, C20:1 and all the identified polyunsaturates including C18:2.

4.2.5.1 Saturated Fatty Acids

Saturated fatty acids as shown in Table 8 constituted 41.82% of the total fatty acids in the donor breast milk lipids with levels ranging from 34.97% for donor 4 to 49.56% for donor 9. Palmitic acid (C16:0), the largest constituent of the saturates, ranged from 15.78% to 24.48% of the total fatty acids. Medium chain saturates (C10:0, C12:0, and C14:0) accounted for 33.09% of the saturated fatty acids and 13.84% of the total fatty acids.

Levels of saturated fatty acids in the present study were found to be within previously reported ranges for mature breast milk. Total saturates accounted for 51% of total fatty acids in Indian mothers' milk at seven to 12 months lactation (Belavady, 1978). Levels of C12:0 and

TABLE 8

Saturated Fatty Acids of Mature Donor Milk†

	Fatty Acid								
Donor	C8:0	C10:0	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0	Total
1	0.06	1.72	9.14	9.66	18.06	0.43	5.77	0.65	45.49
2	0.07	1.64	6.14	6.55	20.17	0.48	6.32	0.61	41.98
3	0.13	1.91	7.96	9.12	20.18	0.50	8.81	0.71	49.32
4	0.00	0.70	3.30	3.69	19.30	0.50	6.77	0.71	34.97
5	0.02	1.50	7.36	7.38	18.30	0.48	7.86	0.70	43.60
6	0.00	1.69	7.91	6.76	15.78	0.38	4.85	0.72	38.09
7	0.08	1.21	3.71	4.12	16.83	0.90	6.96	1.32	35.13
8	0.05	1.23	5.26	5.12	16.09	0.36	6.82	0.77	35.70
9	0.00	1.61	5.76	8.70	24.48	0.71	7.61	0.69	49.56
10	0.05	1.07	7.16	7.96	18.11	0.50	7.54	0.76	43.15
11	0.08	1.29	5.82	5.81	15.98	0.37	5.46	0.59	35.40
12	0.06	1.08	4.59	8.32	23.48	0.70	7.79	0.88	46.90
13	0.08	1.64	7.10	7.98	21.68	0.57	7.66	0.41	47.12
14	0.08	1.32	4.76	7.16	21.94	0.74	8.54	0.76	45.30
15	0.08	1.01	4.91	4.47	18.53	0.38	8.34	0.56	38.28
16	0.11	1.44	5.48	7.14	21.24	0.52	6.46	0.79	43.18
17	0.08	1.91	3.36	3.96	19.09	0.52	7.22	0.80	36.94
18	0.00	1.31	4.99	5.26	22.76	0.49	7.36	0.53	42.70
Mean	0.06	1.40	5.82	6.62	19.56	0.53	7.12	0.72	41.82
SEM	0.01	0.08	0.39	0.44	0.62	0.03	0.25	0.04	1.18
C.V.	68.29	23.31	28.68	28.09	13.44	27.60	14.92	26.00	11.94

† Wt.% of Methyl Esters

C14:0 at 8.7% and 14.2%, respectively, were higher than those found for the donors' milk in the present study which averaged 5.82% and 6.62%, respectively. Lauber and Reinhardt (1979) observed even higher levels of C12:0 and C14:0 for Ivory Coast women, at 9.31% and 15.20%, respectively. Lower amounts of these fatty acids have been reported for Australian (Gibson and Kneebone, 1981) and American women (Clark et al, 1982; Bitman et al, 1983).

Concentrations of medium chain fatty acids have important implications for preterm and sick infants for whom most milk banks are designed. Such fatty acids have been shown to be more readily absorbed than longer chain fatty acids (Tantibhedhyangkul and Hashim, 1975). Total medium chain fatty acids found for term milk expressed during the first month of lactation by other Canadian women were reported to be 8.1% (Lepage et al, 1984). However, these authors defined "medium-chain" as saturated fatty acids containing six to 12 carbons. Therefore, C14:0 at 7.1% in their study was not included whereas a small amount of C8:0 (caprylic acid) was. Caproic acid (C6:0) was not identified in the milk samples in the present study. However, based on the definition used by Lepage et al (1984), a level of 7.28% was calculated for acids containing eight to 12 carbons.

Concentrations of the other saturated fatty acids presented in Table 8 were found to be fairly similar to other reported values. For example, C16:0 averaged 19.56% for the donor milks. The data of Belavady (1978) revealed a level of 23.9% for Indian mothers. Clark et al (1982) reported 21.2% for 10 American mothers at week 16 of lactation and Lepage et al (1984) found a level of 20.3% for the eight Canadian

mothers mentioned above. Stearic acid (C18:0) averaged 7.12% in the present study. A slightly higher value of 7.7% was found for term mothers at day 42 of lactation as reported by Bitman et al (1983). Values of 7.8% (Lepage et al, 1984), 8.4% (Jansson et al, 1981) and 9.2% (Gibson and Kneebone, 1981) have also reported.

4.2.5.2 Monounsaturated Fatty Acids

Total unsaturated fatty acids accounted for 56.83% of the fatty acids in the donor milks. Presented in Table 9 are the monounsaturated fatty acids of the individual milks. These comprised most of the unsaturates, contributing 41.94% to the total fatty acids. Oleic acid (C18:1) accounted for 87.51% of the monounsaturates and 36.70% of the total fatty acids. Levels of C18:1 ranged from 30.41% for donor 3 to 43.90% for donor 15.

Concentrations of all the identified monounsaturates were found to be quite similar to published values for mature milk. Many authors report values for palmitoleic acid (C16:1) and oleic acid (C18:1) only. Levels of C16:1 have ranged from 2.9% for the Canadian women in the Lepage et al (1984) study to 4.1% for American mothers as reported by Guthrie et al (1977). Mean C16:1 level in the present study was 3.83%, equal to that observed by Bitman et al (1983) for term mothers at day 42 of lactation.

Most published values for C18:1 have been found to vary little. Levels of C18:1 found in the present study were consistent with the data of Harzer et al (1983) for German and British women on day 22 of lacta-

TABLE 9

Monounsaturated Fatty Acids of Mature Donor Milk†

	Fatty Acid				
Donor	C14:1	C16:1	C18:1	C20:1	Total
1	0.63	3.53	33.69	0.96	38.81
2	0.72	4.47	34.70	0.52	40.41
3	0.59	2.91	30.41	0.60	34.51
4	0.42	4.48	40.14	0.81	45.85
5	0.57	3.34	39.13	0.77	43.81
6	0.47	3.82	36.19	1.45	41.93
7	0.63	3.53	31.42	1.69	37.27
8	0.29	3.58	42.50	0.02	46.39
9	1.26	5.01	32.77	0.47	39.51
10	0.71	3.06	39.46	0.91	44.14
11	0.35	2.80	39.90	0.69	43.74
12	1.14	4.67	32.68	0.65	39.14
13	0.67	4.02	37.14	0.55	42.38
14	0.97	3.89	31.87	0.87	37.60
15	0.33	2.96	43.90	0.65	47.84
16	0.69	3.56	35.42	0.86	40.53
17	0.38	4.07	39.23	1.00	44.68
18	0.51	5.24	40.08	0.61	46.44
Mean	0.63	3.83	36.70	0.78	41.94
SEM	0.06	0.17	0.95	0.09	0.87
C.V.	42.70	18.82	10.99	46.90	8.83

† Wt.% of Methyl Esters

tion. They reported a range of 30.4% to 43.9% with a mean level of 36.0%. Concentrations outside the typical levels have ranged from a low of 25.4% for milk from Ivory Coast mothers (Lauber and Reinhardt, 1979) to a high of 39.6% for milk from Tanganyikan mothers in the study of Read et al (1965a).

Other monounsaturates observed in the donor milks included C14:1 (myristoleic acid) and C20:1 (gadoleic acid) at 0.63% and 0.78%, respectively. Gibson and Kneebone (1981) found C14:1 and C20:1 in mothers' milk at concentrations of 0.43% and 0.06%. Levels of C14:1 and C20:1 at 0.50% and 0.81%, respectively were observed in the milk analyzed by Harzer et al (1983). The authors of these latter two studies also observed small quantities of C17:1 (?), C22:1 (erucic acid) and C24:1 (nervonic acid). Such monounsaturates were not identified in the donors' milk in the present study.

4.2.5.3 Polyunsaturated Fatty Acids

The polyunsaturated fatty acids (PUFA) of the donor milk samples are presented in Table 10. Total PUFA contributed 14.89% to the total milk fatty acids. Linoleic acid (C18:2) constituted 88.11% of total PUFA and 13.12% of the total acids whereas linolenic acid (C18:3) contributed a smaller but measureable proportion to the milk fatty acids (1.03%).

The range in C18:2 content in the donor milks was considerable. The milk of donor 9 had a level of 7.41% whereas that of donor 7 was 22.89%. Gibson and Kneebone (1981) reported an even greater range for C18:2 at 4 to 26% for 61 mature milk samples. The average content, at 10.8% was,

TABLE 10

Polyunsaturated Fatty Acids of Mature Donor Milk†

	Fatty Acid				
Donor	C16:2	C18:2	C18:3	C20:4	Total
1	0.33	12.29	1.10	0.34	14.06
2	0.38	14.95	0.91	0.34	16.58
3	0.26	13.09	1.48	0.27	15.10
4	0.38	17.04	0.53	0.30	18.25
5	0.33	10.22	0.94	0.26	11.75
6	0.22	15.17	2.75	0.46	18.60
7	0.73	22.89	1.68	0.62	25.92
8	0.23	14.55	0.97	0.35	16.10
9	0.54	7.41	0.75	0.29	8.99
10	0.29	10.56	0.86	0.08	11.79
11	0.22	18.89	0.61	0.33	20.05
12	0.55	9.77	1.08	0.33	11.73
13	0.43	8.22	0.41	0.35	9.41
14	0.60	13.04	0.67	0.33	14.64
15	0.27	12.02	0.80	0.22	13.31
16	0.35	12.58	0.86	0.53	14.32
17	0.41	15.21	1.68	0.52	17.82
18	0.42	8.33	0.45	0.43	9.63
Mean	0.39	13.12	1.03	0.35	14.89
SEM	0.03	0.93	0.13	0.03	1.01
C.V.	36.90	30.14	55.28	35.35	28.76

† Wt.% of Methyl Esters

however, lower than that in the present study. Smaller ranges of 9.2 to 14.0% for mothers milk at day 22 of lactation (Harzer et al, 1983) and 14 to 16% for pooled samples collected on day 42 of lactation from mothers who delivered at different gestational ages (Bitman et al, 1983) have been reported. These latter two ranges are among data that represent the constant fatty acid patterns (as compared to total lipid content) typically reported for pooled samples. The mean C18:2 level of 9.7% for milk from term mothers in the Canadian study of Lepage et al (1984) was close to the average levels found by Harzer et al (1983) for British and German mothers and mothers from Australia (Gibson and Kneebone, 1981). The average C18:2 concentration of 13.12% in the present investigation was closer to the 14.67% for American mothers' milk at 16 weeks of lactation in the Clark et al (1982) study but considerably greater than the 5.7% reported by Lauber and Reinhardt (1979) for milk from Ivory Coast women.

Also presented in Table 10 are the polyunsaturates C18:3 (linolenic acid) and C20:4 (arachidonic acid). The donor milks varied considerably in C18:3 with levels ranging from 0.41% for donor 13 to 2.75% for donor 6. This range of values was only slightly larger than the 0.48 to 2.07% reported by Harzer et al (1983) for mothers' milk at day 22 of lactation. They observed an average C18:3 content of 0.70% as did Lepage et al (1984) for eight term mothers who expressed milk during their first month of lactation. Mean C18:3 concentration for donor milks in the present study was 1.03%, identical to that found by Bitman et al (1983) for term mothers' milk at day 42 of lactation. Considerably higher levels of C18:3 have been reported for milk from both vegetarian (1.59%) and non-vegetarian (1.51%) mothers (Finley et al, 1985a).

Levels of C20:4 in the present study ranged from 0.08% for donor 10 to 0.62% for donor 7. The low C20:4 value found in the milk of donor 10 was very much below the smallest concentration reported by Harzer et al (1983) for mature milk. They observed levels ranging from 0.26% to 0.57% with an average level of 0.36%. Mean C20:4 level for donor milks in the present investigation was 0.35%. Higher levels of C20:4 at 0.60% have been reported for American mothers in the earlier works of Insull and Ahrens (1959) and Bitman et al (1983).

4.2.5.4 Fatty Acid Correlations

Pearson's correlation coefficicents (r) were calculated for the association between selected fatty acids listed in Tables 8, 9, and 10 and two subject variables. These are presented in Table 11.

The donors had been breastfeeding from 11 to 71 weeks, 31.7 weeks being the average length of lactation. The present investigation failed to reveal any significant association between the various fatty acids listed in Table 11 and weeks lactation. This was not surprising given the fact that milk samples were not collected over time. Fatty acids reported to decrease with time have included C16:0 (Underwood et al, 1970; Finley et al, 1985a) and C18:1 (Underwood et al, 1970; Lauber and Reinhardt, 1979). Myristic acid has been found by others to increase with length of lactation (Underwood et al, 1970; Finley et al, 1985a).

The only fatty acid in the donor milks found to be significantly related to parity was C14:0 ($r = 0.48$, $p = 0.04$). Finley et al (1985a) found a slightly lower, although also significant correlation between

TABLE 11

Pearson's Correlation Coefficients (r) Between Selected Fatty Acids and Subject Variables

	Subject Variable	
Fatty Acid	Weeks Lactation	Parity
C10:0	0.19 (0.23)†	0.45 (0.06)
C12:0	0.30 (0.15)	0.41 (0.09)
C14:0	0.35 (0.15)	0.48 (0.04)*
C16:0	-0.21 (0.41)	0.31 (0.22)
C18:0	-0.30 (0.23)	0.27 (0.28)
C16:1	-0.17 (0.50)	0.06 (0.80)
C18:1	-0.37 (0.13)	-0.41 (0.09)
C18:2	0.22 (0.38)	-0.30 (0.23)
C18:3	0.31 (0.21)	0.02 (0.94)
C20:4	0.03 (0.90)	-0.13 (0.60)

† Probability (p) in parentheses
* Statistically significant at $p < 0.05$

these two variables (r = 0.15). They also reported other fatty acids, C8:0, C10:0, and C12:0, to be significantly correlated with parity.

The milk saturated fatty acids, C8:0 through C14:0 have been shown to be synthesized de novo in the mammary gland by chain elongation from acetyl CoA. The fatty acids, C16:0 and C18:0, are derived primarily from the circulating fatty acids in the blood, although C16:0 can be synthesized within the mammary gland (Vorherr, 1974). Levels of C12:0 and C14:0 in the donor samples were most highly correlated with their immediate precursor(s) as shown in Table 12. This finding is consistent with that reported by Finley et al (1985a) for 172 milk samples from 57 women. Finley et al (1985a) also found levels of C8:0 to be negatively correlated with levels of each of the three fatty acids derived from it (C10:0, C12:0, and C14:0). They suggested this was an indication of differing control mechanisms for levels of C8:0 as compared to C10:0, C12:0, and C14:0. The present study did not find levels of C8:0 to be correlated with these three fatty acids.

The monounsaturated fatty acids, C16:1 and C18:1, have been shown to be derived in two ways; from the blood or through production in the mammary gland by desaturation of the respective saturated fatty acid (C16:0 or C18:0) via acyl desaturase (Smith and Abraham, 1975). Level of C16:1 in the donor milks was significantly correlated with the level of C16:0 in the milk ($r = 0.67$, $p = 0.002$) as shown in Table 12. Level of C16:1 was negatively correlated with level of C8:0 ($r = -0.57$, $p = 0.01$). Finley et al (1985a) reported a slightly lower correlation between level of C16:1 and C16:0 ($r = 0.53$, $p \leq 0.05$). They also observed negative associations between levels of C16:1 and that of

TABLE 12

Pearson's Correlation Coefficients (r) Between Level of Fatty Acid† in Donor Milks and That of Respective Precursor(s)

Milk Fatty Acid	Prescursor Fatty Acid C8:0	C10:0	C12:0	C16:0
C10:0	0.30 (0.23)‡			
C12:0	0.08 (0.75)	0.51* (0.03)		
C14:0	0.17 (0.51)	0.44 (0.06)	0.77* (0.0002)	
C16:1				0.67* (0.002)

† Weight percent of methyl esters
‡ Probability (p) in parentheses
* Statistically significant, $p < 0.05$

C12:0 ($r = -0.24$, $p \leq 0.05$) and C10:0 ($r = -0.18$, $p \leq 0.05$) but did not report any such finding with C8:0. Level of C18:1 in the donor milks was not significantly correlated with level of C18:0. It was, however, negatively correlated with C14:0 ($r = -0.56$, $p = 0.01$). Milk C18:0 levels in the study of Finley et al (1985a) were also found to decrease as C14:0 increased.

Several fatty acids are sequentially produced from C18:2 via chain elongation and desaturation including C20:4 (Gibson and Kneebone, 1984; Finley et al, 1985a). Level of C20:4 was positively related to C18:2 (r

= 0.48, p = 0.04). This was in contrast to that data reported by Gibson and Kneebone (1984) but in agreement with the data of Finley et al (1985a).

4.2.5.5 Absolute Amounts of Fatty Acids

Fatty acid data in the literature are usually presented as weight percent of total methyl esters. However, for the benefit of medical and nutritional personnel who require data on absolute amounts of fatty acids in a given volume of food, Posati et al (1975) derived conversion factors (Appendix E) and a mathematical formula for converting weight percents of methyl esters to grams fatty acid per 100 g food. The equation of Posati et al (1975) was adapted to calulate absolute amounts of selected fatty acids in the individual donor milks. This data is presented in Table 13.

Total fatty acids for milks in the present study, derived from Tables 8 to 10, were calculated at 3.04 g/dL, slightly lower than the 3.20 g/dL found by Lepage et al (1984) for eight term milks from Canadian mothers. Underwood et al (1970) reported amounts slightly higher than those in the present study, 3.1 g/dL at 9 months and 4.6 g/dL at 24 months.

The donor milks in the present study had an average amount of C12:0 of 0.18 g/dL which was higher than the 0.15 g/dL calculated for days 22 and 36 lactation for German and British milks (Harzer et al, 1983). These authors reported a similar C14:0 concentration of 0.21 g/dL as found in the present study but higher levels of C16:0, C18:0, and C18:1.

TABLE 13

Absolute Amounts (g/dL) of Selected Fatty Acids in Mature Donor Milks

	Fatty Acid†							
Donor	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:4
1	0.44	0.47	0.90	0.29	1.67	0.61	0.05	0.02
2	0.14	0.15	0.45	0.14	0.78	0.34	0.02	0.01
3	0.30	0.34	0.76	0.34	1.16	0.50	0.06	0.01
4	0.05	0.06	0.30	0.11	0.63	0.27	0.01	0.00
5	0.13	0.13	0.32	0.14	0.68	0.18	0.02	0.00
6	0.47	0.40	0.94	0.29	2.18	0.91	0.16	0.03
7	0.14	0.16	0.65	0.27	1.23	0.89	0.07	0.02
8	0.13	0.13	0.40	0.17	1.05	0.36	0.02	0.01
9	0.08	0.12	0.35	0.11	0.47	0.11	0.01	0.00
10	0.11	0.13	0.29	0.12	0.64	0.17	0.01	0.00
11	0.13	0.13	0.37	0.13	0.93	0.44	0.01	0.01
12	0.24	0.44	1.24	0.41	1.74	0.52	0.06	0.02
13	0.19	0.22	0.59	0.21	1.02	0.22	0.01	0.01
14	0.20	0.30	0.93	0.36	1.35	0.55	0.03	0.01
15	0.14	0.13	0.55	0.25	1.31	0.36	0.02	0.01
16	0.18	0.24	0.72	0.22	1.20	0.43	0.03	0.02
17	0.13	0.16	0.77	0.29	1.59	0.61	0.07	0.02
18	0.05	0.05	0.23	0.08	0.41	0.09	0.00	0.00
Mean	0.18	0.21	0.60	0.22	1.11	0.42	0.04	0.01
SEM	0.03	0.03	0.07	0.02	0.11	0.06	0.01	0.00
C.V.	64.60	61.78	47.62	45.88	42.95	57.26	102.17	66.06

† Wt.% of methyl esters converted to g fatty acid/dL milk according to the method of Posati et al (1975)

As shown in Table 13, the group means for C18:2 and C18:3 were 0.42 g/dL and 0.04 g/dl, respectively. Mean C20:4 was calculated at 0.01 g/dL. Harzer et al (1983) reported a value for C18:2 of 0.35 g/dL at day 22 lactation and 0.42 g/dL at day 36 lactation. Calculations for C18:3 were not reported. A level of C20:4 similar to that in the present study was obtained on day 22 lactation. Day 36 lactation revealed a level of 0.02 g/dL (Harzer et al, 1983).

Variability in total lipid content between milk samples can have a pronounced effect on the absolute amount of a fatty acid in 100 mL of milk. The large coefficents of variability (C.V.) for each fatty acid in Table 13 reflect the variability of both fatty acid (wt.%) and total fat content between donors' milks. For example, the C.V. for C18:2 in Table 13 was 57.26% but 30.14% when expressed in weight percent (Table 10). The range of values for the fatty acids expressed as weight percents did not reveal any consistent pattern between donors. However, when lowest and highest values of absolute amounts of fatty acids were calculated, several patterns became evident. Presented in Table 14 are the range of values for selected fatty acids expressed in absolute amounts (g/dL) and weight percent of methyl esters (Wt.%). In parentheses below each value is the corresponding donor identification number.

The milk of donor 18 contained the lowest amounts (g/dL) of all fatty acids. The milk of donor 6 had the highest absolute amounts (g/dL) for five out of eight fatty acids listed in Table 14. These patterns for donors 1 and 6 were due to the level of total fat in their milks (Table 6). The milk of donor 18 had the smallest amount of total fat (1.07

TABLE 14

Ranges of Variation for Selected Fatty Acids in Donor Milks

Fatty Acid	g/dL	Wt.%†
C12:0	0.05 - 0.47 (18)* (6)	3.30 - 9.14 (4) (1)
C14:0	0.05 - 0.47 (18) (1)	3.69 - 9.66 (4) (1)
C16:0	0.23 - 1.24 (18) (12)	15.78 - 24.48 (6) (9)
C18:0	0.08 - 0.41 (18) (12)	4.85 - 8.81 (6) (3)
C18:1	0.41 - 2.18 (18) (6)	30.41 - 43.90 (3) (15)
C18:2	0.09 - 0.91 (18) (6)	7.41 - 22.89 (9) (7)
C18:3	0.00 - 0.16 (18) (6)	0.41 - 2.75 (13) (6)
C20:4	0.00 - 0.03 (18) (6)	0.08 - 0.62 (10) (7)

† Weight percent of methyl esters
* Donor number in parentheses

g/dL), whereas that for donor 6 was the largest among the 18 donors at 6.27 g/dL. These observations have important implications for medical personnel calculating nutritional requirements for infants receiving milk from human donors. Absolute levels of readily absorbed medium chain fatty acids and essential polyunsaturates will be available in greater amounts in higher fat donor milks.

4.2.6 TRANS FATTY ACID COMPOSITION

Three trans-fatty acids were resolved in the donor milk samples as shown in Table 15. In 13 of the 18 samples, trans-C16:1 was separated from its cis-isomer. Levels of trans-C18:1 and trans-C18:2 were resolved in 14 and four of the 18 samples, respectively. Levels of total trans-fatty acids were also calculated. Total trans-fatty acids ranged from 0.00% to 5.86%.

Trans-C16:1 represented 0.24% of all the fatty acids identified on the methyl ester chromatogram. This level was slightly less than previously reported values. For example, Aitchison et al (1977) found a level of 0.78% in a pooled sample, Finley et al (1985a) reported a mean of 0.57% for 115 samples and a level of 0.39% was observed by Clark et al (1980) in the milk of 11 mothers, 4 to 48 weeks postpartum. Trans-C18:1 represented 89.30% of the total trans-fatty acids and 6.59% of total C18:1 in the donor milk samples. Levels ranged from 0.00% to 5.36% of total fatty acids and an average of 2.24% was observed. Smaller ranges of trans-C18:1 have been reported by Piccano and Perkins (1977) for 24 h pooled samples (2.1 to 4.0%) and Aitchison et al (1977) for milk from six mothers who expressed early morning samples (2.6 to

TABLE 15

Trans Fatty Acids of Mature Donor Milk†

	Fatty Acid			
Donor	C16:1t	C18:1t	C18:2t	Total
1	0.50	4.08	0.05	4.53
2	0.48	1.68	0.06	2.22
3	0.46	3.22	0.00	3.68
4	0.54	4.49	0.00	5.03
5	0.50	5.36	0.00	5.86
6	0.10	2.31	0.00	2.41
7	0.00	0.00	0.00	0.00
8	0.45	4.98	0.00	5.43
9	0.17	1.60	0.00	1.77
10	0.00	0.00	0.00	0.00
11	0.37	3.25	0.07	3.69
12	0.00	0.00	0.00	0.00
13	0.09	2.76	0.00	2.85
14	0.00	0.00	0.00	0.00
15	0.05	3.56	0.00	3.61
16	0.00	1.42	0.00	1.42
17	0.04	2.60	0.69	3.33
18	0.52	2.28	0.00	2.80
Mean	0.24	2.42	0.05	2.71
SEM	0.05	0.41	0.04	0.45
C.V.	95.99	71.31	334.76	70.41

† Wt.% of Methyl Esters

4.5%). Average levels of 4.5% (Clark et al, 1980), 3.4% (Hundrieser et al, 1983), and 3.7% (Finley et al, 1985a) have been found. Values for trans-C18:2 appeared in four of the 18 donor samples, ranging from 0.05 to 0.69%. Trans-C14:1 was not identified in any of the milk samples. Levels of 0.08 to 0.16% were reported for trans-C14:1 by Aitchison et al (1977) and 0.11% for samples expressed on day 22 lactation in the study of Harzer et al (1983).

4.2.7 ALPHA-TOCOPHEROL CONTENT

Considerable variation between the donor milk samples was observed for α-tocopherol. Table 16 presents α-tocopherol concentrations in the 18 individual samples. To allow for straight-forward comparison to previously published values, this data is expressed in both conventional units (μg/dL) and SI units (μmol/L).

Most notable in Table 16 was the degree of variation among milk samples. Levels ranged from 85.00 μg/dL (1.97 μmol/L) in the milk from donor 18 to 732.50 μg/dL (16.99 μmol/L) in the milk from donor 1. Earlier investigations have reported smaller ranges in both total and α-tocopherol content. Herting and Drury (1969) reported α-tocopherol levels of from 52 to 166 μg/dL in 8 frozen milk samples. Mature milk from Japanese women ranged from 83 to 259 μg/dL (Kobayshi et al, 1975). The extent of variability in α-tocopherol content in the donor milks from the present study was also evident by the large coefficient of variation (C.V.). The C.V., 53.28%, as shown in Table 16 was considerably larger than the 32% reported by Vaisman et al (1985) for milk from 23 Israeli mothers at least four weeks post delivery.

TABLE 16

Alpha-Tocopherol Content of Mature Donor Milks

Donor	C U † µg/dL	S I Units µmol/L
1	732.50	16.99
2	350.00	8.12
3	287.50	6.67
4	145.00	3.36
5	192.50	4.47
6	592.50	13.75
7	460.00	10.67
8	242.50	5.63
9	165.00	3.83
10	117.50	2.73
11	245.25	5.60
12	190.00	4.41
13	347.50	8.06
14	362.50	8.41
15	363.75	8.44
16	417.75	9.69
17	417.50	9.69
18	85.00	1.97
Mean	317.24	7.36
SEM	39.84	0.92
C.V.	53.28	53.28

† C U = Conventional Units

Mean α-tocopherol content was 317.24 μg/dL or 7.36 μmol/L. Compared to several studies of the 1960's and 1970's which reported values for α-tocopherol, the concentration in the present study was decidedly greater. For example, presented in Table 3 are the results of studies by Woodruff et al (1964) who reported a mean of 98 μg/dL for the milk of 20 mothers six weeks postpartum, by Herting and Drury (1969) whose eight samples had a mean content of 99 μg α-tocopherol/dL, and by Kobayshi et al (1975) who observed mature Japanese samples to have an average α-tocopherol content of 148 μg/dL. Slightly higher levels of total tocopherol, 360 μg/dL, were reported by Lauber and Reinhardt (1979) for milk from Ivory Coast women. The data of Hashim and Asfour (1968) revealed a very high level of α-tocopherol at 1200 μg/dL in the milk from five women. They used a colorimetric method of analysis which, in later studies, was found to include other reducing substances that could result in over estimated values. Quite similar to the average α-tocopherol content in the present study was the 7.2 μmol/L (310 μg/dL)observed in the milk of 24 Swedish mothers collected from the 12th day to the 5th month postpartum (Jansson et al, 1981).

Outlined in statements by both the American Academy of Pediatrics (1977, 1985) and the Canadian Paediatric Society (1981) was the recommendation that formulas designed for low-birth-weight infant provide a minimum of 0.7 IU vitamin E (0.5 mg α-tocopherol equivalents) per 100 Kcal. If the donor milks in the present study had been pooled as a composite sample, they would have met this recommendation (0.497 mg α-tocopherol equivalents/ 100 kcal). However, when α-tocopherol equivalents/100 Kcal were calculated for the individual samples, 10 out

of the 18 (55.6%) failed to meet it. This data is presented in Appendix H (Table 31).

4.2.7.1 Ratios of α-Tocopherol to Total Lipid and Linoleic Acid

Presented in Table 17 are α-tocopherol/lipid and α-tocopherol/ linoleic acid ratios found for mature human milk in previous studies and in the present investigation. The ratios from the present study were calculated from mean α-tocopherol content (Table 16), total lipid content (Table 6), and absolute amounts of linoleic acid (g/dL) (Table 13). The ratios are expressed in α-tocopherol equivalents (T.E.)/ g total lipid and α-tocopherol equivalents (T.E.)/ g linoleic acid (where 1 α-tocopherol equivalent = 1 mg d-α-tocopherol). Ratios for the individual milk samples from the present study are shown in Appendix H (Table 32).

A correlation coefficient (r) of 0.74 ($p = 0.0004$) between α-tocopherol content and total lipid levels was found for the donor samples in the present study. Jansson et al (1981) reported a smaller correlation of 0.52 ($p < 0.01$) between total tocopherol and total milk lipid content, whereas in the earlier study of Harris et al (1952), a correlation of 0.82 ($p \leq 0.05$) was observed.

As shown in Table 17, the earlier studies of Herting and Drury (1969) and Kobayshi et al (1975) found lower α-tocopherol/ lipid ratios than that calculated for milk samples in the present study. An α-tocopherol/ lipid ratio of 0.10 was found for the donor samples. This is identical to that reported by Jansson et al (1981) for 24 mature samples from Swedish mothers.

TABLE 17

Comparison of Ratios of α-Tocopherol Equivalents (T.E.)/Total Lipid and α-Tocopherol Equivalents (T.E.)/Linoleic Acid in Mature Milks

mg α-T.E.	Reference
Per g total lipid	
0.04	Herting and Drury (1969)
0.06	Kobayshi et al (1975)
0.10	Jansson et al (1981)
0.10	Present Study
Per g linoleic acid	
0.50	Herting and Drury (1969)
0.79	Jansson et al (1981)
0.27	Gross and Gabriel (1985)
0.85	Present Study

The requirement for vitamin E, particularly α-tocopherol, in the small preterm infant has been shown to be higher than that for the term infant due to poorer absorption of the vitamin (Committee on Nutrition, Am. Acad. Ped., 1977, 1985). Vitamin E deficiency may be exacerbated by several factors including high intakes of polyunsaturated fatty acids (PUFA). Higher intakes of PUFA (C18:2), in turn, increase the vitamin E requirements of infants fed breast milk (Dallman, 1974). Therefore, the ratio of vitamin E to PUFA (C18:2) should be noted when measurements of vitamin E in human milk are made.

The α-tocopherol content of the donor milks (μmol/L) varied closely with the linoleic acid content (mmol/L) ($r = 0.748$, $p = 0.0004$) (Figure 3). A similar correlation ($r = 0.70$, $p < 0.01$) was reported by Jansson et al (1981) for mature milk. Chappell et al (1985b) failed to find such a relationship in serial milk samples (both term and preterm milk) obtained during the first six weeks of lactation. When linoleic acid was expressed as a percentage of total fatty acid content in the present study, it was not correlated to α-tocopherol content ($r = 0.324$, $p = 0.189$).

The American Academy of Pediatrics (1977, 1985) has recommended that low-birth-weight infants receive at least 1.0 IU of vitamin E per gram of linoleic acid or 0.67 mg α-tocpherol (where 1.49 IU vitamin E = 1 mg d-α-tocopherol (Health and Welfare Canada, 1983). The Nutrition Committee of the Canadian Paediatric Society (1981) has reiterated this recommendation. Presented in Table 17 are several α-tocopherol/ linoleic acid ratios found for mature human milk including that calculated for the donor milks in the present study. The smallest of these, 0.27, reported by Gross and Gabriel (1985) was for 10 pooled milk samples. The ratio observed for the present study, 0.85, was slightly higher than the 0.79 found by Jansson et al (1981) for Swedish milks. The milks in two of the four studies listed in Table 17 exceeded the recommended 0.67 mg α-T.E./ g C18:2. As seen in Appendix H (Table 32), seven of the 18 (38.8%) individual milks in the present investigation failed to meet this recommendation.

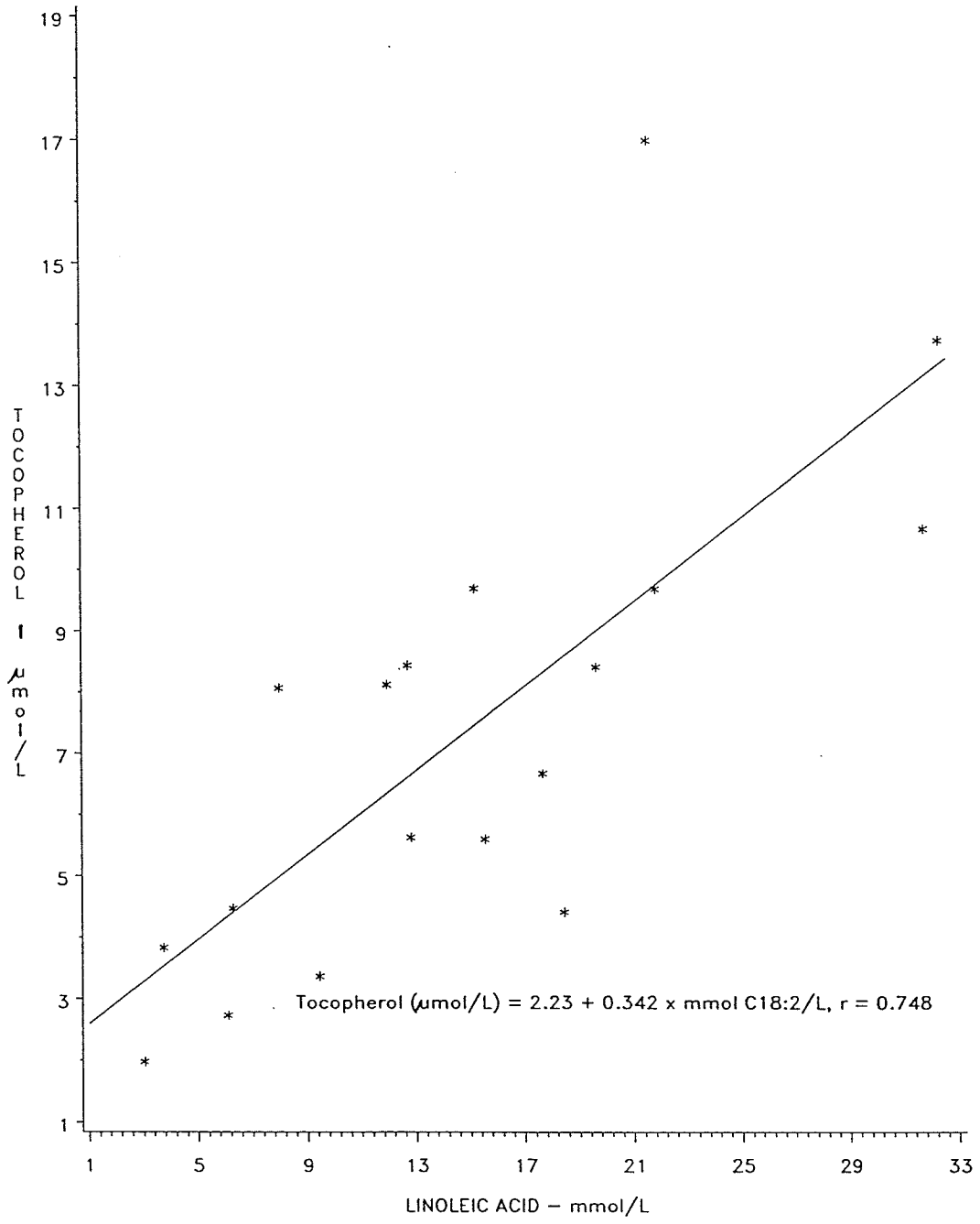

Figure 3: The Relationship Between α-tocopherol (µmol/L) and Linoleic Acid (mmol/L) in 18 Donor Samples

4.3 DIETARY INTAKES OF DONORS

4.3.1 ENERGY AND MACRONUTRIENT INTAKE

Dietary information was collected from the donors using three-day food records. Mean intakes for the three days were tabulated for each donor. Intakes of protein, fat, carbohydrate, and energy are shown in Table 18 for the individual donors. The percentage of kilocalories derived from each macronutrient was also calculated. This information is presented in Table 19.

Mean protein intake for the group of donors was 92.52 g/day. Values ranged from 58.44 g/day for donor 18 to 136.65 g/day for donor 16. Fat intakes ranges from 62.88 g/day for donor 18 to 145.87 g/day for donor 10. Intakes of fat averaged 94.66 g/day. Carbohydrate intakes varied over a wide range with the smallest intake at 198.25 g/day (donor 8) and the largest intake found to be 520.37 g/day (donor 10). Average carbohydrate intake was 309.93 g/day. Mean energy intake was 2424 kcal/day with intakes ranging from 1872.14 kcal/day for donor 15 to 3747.87 kcal/day for donor 10. The coefficient of variation for energy intake was 19.49%, similar to that reported by Butte et al (1984b) for 45 American mothers. The proportion of total energy intake contributed by protein, fat, and carbohydrate in the individual donors' diets is shown in Table 19. Mean contribution from protein, fat, and carbohydrate was 15.34%, 35.05%, and 51.08%, respectively. Such proportions compare favorably to nutrition recommendations for Canadians (Murray and Rae, 1979). Sources of macronutrients and energy from the seven food groups described in Chapter III are summarized in Appendix I (Tables 33 and 34). The largest source of fat and energy intakes were the milk and milk products

TABLE 18

Three-day Mean Macronutrient and Energy Intakes of Donors

Donor	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Energy (Kcal/day)
1	108.82	85.82	335.51	2493.13
2	106.10	112.45	305.04	2617.19
3	98.91	117.19	325.29	2696.37
4	87.23	81.35	295.82	2278.07
5	107.29	124.92	363.40	2929.18
6	70.84	66.89	328.05	2154.89
7	114.15	107.90	227.10	2294.90
8	82.14	83.87	198.25	1886.88
9	69.79	68.07	335.81	2201.64
10	114.02	145.87	520.37	3747.87
11	114.72	133.17	250.70	2660.14
12	78.63	91.21	259.40	2142.15
13	84.11	86.98	385.61	2606.31
14	92.66	63.65	279.38	2004.70
15	74.13	84.50	214.15	1872.14
16	136.65	109.99	360.24	2930.48
17	66.82	77.21	224.60	1899.22
18	58.44	62.89	370.03	2217.69
Mean	92.52	94.66	309.93	2424.05
SEM	4.99	5.87	18.43	111.37
C.V.	22.89	26.32	25.23	19.49

TABLE 19

Percent Contribution of Energy Intake From Protein, Fat, and Carbohydrate Intakes

Donor	Protein (% Kcal)	Fat (% Kcal)	Carbohydrate (% Kcal)
1	17.46	30.98	53.83
2	16.22	38.67	46.62
3	14.67	39.12	48.26
4	15.32	32.14	51.94
5	14.65	38.38	49.62
6	13.15	27.93	60.89
7	19.90	42.32	39.58
8	17.41	40.01	42.03
9	12.68	27.82	61.01
10	12.17	35.03	55.54
11	17.25	45.05	37.70
12	14.68	38.32	48.44
13	12.91	30.04	59.18
14	18.49	28.58	55.74
15	15.84	40.62	45.75
16	18.65	33.78	49.17
17	14.07	36.58	47.30
18	10.54	25.52	66.74
Mean	15.34	35.05	51.08
SEM	0.60	1.34	1.84
C.V.	16.51	16.22	15.26

and bread and cereals groups, respectively. Range of intakes from the food groups are also presented in Appendix I.

Protein and fat intakes of the donors in the present study were similar to those of 61 American lactating women studied by Sims (1978). She reported protein intakes to range from 50 to 150 g/day with an average intake of 90 g/day or 17.4% of energy intake. Fat intakes of these women averaged 98 g/day. Dietary fat varied over a wide range, at 35 to 309 g/day. Average percent of fat contribution to kilocalories was 41% compared to 35% in the present study. At 12 weeks postpartum, the 10 women in the Clark et al (1982) study had 13.8% of their kilocalories derived from protein intake. Fat contributed 38.6% to the energy content of their diets. Vuori et al (1982) found Finnish women to consume an average of 90 g of protein/day. Fat intakes averaged 96 g/day for these women. The lactating women in the study of Stuff et al (1983) consumed an average of 83.0 g protein/ day and 86.5 g fat/day as determined by three-day dietary record analysis. Protein and fat contributed 16% and 37.8%, respectively, to energy intakes.

Carbohydrate intakes by the women in the Sims (1978) study were lower than in the present study, ranging from 93 to 368 g/day. These mothers consumed a mean intake of 222 g carbohydrate/ day. Carbohydrate contributed, on average, 42% to a total energy intake of approximately 2100 kcal/day. Finley et al (1985b) analyzed the diets of 60 women, including 29 vegetarians. Carbohydrate intakes were 272 g/day and contributed 50% to the energy intake, similar to the proportion of carbohydrate in the present study.

Recent studies have reported energy intakes ranging from approximately 2100 to 2500 kcal/day for mothers during the first six months of lactation. The women in the study of Clark et al (1982) consumed an average of 2430 kcal/day, similar to that found for donors in the present investigation. Intakes of 2430 kcal/day at 6 to 8 weeks postpartum and 2060 kcal/day at 17 to 22 weeks postpartum were reported for Finnish mothers (Vuori et al, 1982). Manning-Dalton and Allen (1983) observed a mean energy of 2178 kcal/day for 27 American women during their first three months postpartum. Nutrient analysis of three-day records in the Butte et al (1984b) study revealed wide variations in energy intake (1099 to 4398 kcal/day) for another group of American mothers. Mean intake, 2186 kcal/day, was however, also within reported ranges. The macronutrient and energy intakes, recorded in the present study were consistent with recent reports in the literature of well-nourished, lactating women.

4.3.2 FATTY ACID COMPOSITION OF DONORS' DIETS

Total saturated, monounsaturated, and polyunsaturated fatty acid information was provided by the 1983 version of NAP of the Department of Foods and Nutrition for over 90% of the food items coded for analysis. Results of these analyses are shown in Table 20. Data represents the mean of three day intakes for each donor.

Saturated fatty acid intake ranged from 24.01 g/day for donor 4 to 55.06 g/day for donor 10. The group mean intake was 35.75 g/day. Just over one third of the fatty acids in the donors' diets were monoenoic (31.88 g/day or 39.51% of total fatty acids). Donor 6 consumed the

TABLE 20

Donor Three-day Mean Intake of Total Saturated, Monounsaturated, and Polyunsaturated Fatty Acids

Donor	Saturates (g/day)	Monounsaturates (g/day)	Polyunsaturates (g/day)
1	24.87	24.85	15.44
2	48.16	41.52	14.00
3	49.85	43.19	20.18
4	24.01	28.40	15.40
5	43.20	36.83	17.32
6	26.78	19.09	8.12
7	39.46	36.71	25.25
8	29.06	24.91	9.43
9	34.78	22.12	4.22
10	55.06	48.97	27.35
11	37.73	58.57	19.55
12	45.75	32.27	5.90
13	34.07	26.08	9.88
14	28.46	21.55	5.83
15	33.04	32.29	9.37
16	35.98	33.72	14.36
17	25.96	20.55	6.66
18	27.27	22.18	6.60
Mean	35.75	31.88	13.05
SEM	2.22	2.56	1.62
C.V.	26.39	34.10	52.69

least amount of monounsaturates (19.09 g/day) whereas donor 11 had the largest intake at 58.57 g/day. Mean polyunsaturated fatty acid intake for the group was 13.05 g/day or 16.18% of total fatty acid intake. Intakes were spread over a fairly wide range. Levels ranged from 4.22 g/day by donor 9 to 27.35 g/day by donor 10.

Dietary intakes of the donors were recorded between mid - 1982 to early 1983. Estimates of fatty acid consumption were reported by Robbins and Robichon-Hunt (1985) for the Canadian population for that time period. Their estimates were based on food consumption patterns from Agricultural Canada and Statistics Canada which did not measure actual consumption of food but indicated amounts of foods and nutrients available to the population. Interestingly though, were the levels of fatty acids available as estimated in the Robbins and Robichon-Hunt (1985) report when compared to the levels of intake by the donors in this study. As expected, daily quantities of total saturated, monounsaturated, and polyunsaturated fatty acids available per capita were considerably higher than that recorded in the present study. However, percent saturated fatty acid contribution to total fat in 1983 available for consumption was 36.4%, similar to the actual consumption of 37.8% by the donors in this study. Monounsaturated fatty acids represented 37.6% of the estimated total fat available in 1983 as reported by Robbins and Robichon-Hunt (1985). Donors intake of monounsaturates contributed 33.68% to fat intake. Polyunsaturated fatty acids (PUFA) contributed 13.78% to the total fat intake of the donors. On a per capita per day basis, Robbins and Robichon-Hunt (1985) estimated percent contribution of PUFA to total fat at 17.9% in 1983.

For the Canadian population as a whole, it was estimated that meats, poultry, and fish supplied 38.5% of the saturated fatty acid intake while dairy products, especially cheese, contributed 24%. In the general Canadian diet, dietary PUFA, particularly C18:2, were estimated to come mainly from fats and oils (primarily of vegetable origin) as was also evident in this study, where the fats and oils group accounted for 36% of the PUFAS. In the present study, milk and milk products were the main source of saturated fatty acids (43%), followed by meat, fish, poultry, and eggs (22%). Further details of these data are outlined in Appendix J (Tables 35 and 36).

Extensive fatty acid intake data for lactating women has not appeared frequently in the literature. Two studies have supplied some fatty acid data which can be compared to the intakes of the donors in the present investigation. The first, reported by Vuori et al (1982), examined the habitual diets of two groups of Finnish mothers, 6 to 8 weeks postpartum and 17 to 22 weeks postpartum. Seven-day food records were kept by the women at each time period. Fatty acids were presented as a percentage of total fatty acids. To allow for comparisons with the intakes in this study, data from Table 20 were converted to percentages of total fatty acids. These data are found in Appendix K (Table 37).

Mean total saturated fatty acid intake of the 20 Finnish women at 6 to 8 weeks postpartum was 51.9% (SD 5.5). At 17 to 22 weeks, the percentage increased slightly to 52.7% (SD 5.9, n = 13) (Vuori et al, 1982). Calculated as a percent of total fatty acids, saturate intake of the donors in the present study was lower than that of the Finnish mothers, at 44.3%. Total monounsaturates of the Finnish women was 33.1%

(SD 2.4) at 6 to 8 weeks and 32.3% (SD 1.8) at 17 to 22 weeks. The donors' intake in the present investigation was slightly higher, at 39.5% of total fatty acids. Intake of total polyunsaturates by the donors was 16.2% of total fatty acids. That of the Finnish women was lower than this at both time periods, the average being 15.0%. Several individual dietary fatty acids were also calculated for the Finnish women. For example, linoleic acid averaged 12.5% and 12.7% at 6 to 8 weeks and 17 to 22 weeks postpartum, respectively (Vuori et al, 1982). A P/S dietary ratio of 0.30 was observed, also for both time periods. In the present study, the dietary P/S ratio ranged from 0.12 to 0.64, with a group mean of 0.36.

The Finley et al (1985a) study reported fatty acid intakes for both vegetarians and non-vegetarians. Saturated fatty acid intakes were higher for the 100 non-vegetarians (35 g/day) than for the 102 vegetarians (25 g/day). The intakes of the present study's donors resembled that of the non-vegetarians at 35.75 g/day. Donor 6 was the only vegetarian in the sample, although she had added eggs and fish to her diet since her pregnancy (Appendix G, Table 30). Her mean saturated fatty acid intake was 26.78 g/day. Total monounsaturated and polyunsaturated fatty acid intakes were not reported by Finley et al (1985a). Intakes of C18:1 and C18:2 were presented however. Vegetarians consumed 30 g of C18:1 and 14 g of C18:2 while the non-vegetarians consumed and average of 34 g of C18:1 and 12 g C18:2 daily.

As mentioned in Chapter III, the database used for diet analysis was very limited with respect to data available to assess C18:2 intakes. Despite this, however, diets of the donors were coded for analysis to

determine the range of C18:2 intakes in the small number of food items (Appendix F, Table 26) in the database with values for this fatty acid. Mean intake was calculated at 6.95 g/day, with values ranging from 0.90 g/day for donor 14 to 19.66 g/day for donor 7. Mean intake of C18:2 by the non-vegetarians in the Finley et al (1985a) study discussed above was almost double that calculated for the donors in the present study. Expressed in terms of percent of total fatty acids, C18:2 intake of the donors was 8.61%. Levels of C18:2 in the diets of the Finnish women described by Vuori et al (1982) were 12.5% and 12.7% of total fatty acids at 6 to 8 weeks and 17 to 22 weeks postpartum, respectively. Had the database contained C18:2 values for foods such as breads, cereals, snack and dessert items, intakes of the donors would have been, in all probability, considerably higher.

Mean intake of C18:2 of mothers interviewed during the summer (9.18 g) was found to be signifcantly greater than the average intake of mothers contacted during the winter months (4.14 g) ($t = 2.330$, $p = 0.028$). Intake of C18:2 by the two groups remained significantly different even when the highest intake (donor 7) was eliminated ($t = 2.231$, $p = 0.041$). Differences between summer and winter diets appeared to be due to intakes of salad oils, and tablespreads.

4.4 TRANS FATTY ACID COMPOSITION OF DONORS' DIETS

The number of available values for trans-fatty acids in the computer database used for dietary analysis was found to be extremely limited. Only 0.78% of the foods in the total database contained total trans-fatty acid values (Health and Welfare Canada, 1983). Diets, therefore,

could not be quantified accurately. However, when food items consumed by the donors in the present study were divided into food groups (Appendix F, Table 26), the number of available values for trans-fatty acids in the food fats and oils group presented a slightly better picture. Eleven out of the 23 foods in this group had values for trans-fatty acids (47.8%). Since the largest percent of these fatty acids are contained in these foods (Enig et al, 1983), dietary analysis was preformed using the limited amount of available data. Results presented represent intake from all food items coded for analysis. Total trans-fatty acids ranged from 0.11 g/day for donor 14 to 6.58 g/day for donor 3. A group mean intake of 2.24 g/day was found.

The limitations of this dietary analysis was immediately evident when intakes calculated by other investigators were compared. Brisson (1981) estimated Canadian intake of trans-fatty acids to average 9.1 g/day. Aitchson et al (1977) analyzed diet collections from 11 lactating women. Average analytical values from these collections ranged from 1.30 to 8.27% for total trans-fatty acids. Mean dietary intake was calculated at 5.03%. Craig-Schmidt et al (1984) estimated intakes of C18:1t by the lactating women in their study to average 7.78% of total fat intake, considerably higher than the 2.36% of total fat intake calculated for the donors in the present investigation.

4.4.1 VITAMIN E INTAKE

Data available in the 1983 nutrient database (Health and Welfare Canada, 1983) was limiting with respect to tocopherol content. Only 39.4% of the food items coded for analysis contained total tocopherol

values (Appendix F, Table 26). Alpha-tocopherol data was available for approximately 8% of the food items. Diets were assessed for total tocopherol content using the small amount of available data. Intakes varied widely, ranging from 2.22 mg/day for donor 12 to 26.12 mg/day for donor 10. Mean intake for all donors was 12.68 mg/day. In an earlier investigation by Bieri and Evarts (1973), tocopherol content of "typical" American meals was found to be generally related to the amount of fat in these meals. Donor 10 not only had the largest intake of total tocopherol but also the greatest fat intake (Table 18). Her daily intake of a soybean oil margarine averaged 39.5 g. She also consumed mayonnaise and salad dressings on each of the three days of recording her intake, which would account for her relatively high intake of tocopherol. Donor 12, on the other hand, consumed a daily average of 10 g of butter and did not eat any salad dressings during the three day period. A positive correlation between total fat intake and dietary tocopherol was found ($r = 0.561$, $p = 0.015$) for the total group of donors.

The study mentioned above by Bieri and Evarts (1973) found α-tocopherol to vary from 0.5 mg to 5.7 mg for the various meals (breakfasts, lunches, dinners) analyzed. Calculated on a daily intake basis, range of intake was found to be 4.4 to 12.7 mg α-tocopherol. Average intake was calculated at 9.0 mg/day. In the same year, Thompson et al (1973) analyzed individual meals and a composite Canadian diet. Calculated from nutrient tables, the diet was found to contain 7.51 mg α-tocopherol/day. Laboratory analysis of this diet found 6.38 mg α-tocopherol/day. The recent study of Anderson and Pittard (1985) reported energy and vitamin E daily intakes to be 3436 kcal and 27 mg,

respectively, for the one mother investigated. Twenty four women in the Chappell et al (1985b) study had an average vitamin E intake of 15 mg/day, only slightly greater than the 12.6 mg total tocopherol/day calculated for the donors in the present study using a limited nutrient database.

A student t-test at $p = 0.05$ was performed to determine if intakes of tocopherol significantly differed for the donors interviewed during the summer from those interviewed during the winter months. Mean intake for the 10 donors contacted in the summer was 16.62 mg/day. The 8 donors interviewed during the following winter had a mean intake of 7.77 mg/day. These intakes were found to be significantly different ($t = 3.201$, $p = 0.006$). Even when the highest and lowest intakes (from donors 10 and 12) were excluded, mean intakes remained sigificantly different between the two time periods ($t = 2.574$, $p = 0.022$). The major differences in food intake between these two groups seemed to center around use of tablespreads (butter versus margarines) and salad dressings. Eight out of the 10 (80%) donors interviewed during the summer used a commerical margarine as their regular tablespread. Four out of the eight (50%) mothers interviewed during the winter used margarines. Six mothers contacted in the summer months regularly consumed mayonnaise whereas only two from the winter group did so. More of the mothers (six) from the summer group used salad dressings with a soybean or canola base compared to the number of mothers from the winter group (one).

4.5 THE EFFECTS OF DIETARY INTAKE ON DONOR MILK COMPOSITION

4.5.1 DIETARY EFFECTS ON FAT CONTENT OF DONOR MILK

This section outlines the results of correlation analysis between macronutrient and energy intakes and fat content of the donor milks. Scatter diagrams of these relationships are shown in Figures 4 to 7.

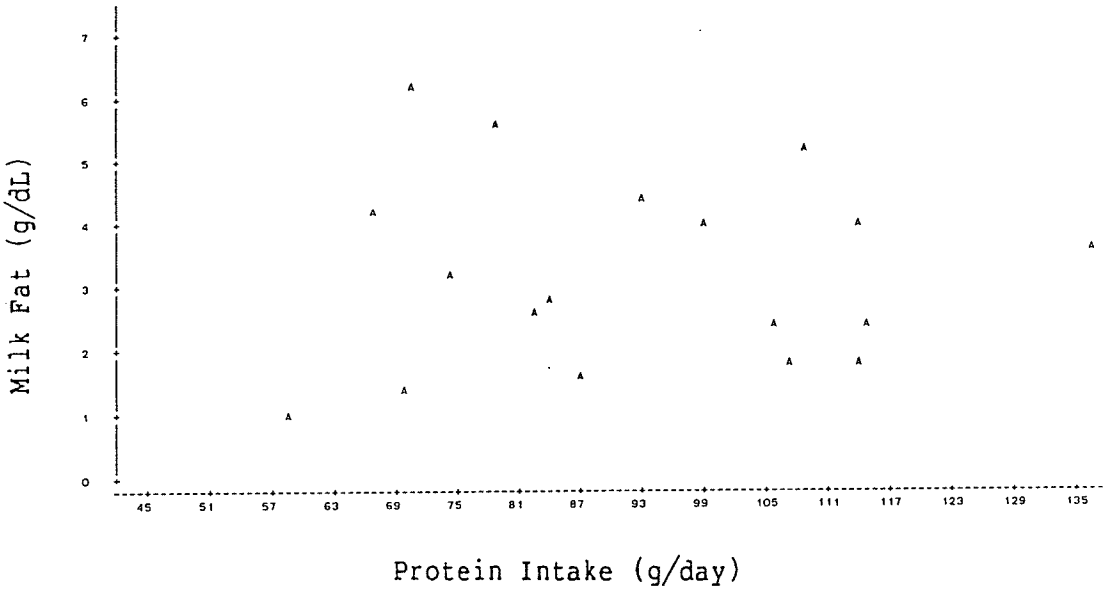

Figure 4: The Relationship Between Dietary Protein (g/day) and Fat Content (g/dL) of Donor Milks

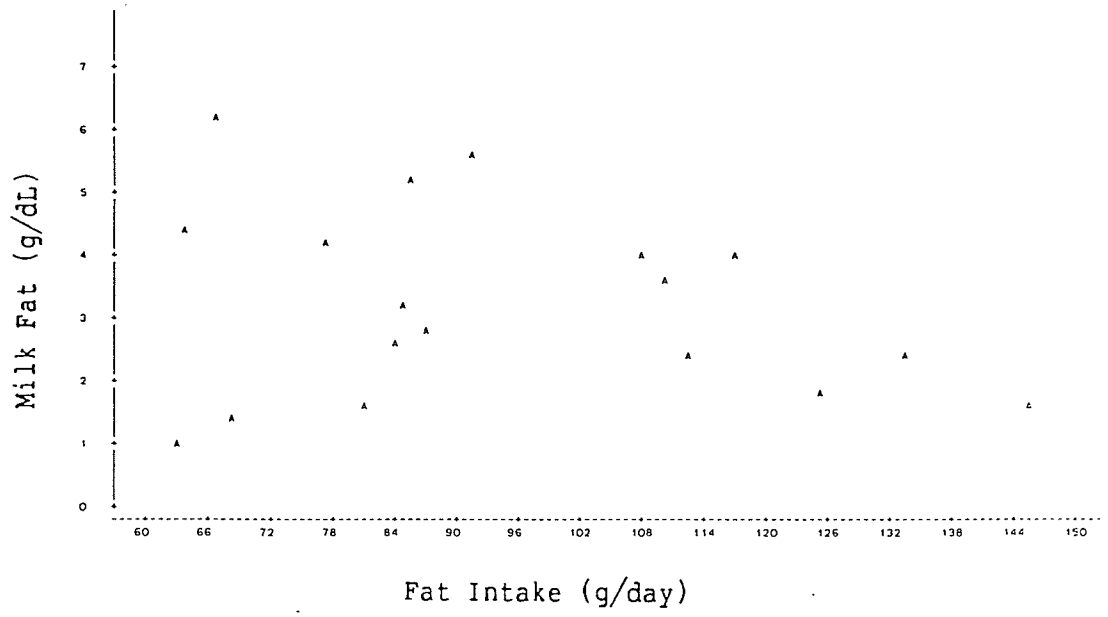

Figure 5: The Relationship Between Dietary Fat (g/day) and Fat Content (g/dL) of Donor Milks

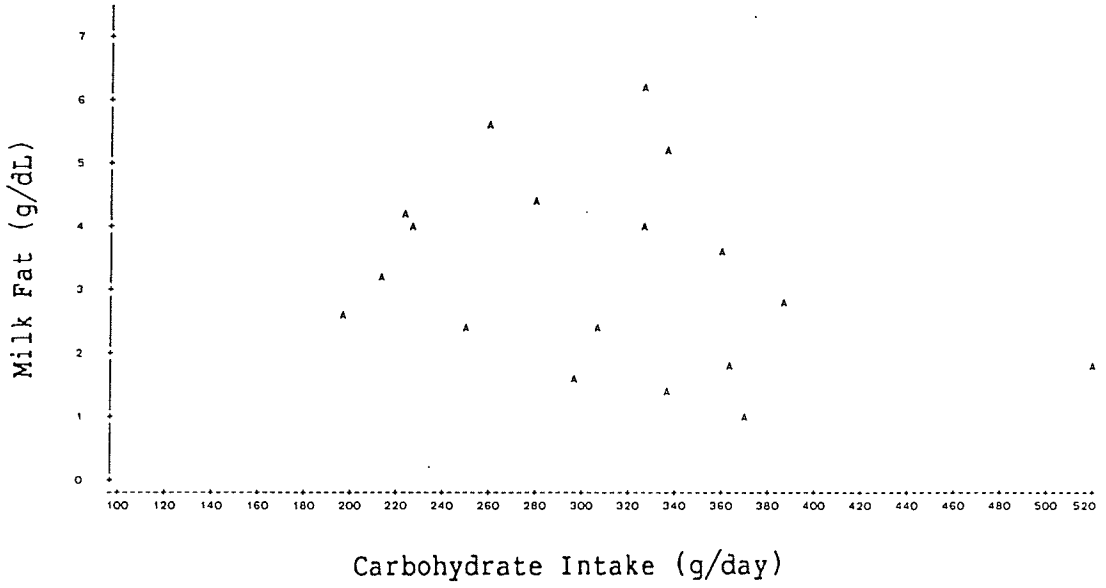

Figure 6: The Relationship Between Dietary Carbohydrate (g/day) and Fat Content (g/dL) of Donor Milks

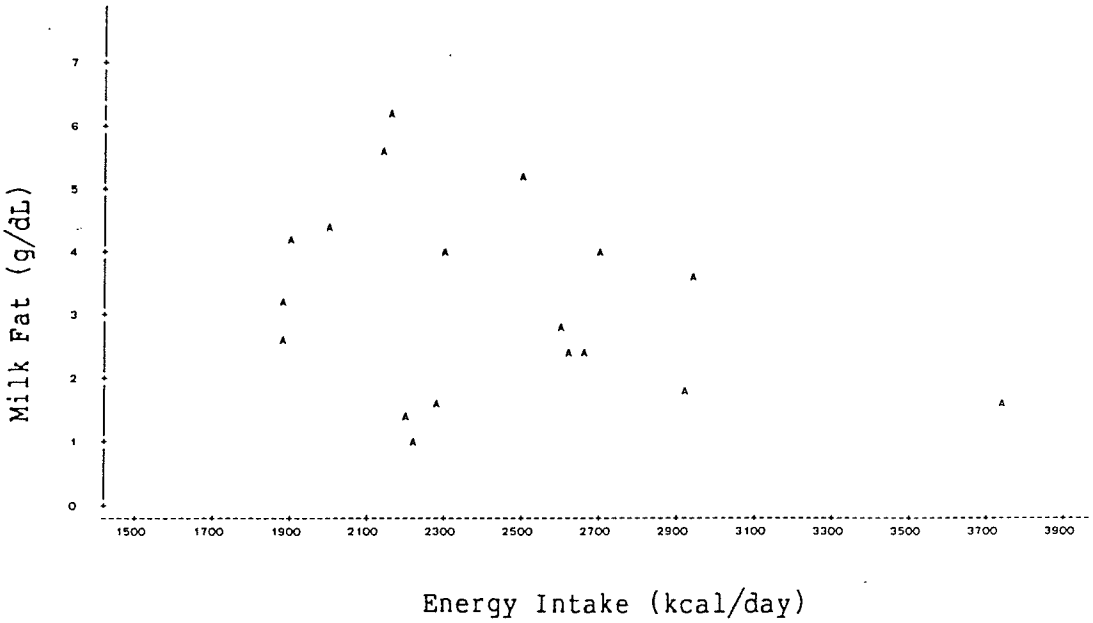

Figure 7: The Relationship Between Dietary Energy (kcal/day) and Fat Content (g/dL) of Donor Milks

Evident in these scatter plots was the lack of association between each macronutrient and energy and total fat content of the milk samples. Pearson's correlation coefficients (r) were calculated between these variables to further substantiate these findings. These are presented in Table 21.

TABLE 21

Pearson's Correlation Coefficients (r) Between Milk Fat and Macronutrient and Energy Intakes

	r	Probability
Fat x Dietary Protein	-0.008	0.972
Fat x Dietary Fat	-0.238	0.342
Fat x Dietary Carbohydrate	-0.308	0.212
Fat x Dietary Energy	-0.313	0.205

Contrary to the above results, the early studies of Deem (1931) and Deb and Cama (1962) reported distinct dietary effects on milk fat levels. However, in both studies, diets were altered in macronutrient and/or in energy content. For example, Deem (1931) observed a slight decrease in milk fat concentration when five subjects consumed a high protein diet (143 to 152 g/day or 14.4 to 19.2% of energy intake). Protein supplementation of women habitually on low protein diets led to marked increases in milk fat levels as reported by Deb and Cama (1962). Increased fat concentrations were found by Deem (1931) after her

subjects consumed very high fat diets (229 to 265 g/day or 51.6 to 55.4% of energy intake). The diets in this study were approximately 2200 calories higher than those utilized by the Deb and Cama (1962).

In a number of later studies no relationship between milk fat and macronutrients or energy were reported. Khin-Maung-Naing et al (1980) for example, failed to observe any effect of nutritional status of Burmese women on proximate composition of their breast milk. In West Africa, the fat content of milk from 120 mothers did not change significantly after taking a high energy supplement (averaging 800 to 900 kcal/day) for 12 months (Prentice et al, 1980). Ad libitum maternal diets were found to have no effect on total fat content of breast milk for Finnish (Vuori et al, 1982) and American women (Finley et al, 1985a; Lipsman et al, 1985). Such results are consistent with those found in the present investigation.

4.5.2 DIETARY EFFECTS ON FATTY ACID COMPOSITION OF DONOR MILK

The effects of diet on the fatty acid composition of the donor milks are discussed under two headings, the effects of macronutrient and energy and the effects of fatty acid intake on milk fatty acid composition.

4.5.2.1 Dietary Macronutrient and Energy Effects on Milk Cis Fatty Acid Composition

Pearson's correlation and simple regression analyses were used to determine the association between dietary macronutrients and energy intake and fatty acid composition of the donor milks. These analyses

were performed with two sets of data, with the milk fatty acids expressed as weight percents of total fatty acid methyl esters (Tables 8, 9, 10) and secondly, with fatty acid data expressed as grams per deciliter milk (Table 13). Correlation coefficients for dietary factors and selected milk fatty acids are found in Tables 22 and 23. It is interesting to note that significant correlations ($p < 0.05$) were only found when fatty acids were expressed on a weight percent basis (Table 22). The equation used to convert fatty acids to absolute amounts (g/dL) included total milk lipid content (section 3.5.6). As discussed in subsection 4.4.1, relationships between total milk lipids and macronutrient and energy intake were not found. Therefore, the lack of association between these dietary parameters and fatty acids expressed as gm/dL (Table 23) may be due to the fact that total milk lipid was inlcuded in the calculation.

Protein intake was not found to affect the fatty acid composition of the donor milks. This is in contrast to the results of Vuori et al (1982) who investigated the relationship between Finnish diets and breast milk composition. The mothers in that study consumed an average of 89.5 g protein/ day. Fatty acids were expressed as percent of total fatty acids. A correlation between protein intake and C16:0 was observed ($n = 33$, $r = 0.364$, $p < 0.05$). Finley et al (1985a) reported a similar association for American mothers. Figures 8 and 9 illustrate the lack of association found in the present study between protein intake and two selected fatty acids (C16:0 and C12:0).

Fat intake by the donors averaged 94.66 g/day. Correlation and regression analysis failed to find a relationship between fat intake and

TABLE 22

Pearson's Correlation Coefficients (r) Between Milk Fatty Acids (Wt.%)† and Macronutrient and Energy Intakes

Fatty Acid Wt.%	Dietary Intake			
	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Energy (kcal/day)
C12:0	0.254 (0.308)*	0.276 (0.266)	0.546 (0.019)**	0.495 (0.036)**
C14:0	0.232 (0.354)	0.175 (0.487)	0.541 (0.020)**	0.440 (0.068)
C16:0	-0.284 (0.253)	-0.354 (0.150)	0.266 (0.367)	-0.082 (0.744)
C18:0	-0.196 (0.433)	-0.024 (0.924)	0.046 (0.858)	-0.036 (0.886)
Total Saturates	-0.006 (0.979)	-0.034 (0.894)	0.504 (0.032)**	0.274 (0.271)
C18:1	-0.248 (0.321)	0.026 (0.920)	-0.074 (0.771)	-0.062 (0.807)
Total Monounsaturates	-0.358 (0.145)	-0.100 (0.693)	-0.051 (0.841)	-0.124 (0.621)
C18:2	0.348 (0.156)	0.221 (0.379)	-0.530 (0.024)**	-0.150 (0.552)
Total Polyunsaturates	0.306 (0.216)	0.172 (0.493)	-0.531 (0.023)**	-0.182 (0.469)

† Wt.% of methyl esters
* Probability in parentheses
** Statistically significant, $p < 0.05$

TABLE 23

Pearson's Correlation Coefficients (r) Between Milk Fatty Acids (gm/dL)† and Macronutrient and Energy Intakes

Fatty Acid gm/dL	Dietary Intake			
	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Energy (kcal/day)
C12:0	0.074 (0.768)*	-0.128 (0.611)	0.034 (0.892)	-0.039 (0.876)
C14:0	0.091 (0.719)	-0.135 (0.593)	-0.004 (0.984)	-0.065 (0.797)
C16:0	-0.033 (0.896)	-0.264 (0.290)	-0.270 (0.279)	-0.307 (0.214)
C18:0	-0.040 (0.873)	-0.206 (0.412)	-0.352 (0.152)	-0.336 (0.173)
Total Saturates	-0.013 (0.958)	-0.216 (0.388)	-0.192 (0.445)	-0.231 (0.356)
C18:1	-0.082 (0.746)	-0.264 (0.291)	-0.364 (0.138)	-0.368 (0.132)
Total Monounsaturates	-0.084 (0.740)	-0.276 (0.266)	-0.351 (0.153)	-0.367 (0.134)
C18:2	0.118 (0.639)	-0.118 (0.639)	-0.451 (0.061)	-0.317 (0.199)
Total Polyunsaturates	0.080 (0.753)	-0.144 (0.566)	-0.422 (0.080)	-0.320 (0.196)

† Wt.% of methyl esters converted to g fatty acids/dL milk.
* Probability in parentheses

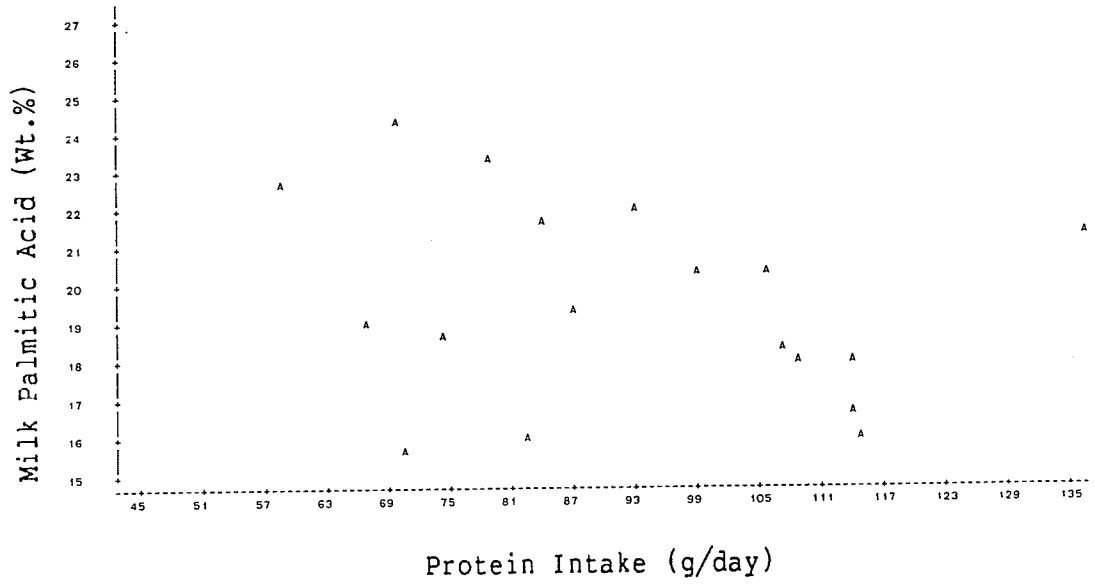

Figure 8: The Relationship Between Dietary Protein (g/day) and C16:0 (Wt.%) of Donor Milks

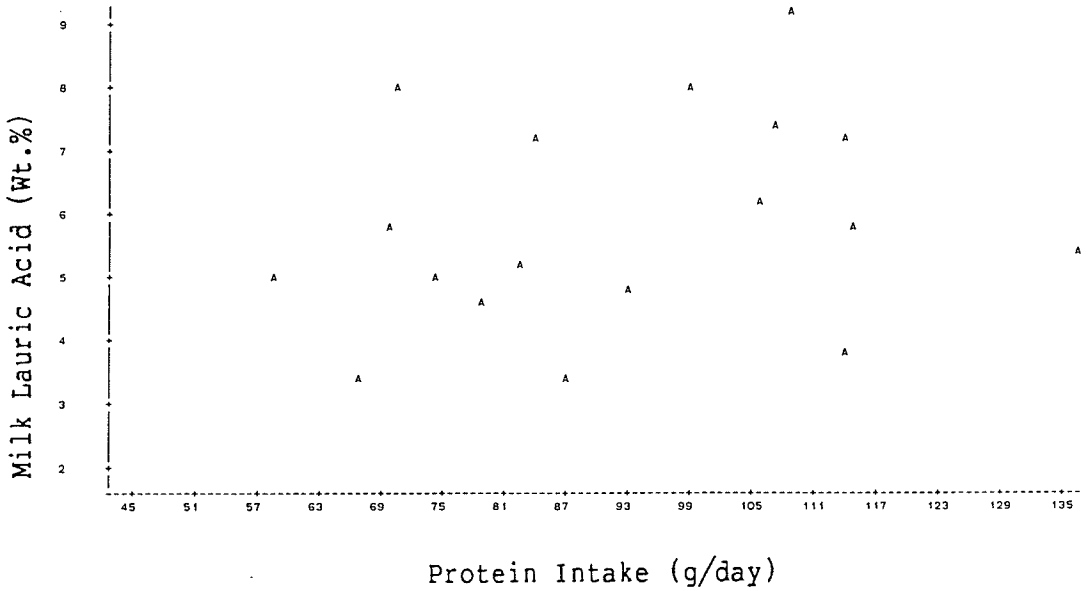

Figure 9: The Relationship Between Dietary Protein (g/day) and C12:0 (Wt.%) of Donor Milks

fatty acid composition of the donor milks (Tables 22 and 23). Simple correlations between total fat intake and both milk C16:0 and C18:0 revealed positive relationships in the study of Finley et al (1985a). These fatty acids are found abundantly in animal fat. Thus, it was not surprising that these authors also observed a positive correlation between animal fat intake and C16:0 ($r = 0.48$, $p \leq 0.05$) and C18:0 ($r = 0.56$, $p \leq 0.05$). Vegetable fat intake was negatively correlated with these fatty acids ($p \leq 0.05$). Earlier, Vuori et al (1982) reported a significant relationship between C16:0 and fat intake ($r = 0.376$, $p < 0.05$). In the present study, correlation analysis revealed the lack of effect of dietary fat on C16:0 (Wt.%) ($r = -0.354$, $p = 0.150$). The scatter between these two variables is illustrated in Figure 10. The scatter diagram between C18:0 (Wt.%) and dietary fat showed a similar lack of relationship (Figure 11). As previously mentioned, fatty acids expressed as absolute amounts (g/day) were not found to be related to any dietary variables. An example of this lack of association is illustrated in Figure 12 with the fatty acid C18:1 (g/dL) serving as the dependent variable.

As shown in Table 22, intake of carbohydrate had a significant effect on several milk fatty acids. Significant positive correlations were found between dietary carbohydrate (g/day) and the saturated fatty acids C12:0 ($r = 0.546$, $p = 0.019$) and C14:0 ($r = 0.541$, $p = 0.020$). Total milk saturates were also positively related to the carbohydrate intake of the donors ($r = 0.504$, $p = 0.032$). The R^2 between dietary carbohydrate and C12:0 was 0.298, indicating that 29.8% of the variation in milk lauric acid was explained by the simple linear regression model

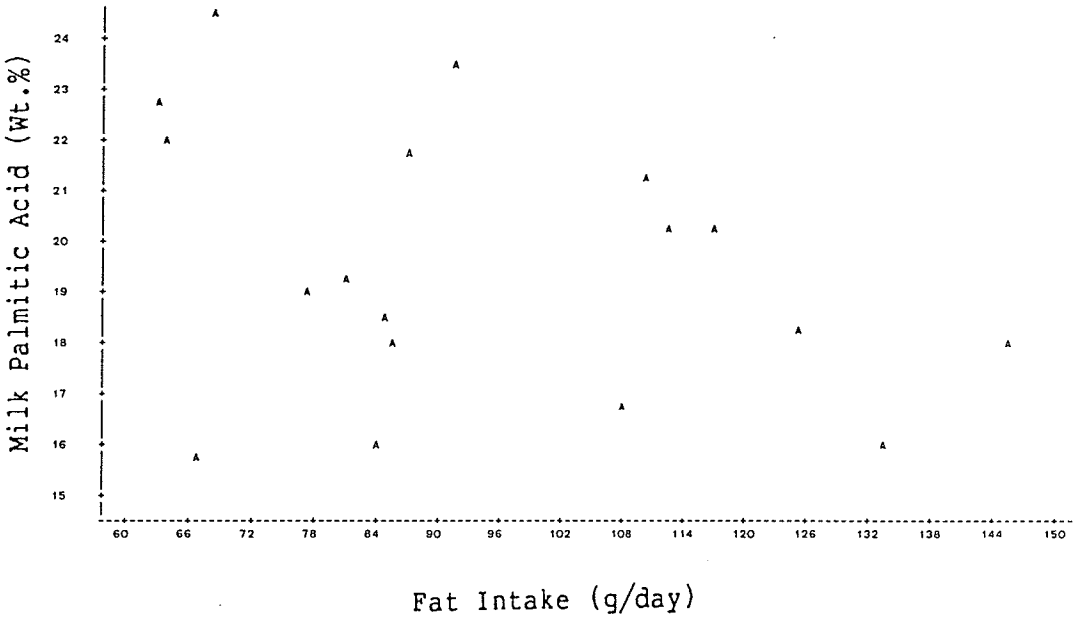

Figure 10: The Relationship Between Dietary Fat (g/day) and C16:0 (Wt.%) of Donor Milks

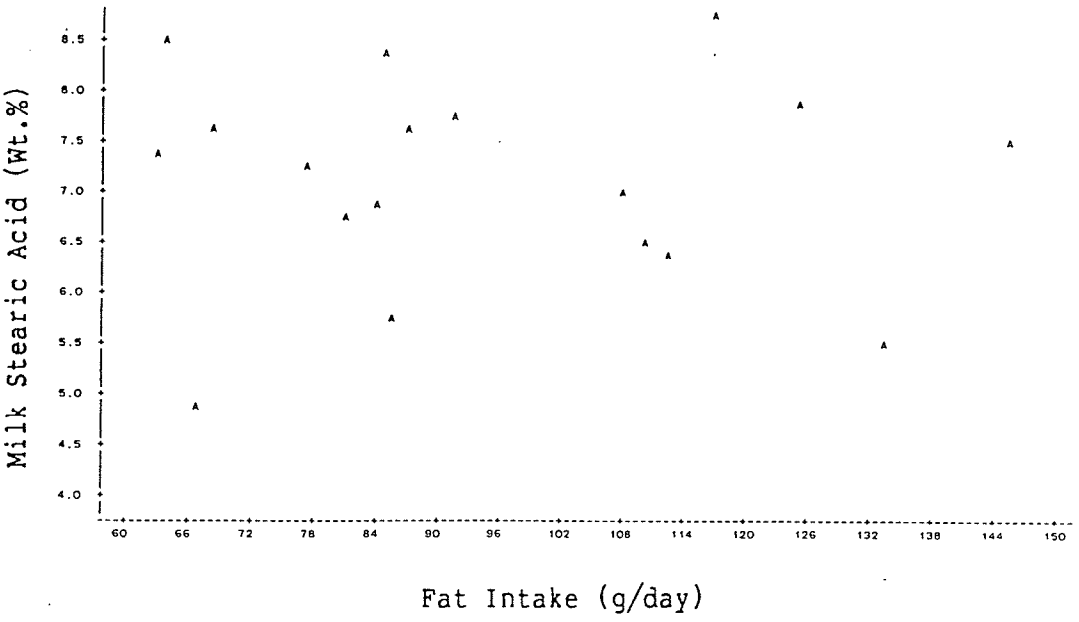

Figure 11: The Relationship Between Dietary Fat (g/day) and C18:0 (Wt.%) of Donor Milks

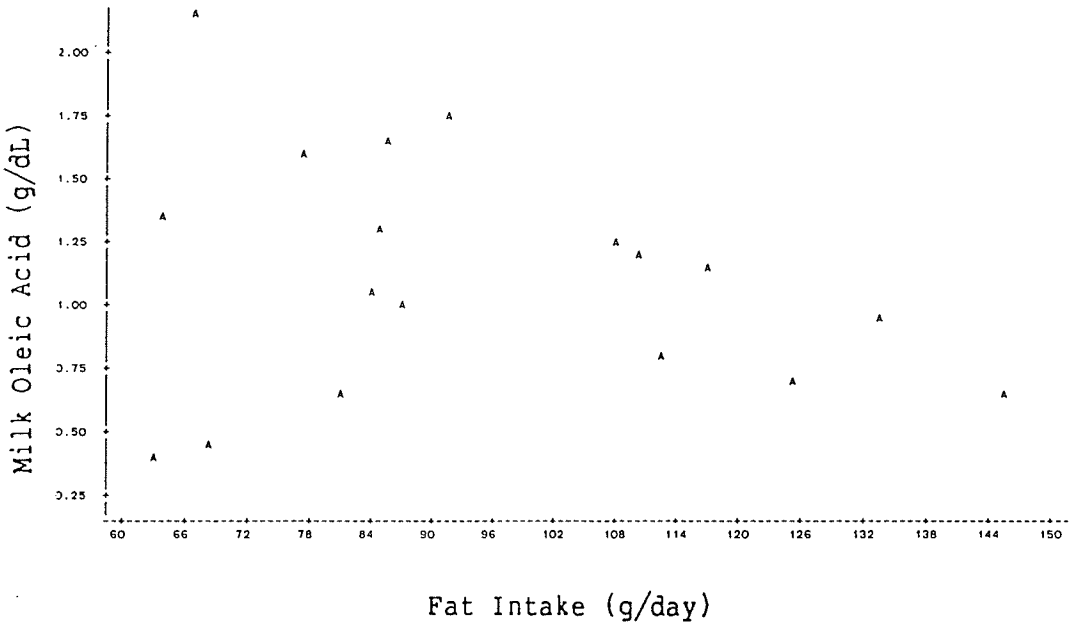

Figure 12: The Relationship Between Dietary Fat (g/day) and C18:1 (g/dL) of Donor Milks

based on carbohydrate intake. Figure 13 depicts this relationship. Figure 14 displays the association between milk C14:0 and dietary carbohydrate. In this case, 29.2% of the variation in milk myristic acid (Wt.%) was explained by dietary carbohydrate ($R^2 = 0.292$). The relationship between total milk saturated fatty acids (Wt.%) and dietary carbohydrate is demonstrated with its regression equation in Figure 15.

The effects of dietary carbohydrate on milk saturated fatty acids is well documented. Most authors who have reported these associations however, have had as their subjects, women consuming carbohydrate well in excess of that ingested by the donors in the present study. Read et al (1965a) found levels of C12:0 and C14:0 to be highest in the milk of

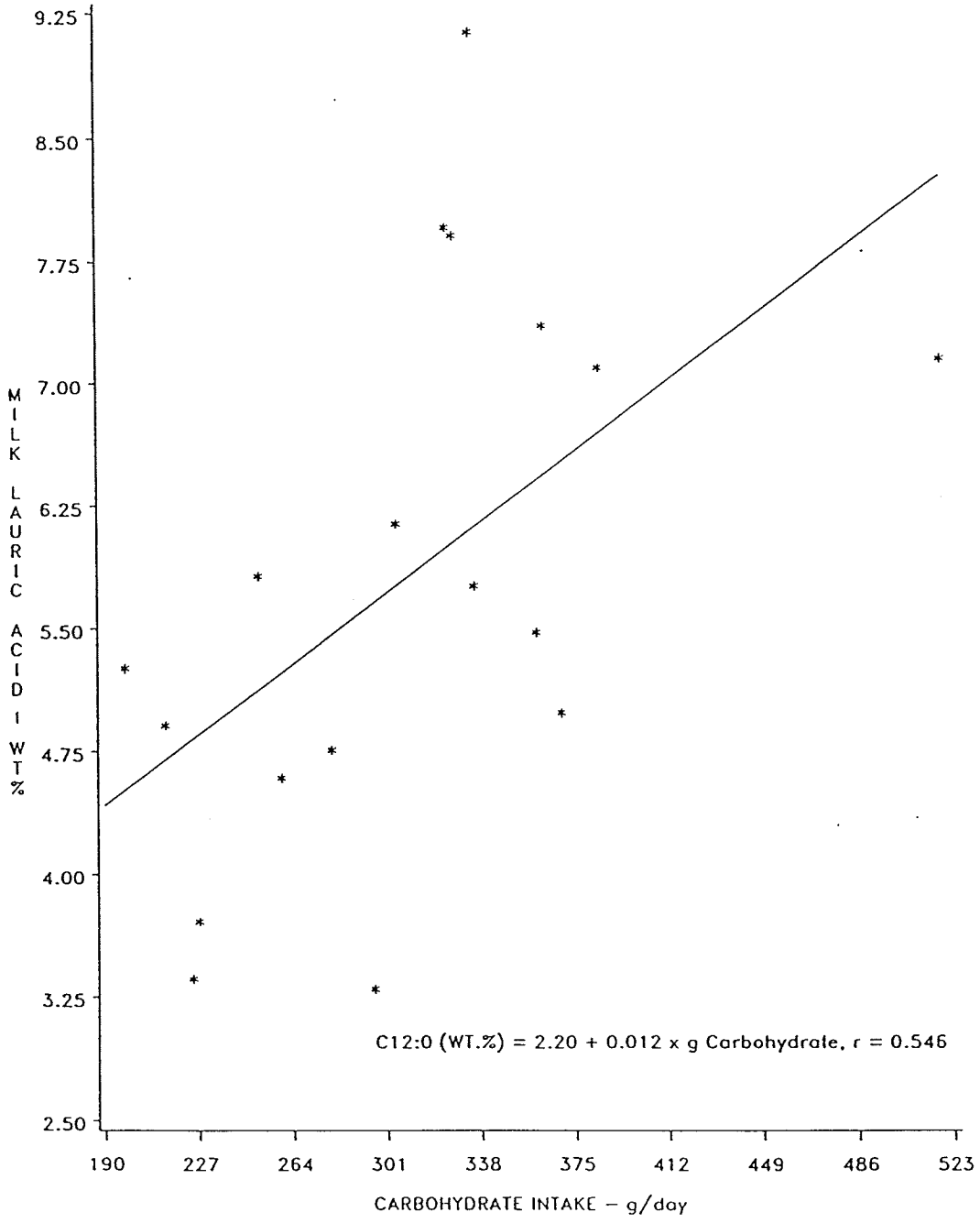

Figure 13: The Relationship Between Dietary Carbohydrate (g/day) and C12:0 (Wt.%) of Donor Milks

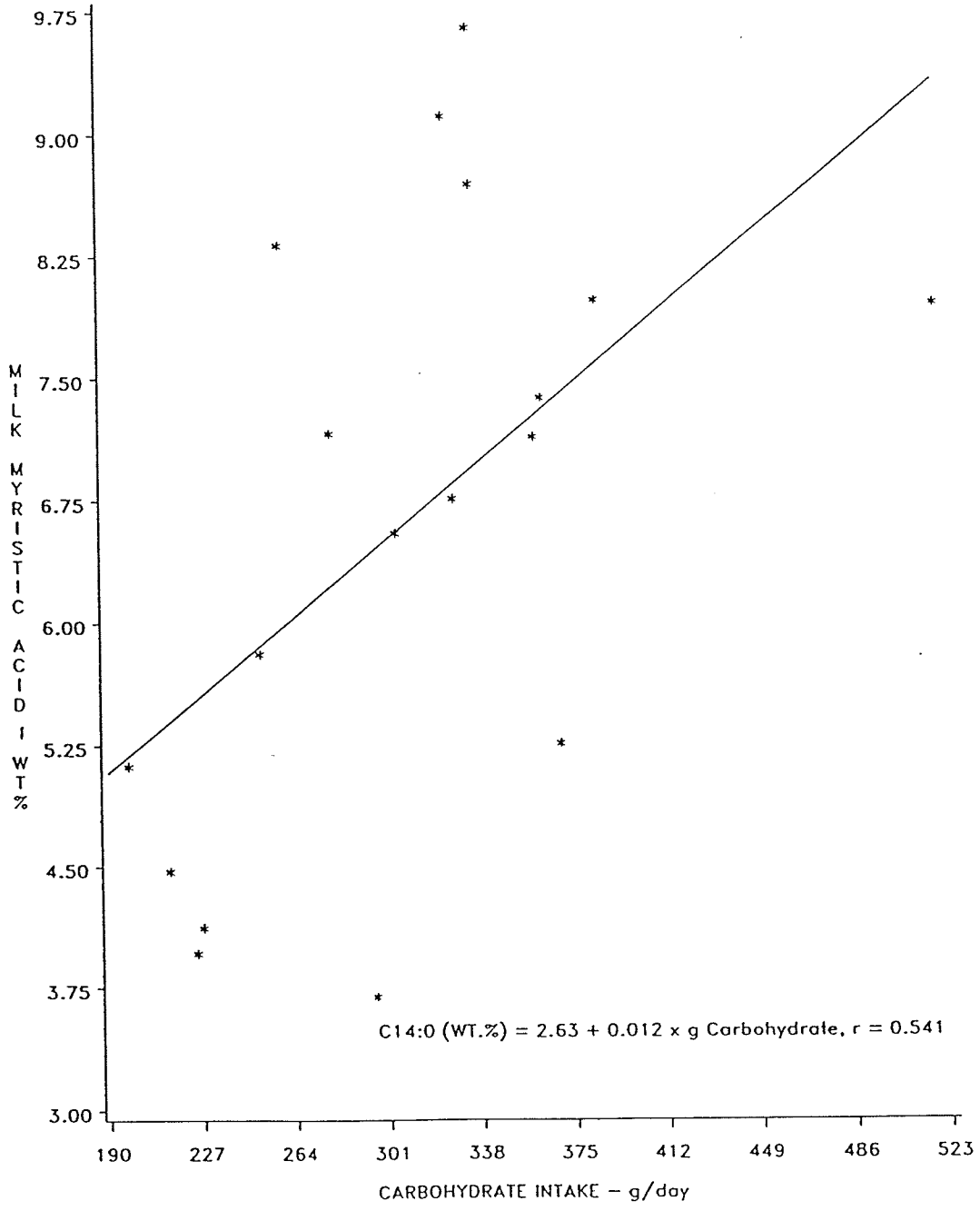

Figure 14: The Relationship Between Dietary Carbohydrate (g/day) and C14:0 (Wt.%) of Donor Milks

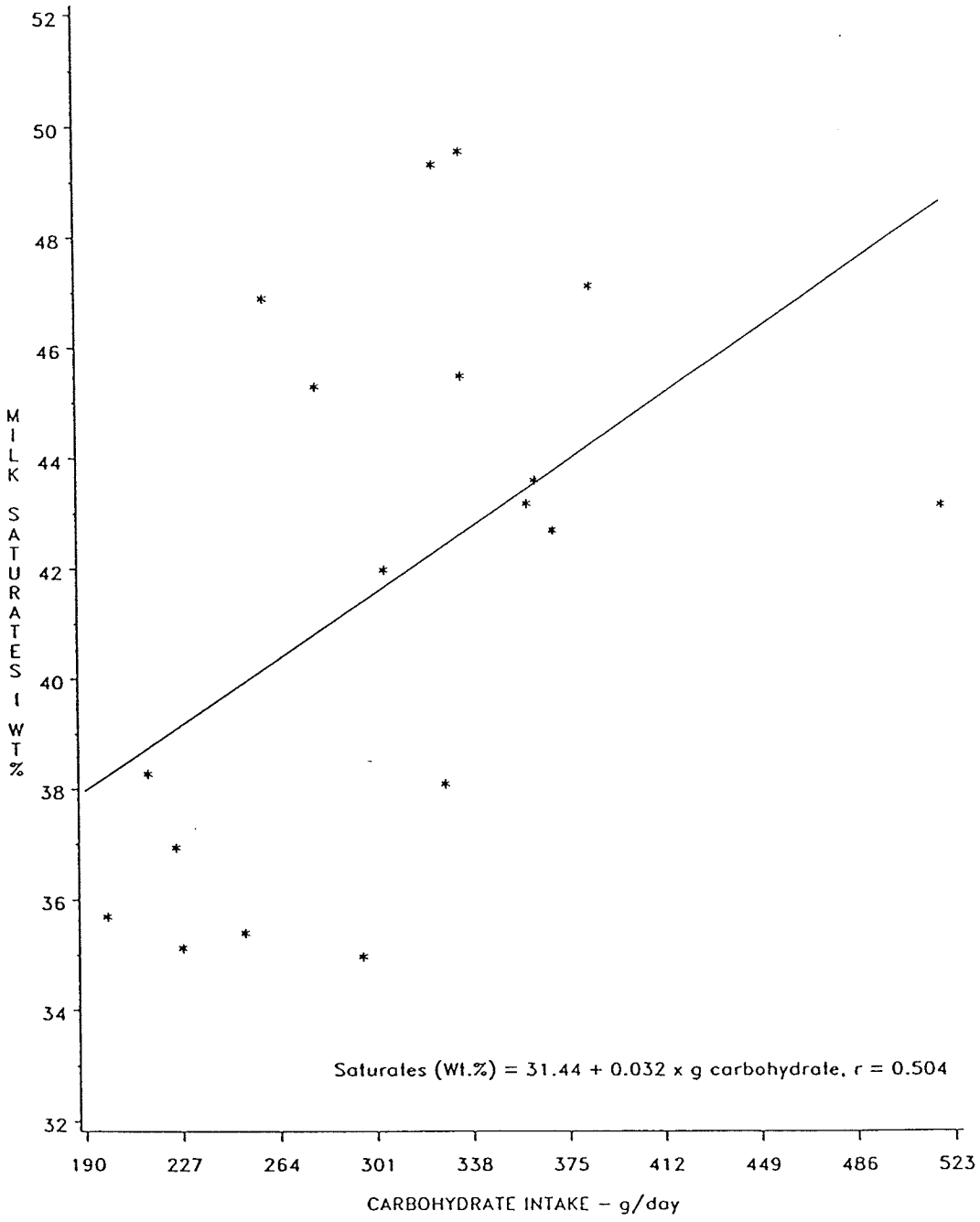

Figure 15: The Relationship Between Dietary Carbohydrate (g/day) and Total Saturates (Wt.%) of Donor Milks

Tanganyikan women compared to other ethnic groups studied. These mothers consumed diets in which carbohydrate contributed 70% to the energy content. Carbohydrate averaged 51% of caloric intake in the diets of the donors in the present study. Lauric acid and C14:0 averaged 5.82% and 6.62%, respectively. The milk of the Tanganyikans contained 16.5% C12:0 and 12.7% C14:0 (Read et al, 1965a). Milk from Ivory Coast mothers also contained high levels of these fatty acids. This was assumed to be due to their carbohydrate consumption which averaged 75% of calorie intake (Lauber and Reinhardt, 1979). Harzer et al (1984) observed milk C12:0 and C14:0 to be higher after consumption of a low-fat/high carbohydrate (65% carbohydrate) than after consumption of a high-fat/ low-carbohydrate (35% carbohydrate) diet. The 'high' levels of these fatty acids were, however, only half the amount of the milk levels of the Tanganyikan women reported in the Read et al (1965a) study. Levels of milk C12:0 and C14:0 in the present study more closely resembled the milk of mothers ingesting 40 to 51% calories from fat as reported by Read et al (1965a). Underwood et al (1970) did not observe amounts of medium chain fatty acids to be as high as those reported by Read et al (1965a). The diets of the Pakistani mothers in this study had a similar amount of carbohydrate as the Tanganyikans (70%).

Vuori et al (1982) did not find any correlations between diet and these fatty acids. The study of Finley et al (1985a) also failed to find a relationship between carbohydrate and C12:0 but simple regession analysis did reveal a positive correlation with C14:0 ($r = 0.15$). Multivariate analysis found dietary carboyhydrate level to be significantly related to C18:0 ($r = -0.22$) in that study. (Finely et al,

1985a). Vuori et al (1982) reported a significant negative correlation with C20:0 ($r = -0.375$, $p < 0.05$). In the present study, carbohydrate intake was not found to be related to levels of C18:0 (Table 22) or to C20:0. Scatter diagrams of these fatty acids (Wt.%) plotted against carbohydrate (g/day) are found in Figures 16 and 17.

Dietary carbohydrate was negatively correlated with C18:2 ($r = -0.530$, $p = 0.024$) and total polyunsaturates ($r = -0.531$, $p = 0.023$). Regression lines and coefficients for these relationships were calculated and are presented in Figures 18 and 19. The data provided by Lauber and Reinhardt (1979) for milk from Ivory Coast women and by Underwood et al (1970) for Pakistani women revealed lower than Western averages for C18:2. Although correlations were not performed on the data, mothers in both studies consumed high carbohydrate diets (70 to 75% of energy) which may suggest an inverse relationship as found in the present study.

The only fatty acid found to correlate with total energy intake was C12:0 (Wt.%). As shown in Table 22, a significant positive relationship was found ($r = 0.495$, $p = 0.036$). This association, demonstrated in Figure 20, was probably a reflection of carbohydrate intake, which averaged 51% of total energy intake. Correlation analysis between dietary carbohyrate and total energy intake revealed a strong relationship ($r = 0.812$, $p = 0.0001$). Kneebone et al (1985) inferred the inverse of this finding. They observed highest levels of both C12:0 and C14:0 in the milks of Malay and Indian mothers who also consumed diets lowest in energy. These women consumed much greater amounts of dietary saturated fatty acids which was reflected in their milks. The mothers in this

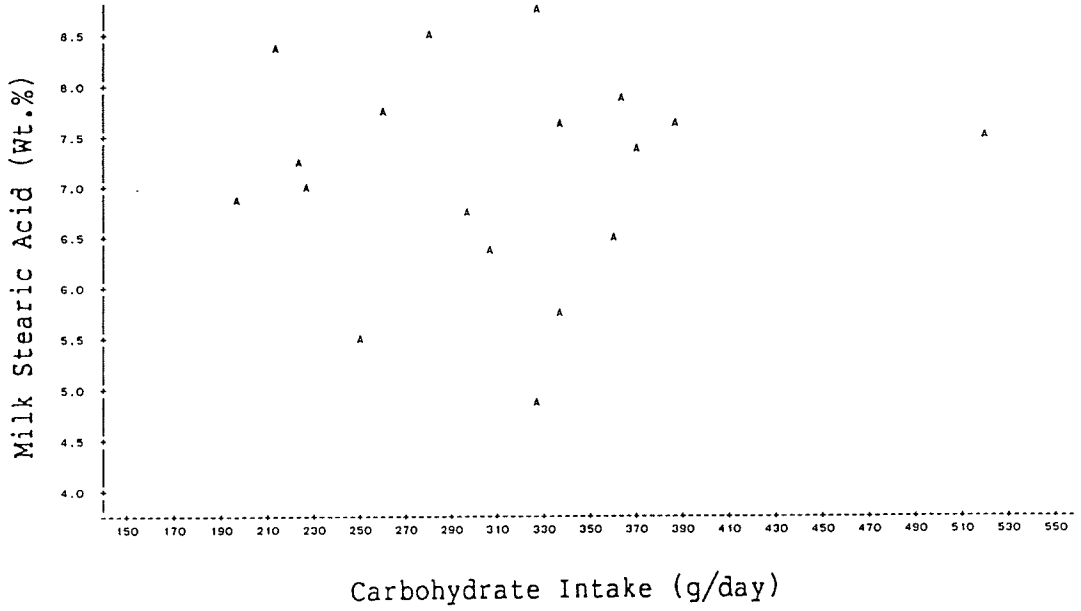

Figure 16: The Relationship Between Dietary Carbohydrate (g/day) and C18:0 (Wt.%) of Donor Milks

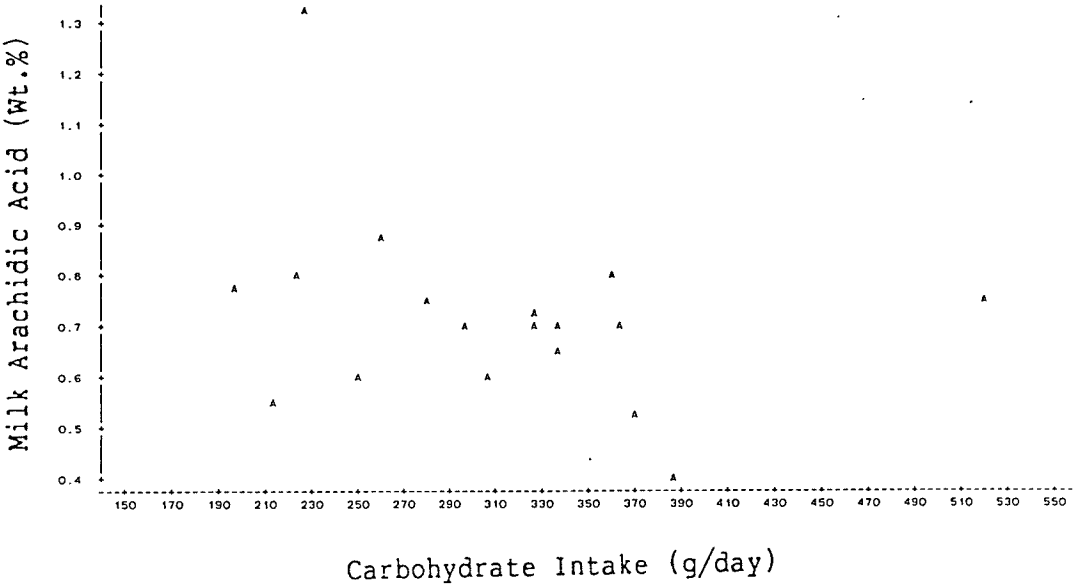

Figure 17: The Relationship Between Dietary Carbohydrate (g/day) and C20:0 (Wt.%) of Donor Milks

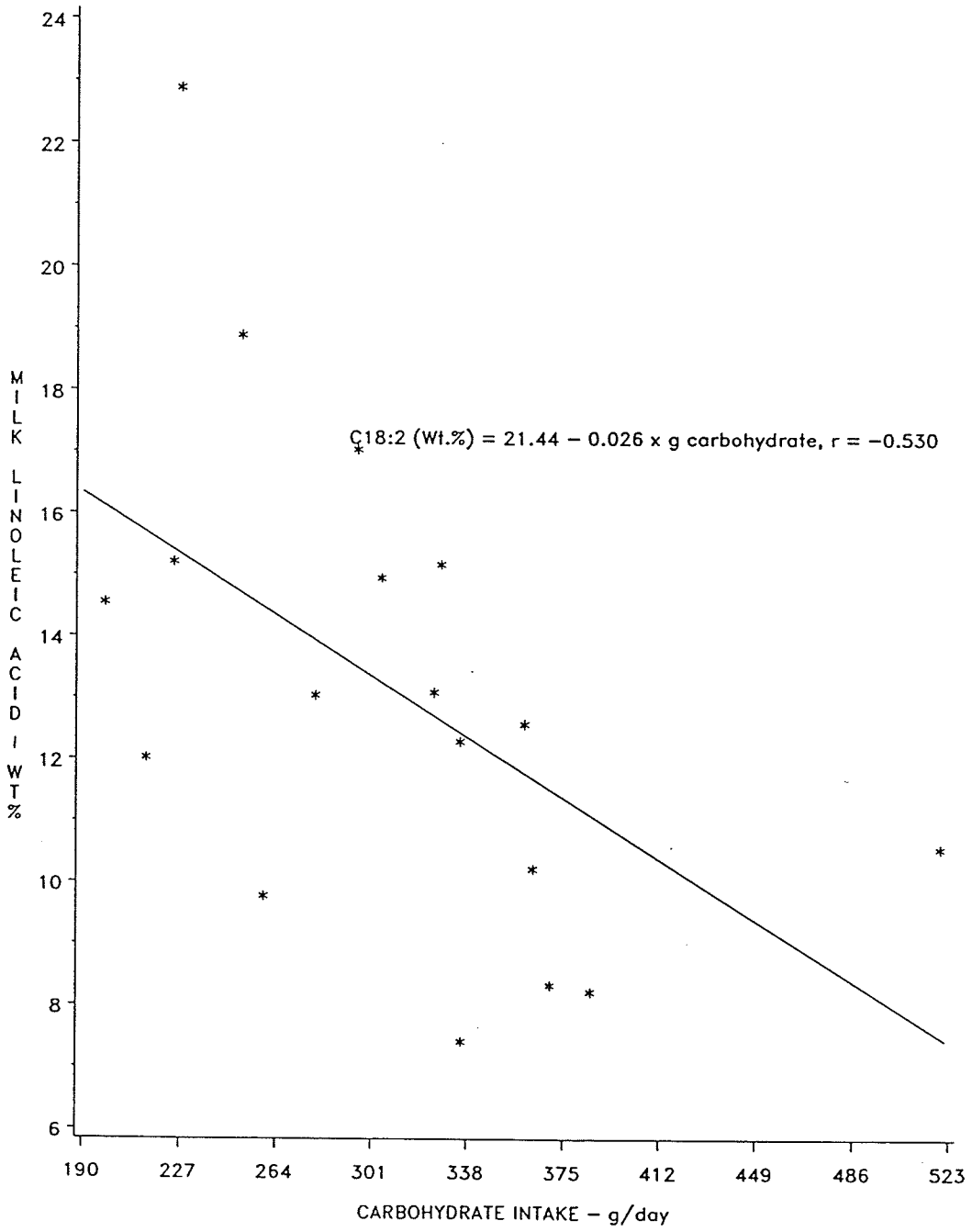

Figure 18: The Relationship Between Dietary Carbohydrate (g/day) and C18:2 (Wt.%) of Donor Milks

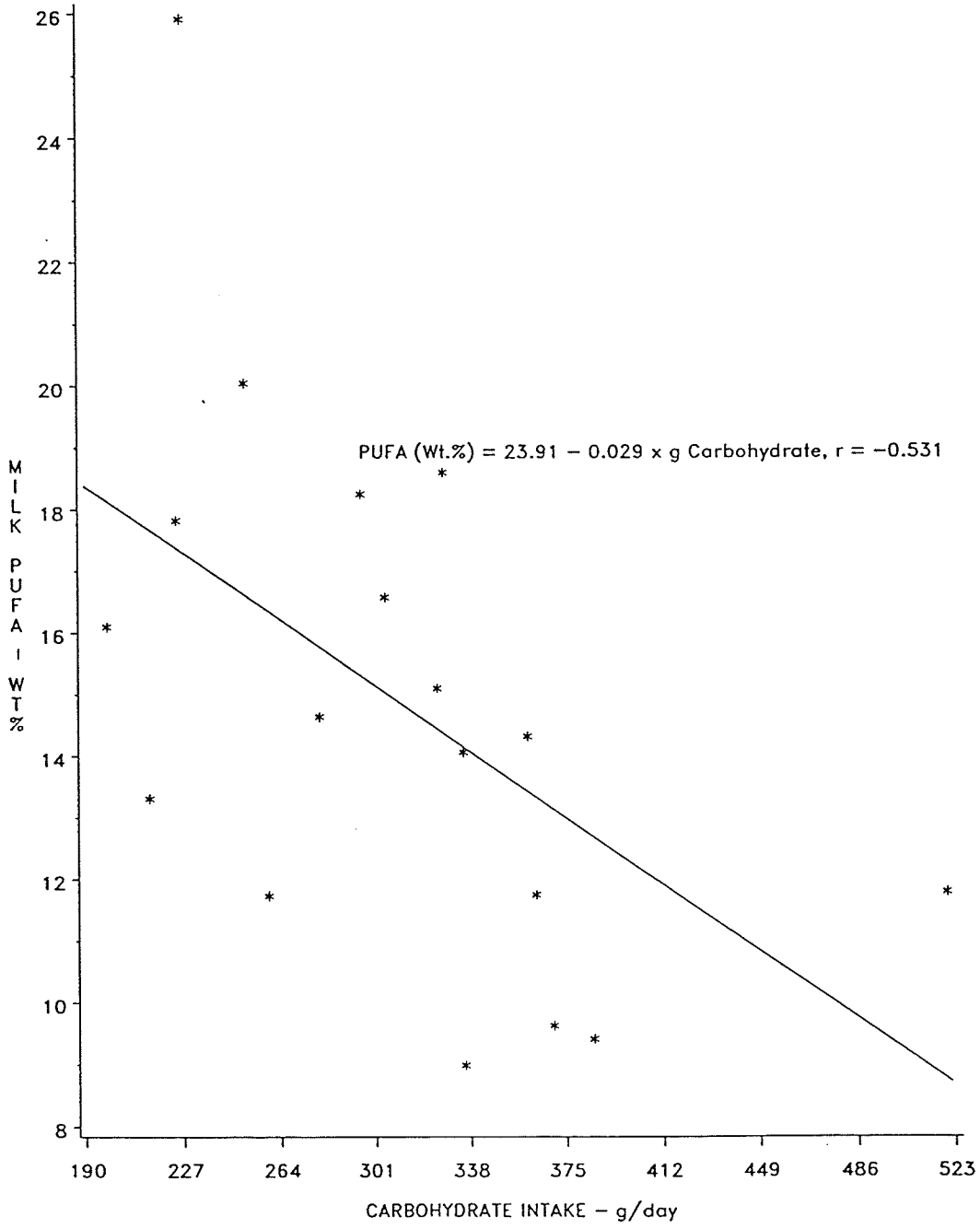

Figure 19: The Relationship Between Dietary Carbohydrate (g/day) and Total Polyunsaturated Fatty Acids (Wt.%) of Donor Milks

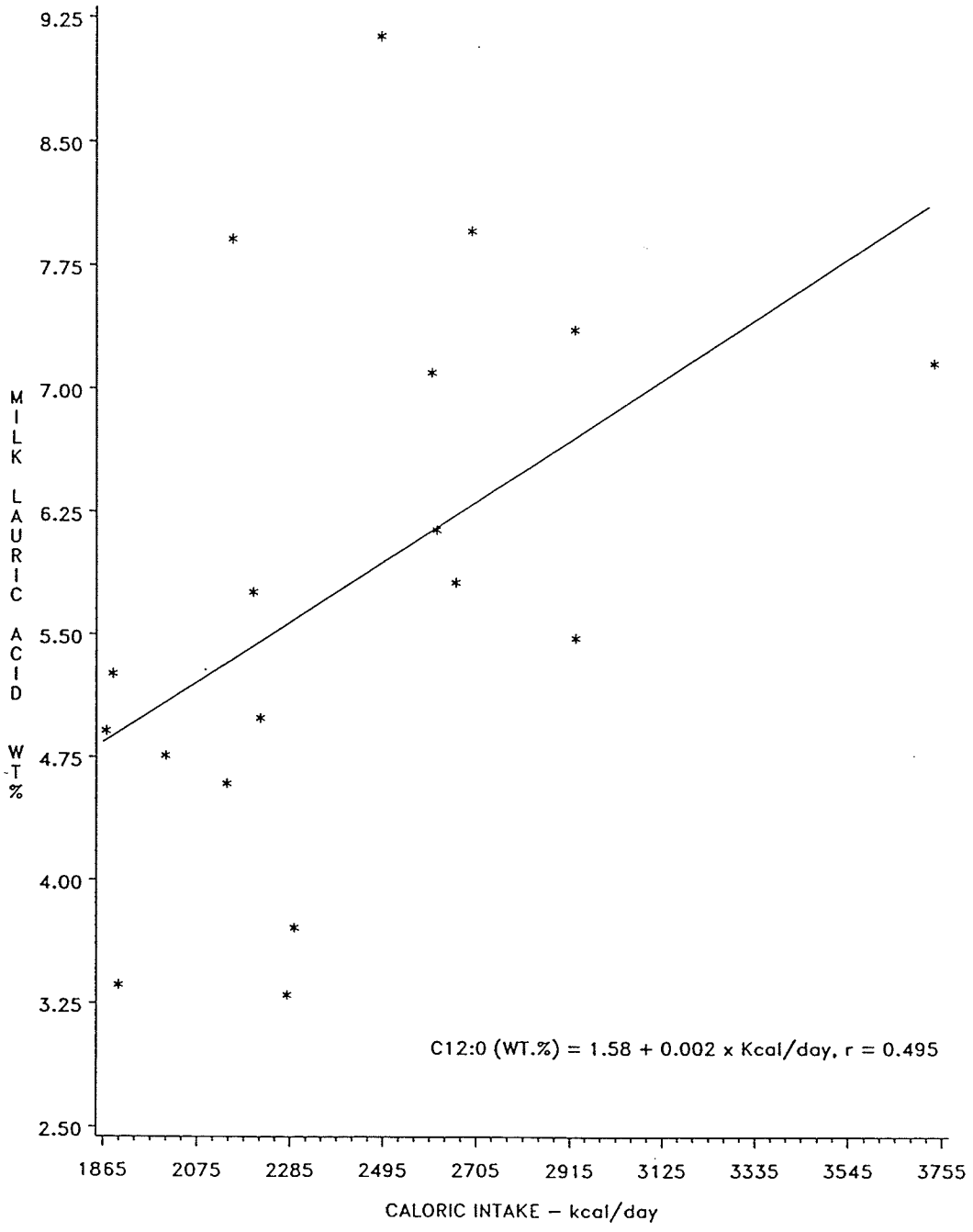

Figure 20: The Relationship Between Energy Intake (kcal/day) and C12:0 (Wt.%) of Donor Milks

study found to have low levels of C12:0 and C14:0, consumed more energy but also high levels of polyunsaturated fatty acids (Kneebone et al (1985). Thus, the type of fat consumed, and not total energy intake, may have,in fact, had more effect on fatty acid composition of the milks.

4.5.2.2 Dietary Fatty Acid Effect on Milk Fatty Acid Composition

Pearson's correlation coefficients were used to determine associations between fatty acid intake and fatty acid composition of the donor milks. Simple regression analysis was used to further substantiate any correlation results found to be significant. Presented in Tables 24 and 25 are the results of the correlation analysis. The milk fatty acid data in Table 24 are expressed as weight percents of total methyl esters. The data in Table 25 are expressed as grams per deciliter milk.

Correlation analysis failed to find significant relationships between milk fatty acids and total dietary saturated and monounsaturated fatty acids. This is in contrast to the results of Vuori et al (1982) who reported a significant correlation between total dietary and milk saturates (Wt.%) ($n = 33$, $r = 0.363$, $p < 0.05$). Figure 21 demonstrates the wide scatter of data points when dietary and milk saturated fatty acids from the present study were plotted. Relationships between milk fatty acids and total dietary monounsaturated fatty acids were not given in the Vuori et al (1982) study. They did observe a positive association between total amount of polyunsaturated fatty acids of maternal diet and breast milk ($n = 33$, $r = 0.448$, $p = < 0.01$). A correlation between these variables was not found in the present study ($r = 0.421$, $p = 0.082$) as evident in Figure 22.

TABLE 24

Pearson's Correlation Coefficients (r) Between Milk Fatty Acids (Wt.%)† and Fatty Acid Components of Donors' Diets

Milk Fatty Acid Wt.%	Dietary Component			
	Saturates (g/day)	Monounsaturates (g/day)	Polyunsaturates (g/day)	C18:2 (g/day)
C12:0	0.258 (0.301)*	0.158 (0.531)	0.236 (0.344)	0.122 (0.631)
C14:0	0.394 (0.106)	0.082 (0.745)	0.024 (0.926)	-0.078 (0.758)
C16:0	0.076 (0.762)	-0.307 (0.214)	-0.514 (0.029)**	-0.514 (0.028)**
C18:0	0.312 (0.206)	-0.038 (0.880)	-0.102 (0.686)	-0.107 (0.671)
Total Saturates	0.354 (0.150)	-0.097 (0.701)	-0.199 (0.428)	-0.257 (0.302)
C18:1	-0.266 (0.284)	0.036 (0.885)	-0.058 (0.819)	-0.212 (0.397)
Total Monounsaturates	-0.334 (0.174)	-0.086 (0.734)	-0.169 (0.502)	-0.316 (0.200)
C18:2	-0.098 (0.699)	0.303 (0.222)	0.450 (0.054)	0.611 (0.007)**
Total Polyunsaturates	-0.108 (0.668)	0.234 (0.347)	0.421 (0.082)	0.601 (0.008)**

† Wt.% of methyl esters
* Probability in parentheses
** Statistically significant, $p < 0.05$

TABLE 25

Pearson's Correlation Coefficients (r) Between Milk Fatty Acids (gm/dL)† and Fatty Acid Component of Donors' Diets

Milk Fatty Acid gm/dL	Dietary Component			
	Saturates (g/day)	Monounsaturates (g/day)	Polyunsaturates (g/day)	C18:2 (g/day)
C12:0	-0.116 (0.645)*	-0.191 (0.446)	-0.036 (0.888)	0.044 (0.863)
C14:0	0.002 (0.991)	-0.181 (0.473)	-0.132 (0.601)	-0.064 (0.802)
C16:0	-0.071 (0.780)	-0.282 (0.256)	-0.284 (0.254)	-0.150 (0.552)
C18:0	-0.002 (0.993)	-0.218 (0.382)	-0.196 (0.436)	-0.044 (0.860)
Total Saturates	-0.061 (0.810)	-0.252 (0.313)	-0.198 (0.429)	-0.072 (0.776)
C18:1	-0.250 (0.318)	0.299 (0.228)	-0.224 (0.371)	-0.086 (0.733)
Total Monounsaturates	-0.238 (0.340)	-0.312 (0.207)	-0.233 (0.352)	-0.092 (0.717)
C18:2	-0.188 (0.455)	-0.121 (0.632)	0.090 (0.720)	0.306 (0.217)
Total Polyunsaturates	-0.190 (0.451)	-0.156 (0.536)	0.056 (0.824)	0.272 (0.273)

† Wt.% of methyl esters converted to g fatty acids/dL milk.
* Probability in parentheses

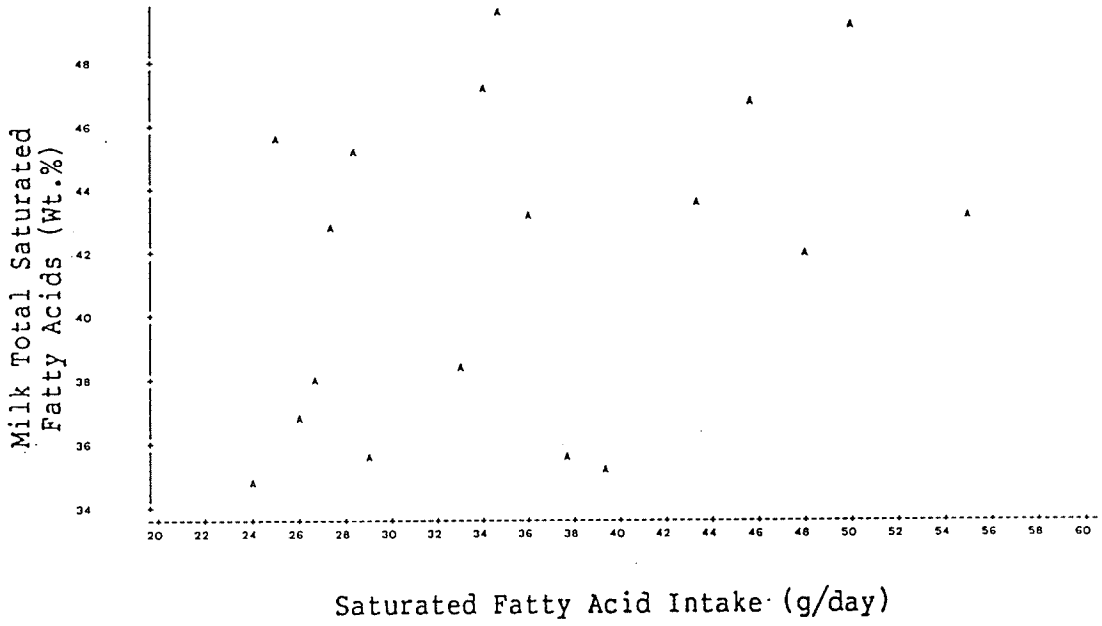

Figure 21: The Relationship Between Dietary Saturated Fatty Acids (g/day) and Total Saturates (Wt.%) of Donor Milks

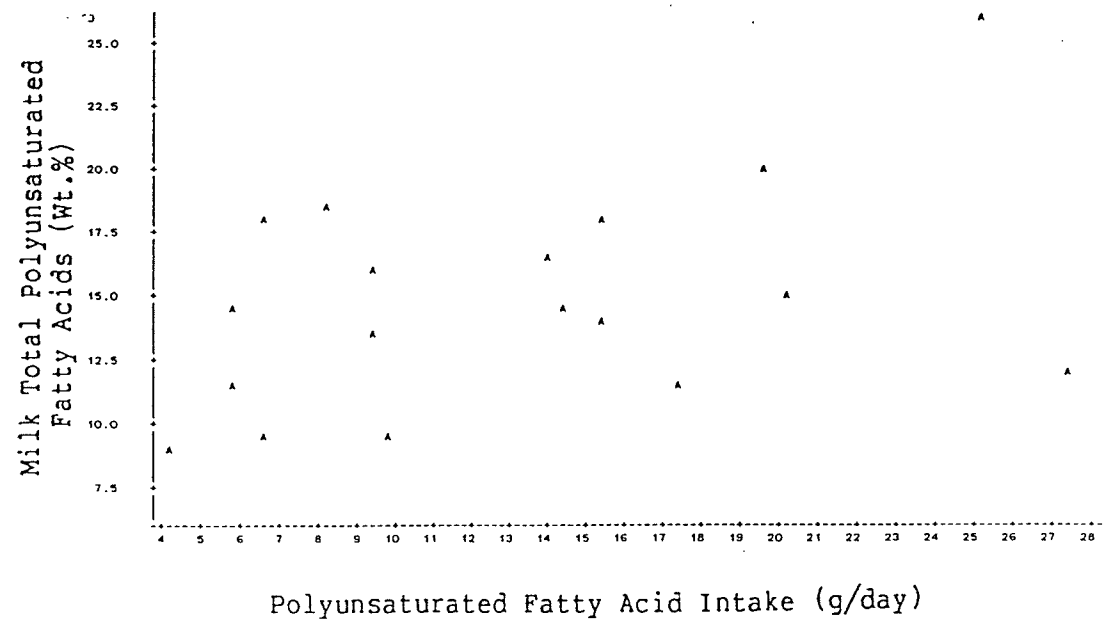

Figure 22: The Relationship Between Dietary Polyunsaturated Fatty Acids (g/day) and Total Polyunsaturated (Wt.%) of Donor Milks

Significant Pearson's correlations were observed only when milk fatty acids were expressed as weight percents of methyl esters (Table 24). Two significant negative associations were found. Milk palmitic acid was negatively correlated with both dietary C18:2 and total polyunsaturates. Presented in Figure 23 is the relationship between total polyunsaturates and C16:0 ($r = -0.514$, $p = 0.029$). Figure 24 displays the association between dietary C18:2 (g/day) and milk C16:0 ($r = -0.514$, $p = 0.028$).

A search through the literature failed to find studies reporting relationships between milk C16:0 and dietary fatty acids. Finley et al (1985a) did, however, observe a negative correlation between C16:0 and vegatable fat intake, a high source of dietary linoleic acid. Had the investigators preformed correlation analysis directly between C16:0 and dietary C18:2, a negative relationship, as observed in the present study, might have been found.

There were only two significant positive relationships found between fatty acid intake and milk fatty acid composition (Table 24). Figure 25 shows the relationship found between dietary C18:2 and total milk polyunsaturates ($r = 0.601$, $p = 0.008$). The association between dietary and milk C18:2 ($r = 0.611$, $p = 0.007$) is presented in Figure 26. Intake of C18:2 explained 37.4% ($R^2 = 0.374$) of the variation in milk C18:2. Dietary C18:2 of Finnish women explained a slightly smaller percentage (27%) of the variation in milk C18:2 levels (Vuori et al, 1982).

Linoleic acid content of breast milk has been shown to respond quickly to increased concentrations of dietary polyunsaturated fatty

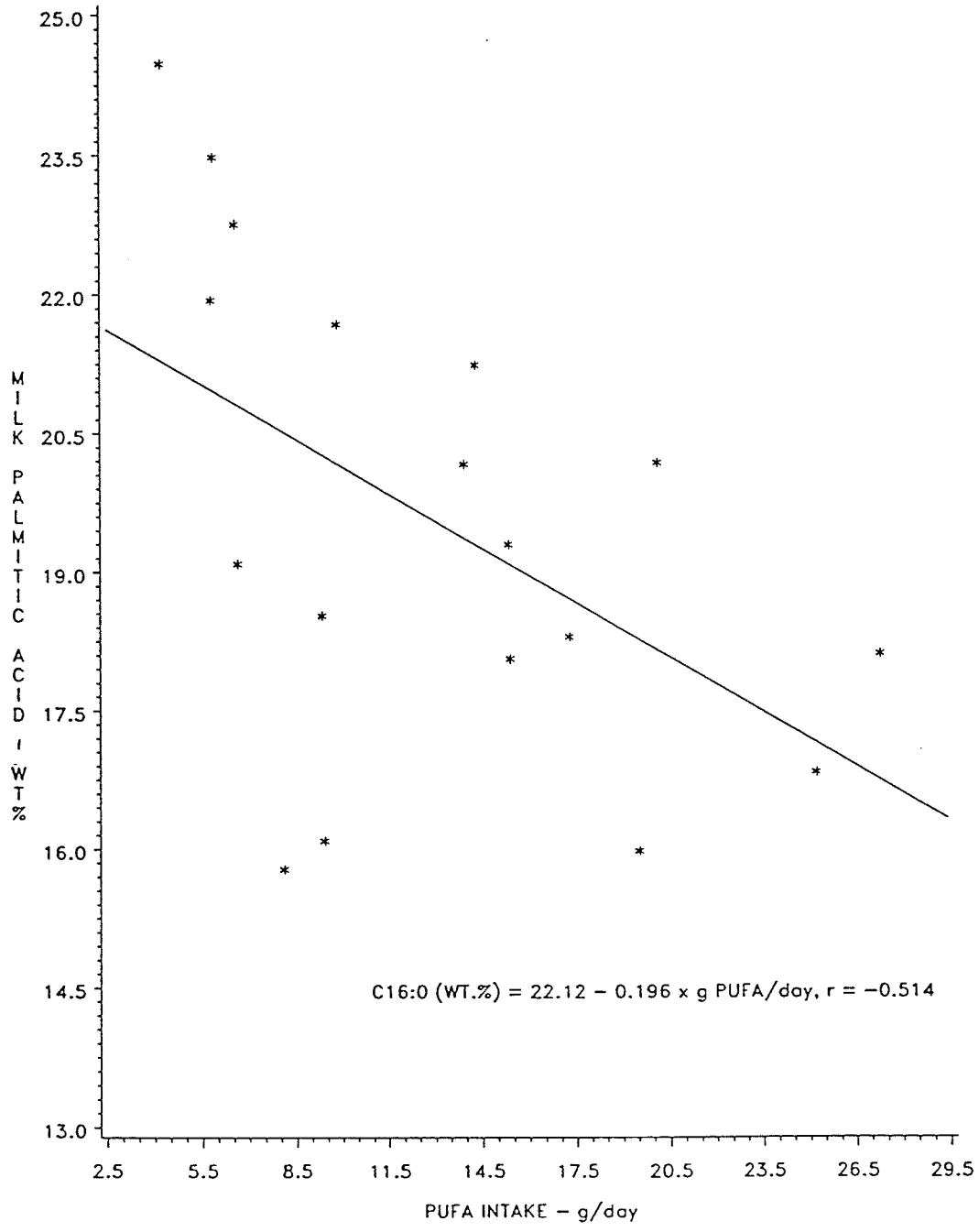

Figure 23: The Relationship Between Dietary Polyunsaturated Fatty Acids (g/day) and C16:0 (Wt.%) of Donor Milks

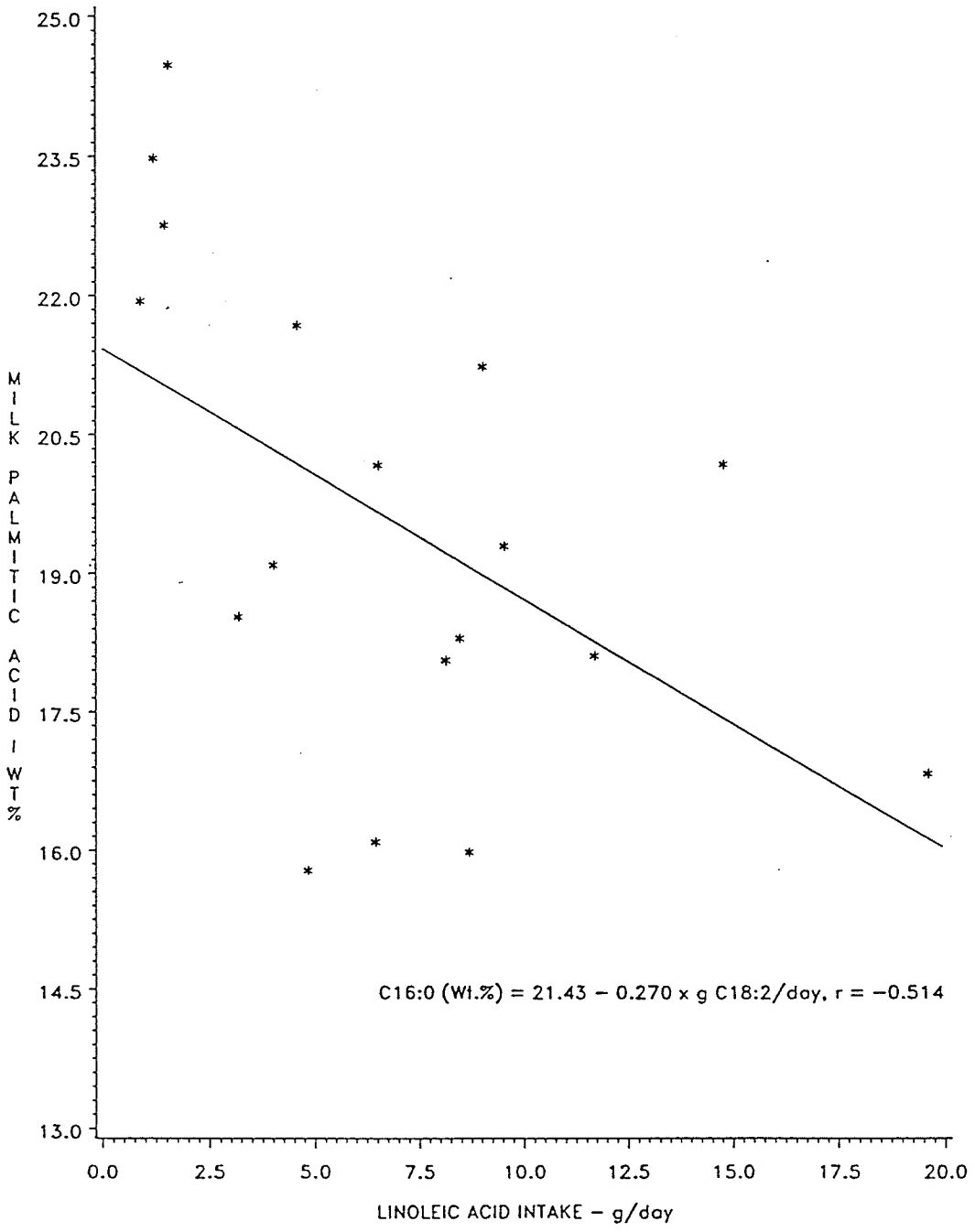

Figure 24: The Relationship Between Dietary C18:2 (g/day) and C16:0 (Wt.%) of Donor Milks

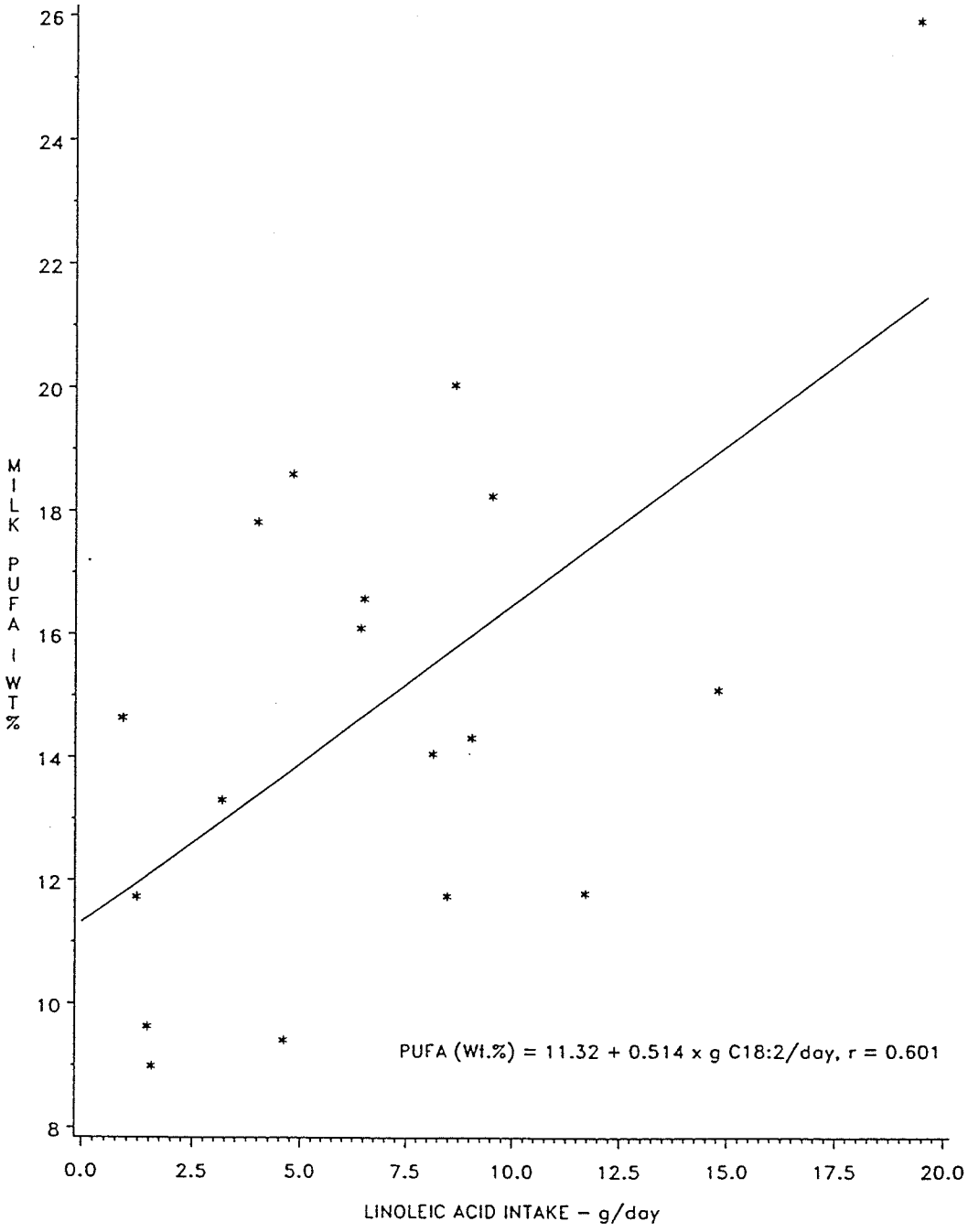

Figure 25: The Relationship Between Dietary C18:2 (g/day) and Total Polyunsaturated Fatty Acids (Wt.%) of Donor Milks

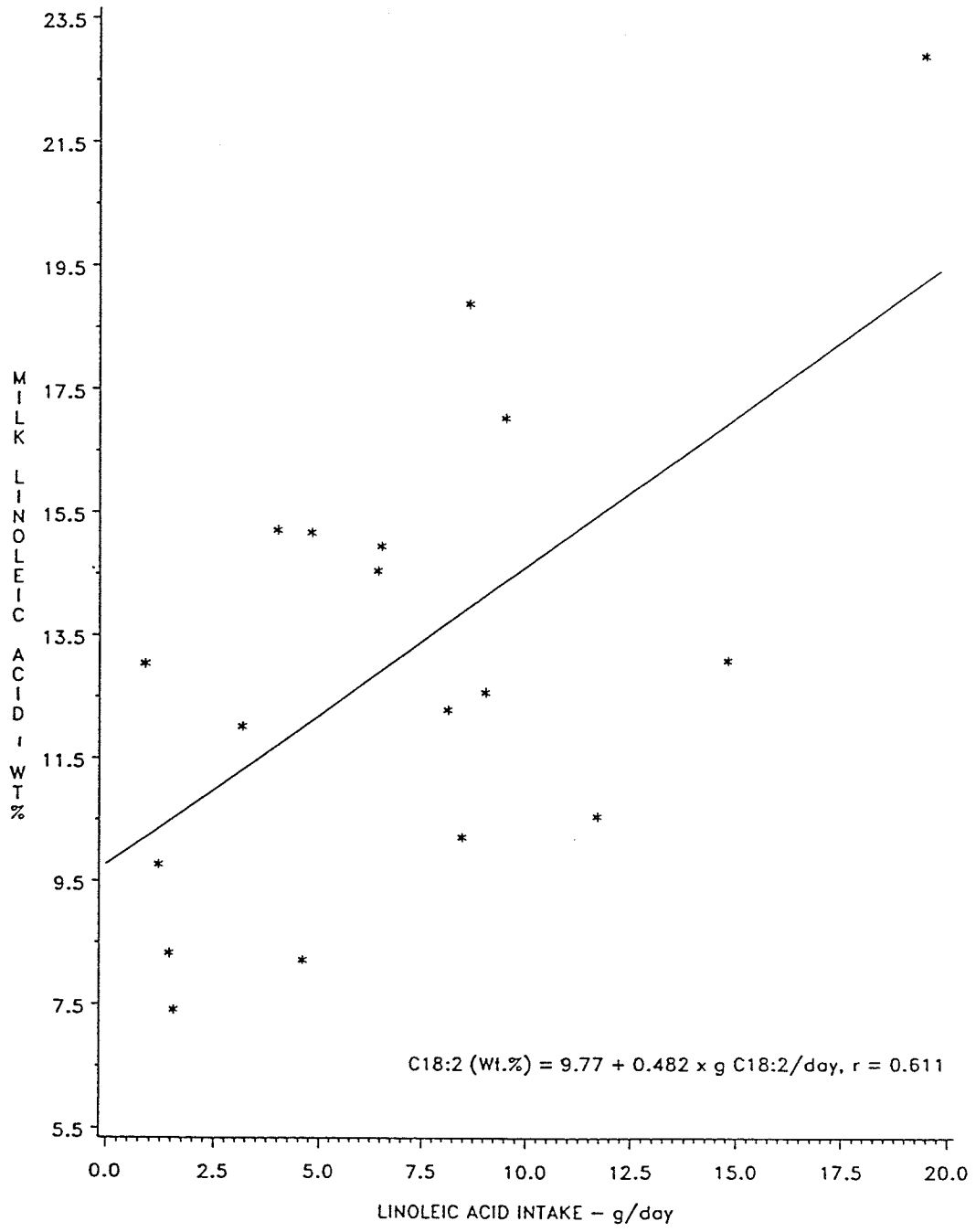

Figure 26: The Relationship Between Dietary C18:2 (g/day) and C18:2 (Wt.%) of Donor Milks

acids (Insull et al, 1959; Welby et al, 1973; Potter and Nestel, 1976; Mellies et al, 1978). Vuori et al (1982) reported a slighlty lower positive correlation between ad libitum intake of C18:2 and milk C18:2 than found in the present study ($n = 33$, $r = 0.515$, $p < 0.01$). Higher proportions of C18:2 have been found in the breast milk of vegetarians compared to that of non-vegetarians, whose dietary intakes differ substantially in linoleic acid intake (Sanders et al, 1978; Finley et al, 1985a). A significant positive correlation between vegetable fat intake and C18:2 ($r = 0.53$, $p \leq 0.05$) was reported by Finley et al (1985a).

It was interesting to note that donor 7 had the highest intake of linoleic acid (19.66 g) and also the largest percentage of C18:2 in her milk (22.89%). As shown in Figure 26, this caused a noticeable outlier when the data points of all donors were plotted together. Not surprisingly, then, removal of donor 7 from correlation analysis resulted in a non-significant relationship between dietary and milk C18:2 ($n = 17$, $r = 0.362$, $p = 0.152$). The R^2 value of this relationship was calculated at 0.132, a reduction of 65% from the previous data. The change in the regression line using this data is illustrated in Figure 27. To clearly illustrate the change in the slope of the line, the length of each axis was unaltered from that used in Figure 26. Therefore, although a significant correlation between dietary and milk C18:2 was found for the 18 donors, the results must be interpreted and compared to other research findings with caution due to the poor dietary estimates for C18:2 and small sample size. Correlation between dietary polyunsaturated fatty acids, which represented a much better estimate of dietary

intake (Appendix F, Table 26), and milk C18:2 was very close to a 5% level of significance ($p = 0.054$).

As previously mentioned in subsection 4.3.2, dietary P/S ratio ranged from 0.12 to 0.64. The P/S ratio for the group averaged 0.36. Milk P/S ratio varied from 0.17 to 0.72, with the average P/S found to be 0.36. This was calculated using the sum of C18:2 + C18:3 + C20:4 (expressed as weight percents of methyl esters) divided by total saturated fatty acids, expressed as weight percents of methyl esters. A significant correlation was found between dietary and milk P/S ratios ($r = 0.564$, $p = 0.014$). This relationship is further illustrated in Figure 28. Aitchison et al (1977) determined the P/S ratios in early morning milk samples and diet using C18:2 and total saturated fatty acid values. A significant correlation was also found ($n = 27$, $r = 0.43$, $p < 0.05$). Calculated using only C18:2 and total saturated fatty acids to determine milk P/S in the present study, a correlation with dietary P/S revealed a coefficient of 0.592, $p = 0.01$. Diet and milk P/S ratios were 0.30 in the study of Vuori et al (1982) and a positive correlation between these ($n = 33$, $r = 0.456$, $p < 0.01$) was also reported.

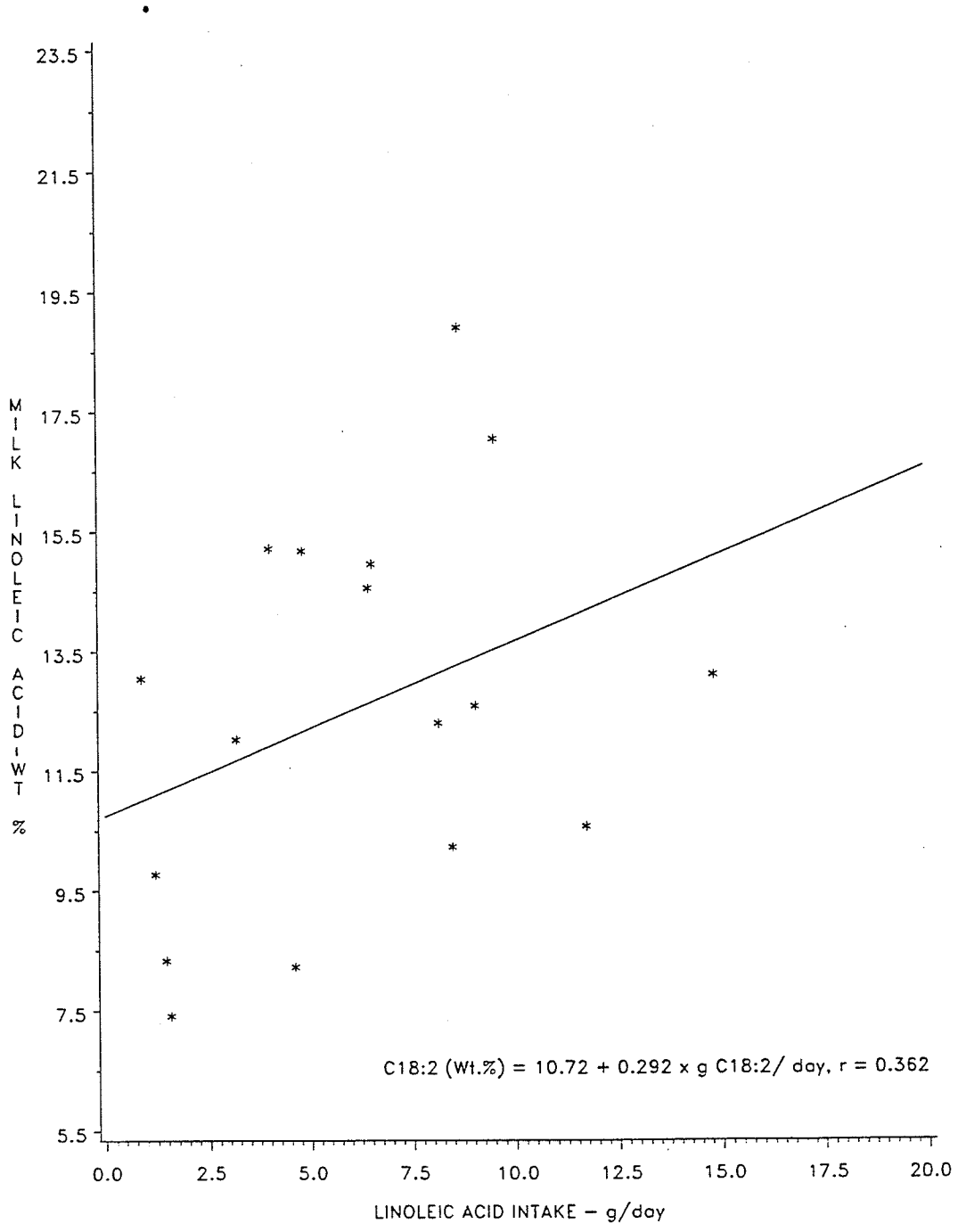

Figure 27: The Relationship Between Dietary C18:2 (g/day) and Milk C18:2 of 17 Donor Milks

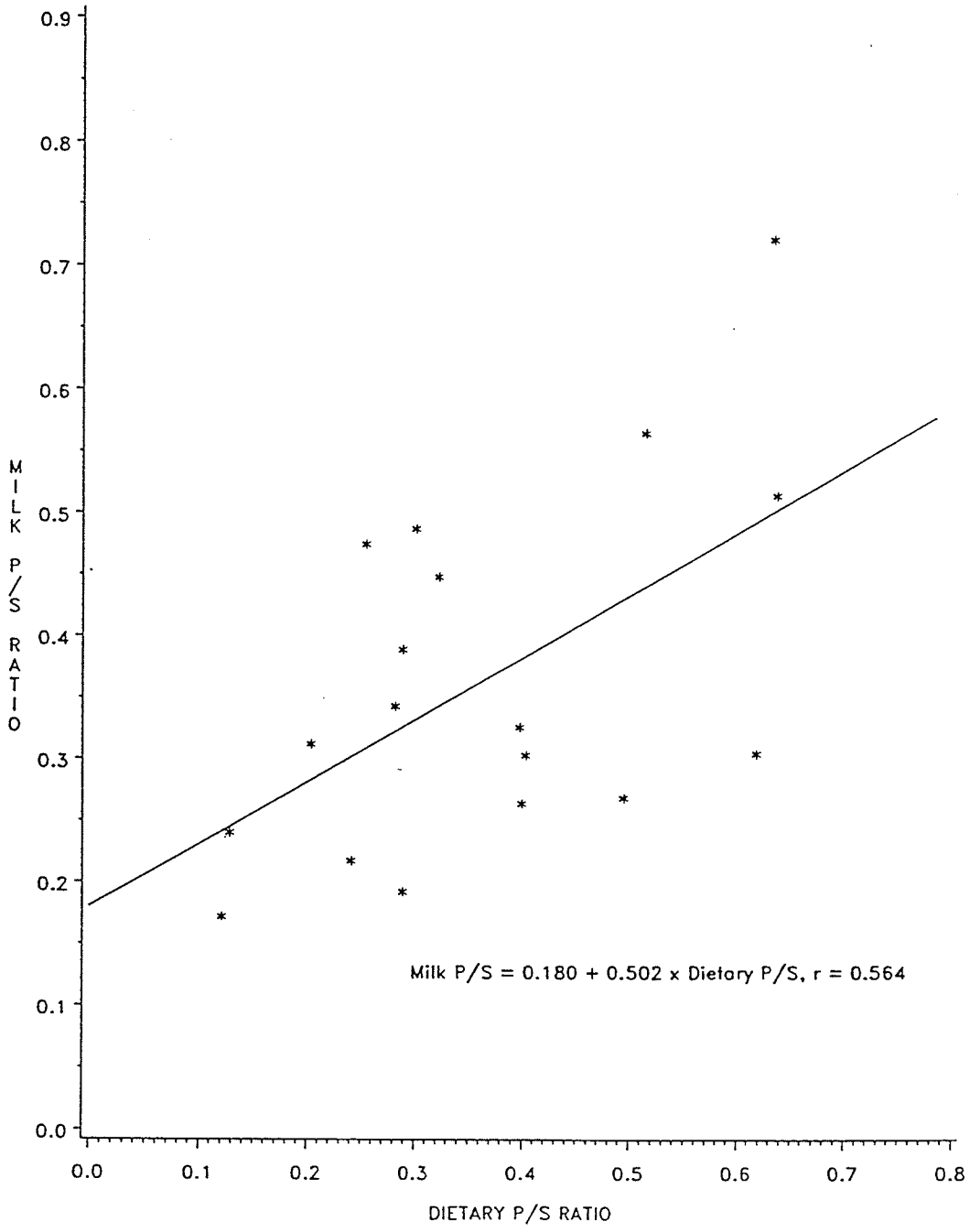

Figure 28: The Relationship Between Dietary and Milk P/S Ratios

4.6 DIETARY EFFECTS ON TRANS FATTY ACID COMPOSITION OF DONOR MILKS

Correlation analysis between dietary and milk total trans-fatty acids failed to reveal a significant relationship ($r = 0.354$, $p = 0.150$). Donors were divided into one of two groups based on use of tablespreads (butter versus margarines) and dietary trans-fatty acid levels were found to be significantly different for these two groups ($t = -3.974$, $p = 0.002$). Percent total milk trans-fatty acid was not, however, different between these groups ($t = -1.876$, $p = 0.079$) nor were there correlations found between each group and milk total trans-fatty acids. These results were not unexpected given both the small sample size and the limitations of dietary analysis. A scatter diagram clearly illustrating the lack of correlation between dietary and milk trans-fatty acids is found in Figure 29.

Associations between dietary and milk trans-fatty acids have been reported in several previous investigations. The sum of percent trans-C16:1 plus trans-C18:1 was significantly correlated ($r = 0.48$, $p \leq 0.001$) with percent margarine consumption in the study of Finley et al (1985a). Aitchson et al (1977) found significant correlations between dietary trans and C18:1t and total trans-fatty acids in milk samples collected the same evening ($p < 0.01$) but not with samples obtained the following morning. Contrary to this, Craig-Schmidt et al (1984) observed milk trans-fatty acids, particularly C18:1t, to be highly correlated ($r = 0.91$) with C18:1t in the diet of the previous day.

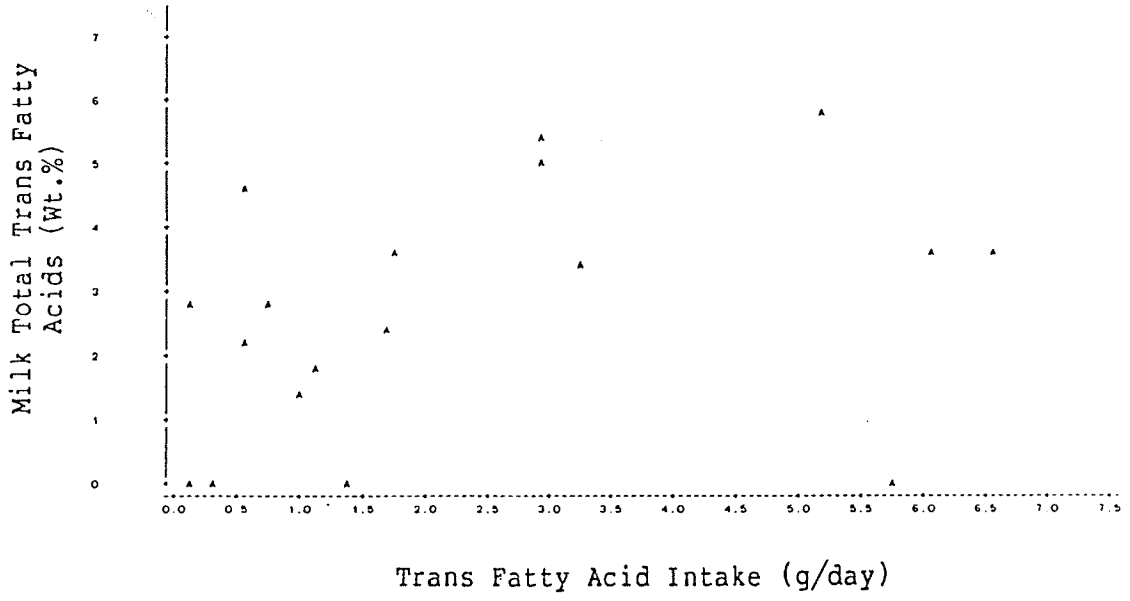

Figure 29: The Relationship Between Dietary Trans Fatty Acids (g/day) and Total Trans Fatty Acids (Wt.%) of Donor Milks

4.7 DIETARY EFFECTS ON TOCOPHEROL CONTENT OF DONOR MILK

Donor intake of total tocopherol averaged 12.68 mg/day as indicated in subsection 4.3.4. This figure was found to be lower than intakes reported for lactating mothers in other investigations, not unexpected, given the limitations of the nutrient database used for analysis. Correlation analysis between dietary total tocopherol and milk α-tocopherol failed to find a significant relationship ($r = 0.156$, $p = 0.536$). Figure 30 demonstrates this lack of association. Correlations between total dietary fat, dietary fatty acids, and percent fat and energy from the various food groups (Appendix F, Table 26) with milk α-tocopherol content also revealed lack of association.

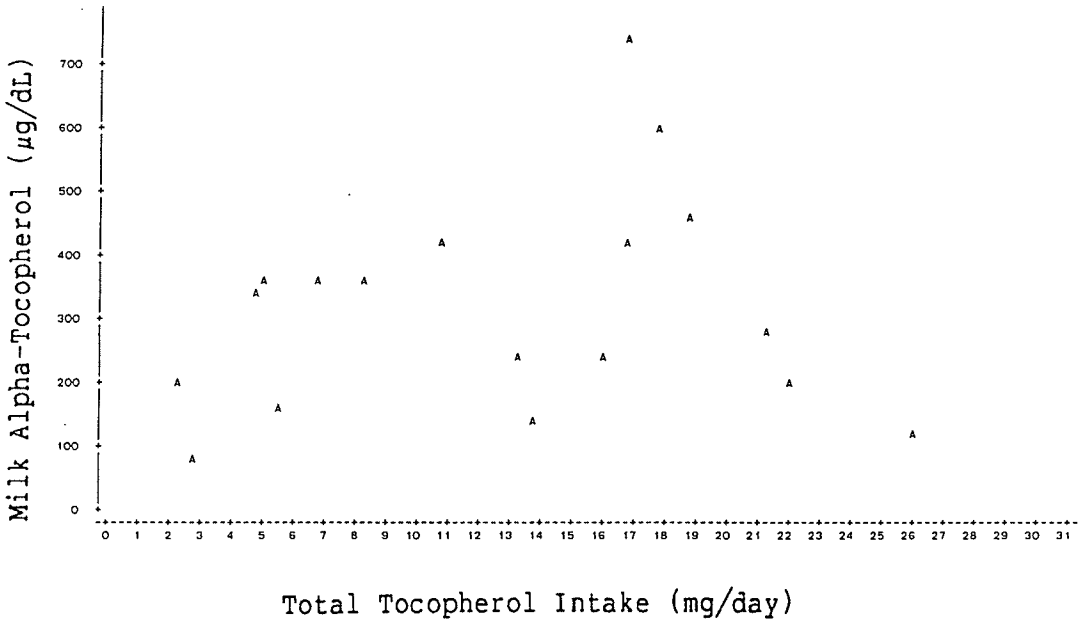

Figure 30: The Relationship Between Dietary Total Tocopherol (mg/day) and α-Tocopherol (µg/dL) of Donor Milks

In 1975, Filer stated that vitamins soluble in milk fat were not believed to be significantly altered by maternal diet. In the present study, total milk fat was not found to be influenced by any dietary variable. Given this finding and the problems with dietary analysis of tocopherol, a fat soluble vitamin, the lack of association between diet and milk was not surprising. Other investigators to date have also failed to find a significant correlation between dietary and milk tocopherol (Chappell et al, 1985b; Ali et al, 1986).

Chapter V

SUMMARY AND CONCLUSIONS

A total of 18 donors from the HSC Breast Milk Programme participated in this study. Only one milk sample was obtained from each donor due to ethical contraints at the time. All milk samples were collected during the first feed of the morning and represented an aliquot of actual milk fed to premature infants at HSC. Food records were kept by each donor for the three days prior to milk collection. This study was designed to examine the variability of fat, fatty acid, and tocopherol content of individual donor milks. The relationships between ad libitum maternal intake and milk composition were also studied.

Levels of macronutrients in the donor milks generally fell within previously reported ranges. Mean lactose content was slightly higher than found in other studies but was found to vary the least compared to other macronutrients.

Fat content varied widely from one donor milk to another but mean concentration was similar to previous reports, many of which obtained samples at different times during the day. As mentioned above, samples in the present study were collected at the first feed of the morning. Early morning samples have been noted for their low fat contents. Sampling procedure for the present study was carried out in a manner designed to obtain representative milk aliquots fed to infants at HSC. Donors were instructed to first express milk for HSC, thoroughly shake

the sample and pour off an aliquot for this study. It was thought that such a procedure would lead to collection of both 'fore' and 'hind' milk. Low fat levels reported for early morning collections in several previous studies may have contained more 'fore'(low fat) than 'hind' milk. Although samples were not collected over time, it was interesting to find a positive correlation between fat content and weeks lactation. Energy content, also highly variable, was observed to be strongly influenced by total fat content of the donor milks.

Donor milks were analyzed for a large number of fatty acids including several trans-fatty acids. Saturated fatty acids constituted approximately 40% of the total fatty acids found in the milks. Levels of individual saturated fatty acids appeared to be within previously published ranges. Monounsaturated fatty acids contributed another 42% to the total fatty acids. Oleic acid (C18:1) accounted for the largest proportion of the monounsaturates and varied little among the individual donor milks in comparison to many other fatty acids. Linoleic acid (C18:2) ranged from 7.4 to 22.8% in the milks. Mean C18:2 content was 13.1%. This level was similar to that observed in a 1982 American report and slightly higher than levels found for British, German, and Australian milks. When converted to absolute amounts using the equation of Posati et al (1975), which includes total lipid, the wide range of milk fatty acids among donors became more pronounced. It is important to note that such variability would have a marked effect on the absolute amounts of fatty acids in milks fed to premature infants.

Donor milks were also analyzed for α-tocopherol content. A large degree of variation between samples was found. Values ranged from 85.0

to 732.5 μg/dL. Mean content of 317.2 μg/dL was greater than in many early studies but fairly similar to a 1981 Swedish investigation. Ten of the 18 individual milks failed to meet the recommendation of the Canadian Paediatric Society (1981) that milks fed to low-birth-weight (LBW) infants contain 0.5 mg α-tocopherol/ 100 kcal. Small preterm infants have high requirements for vitamin E. High intakes of polyunsaturated fatty acids lead to increased requirements. Thus, the ratio of tocopherol to PUFA is important to note in milks fed to preterm infants. A strong correlation was found between α-tocopherol (μmol/L) and C18:2 (mmol/L) in the donor milks. Seven of the 18 individual milks, however, failed to meet the recommendation of the American Academy of Pediatrics (1977, 1985) and the Canadian Paediatric Society (1981) that milks for LBW infants contain at least 0.67 mg α-tocopherol/ g C18:2. Thus, the findings of this study regarding the variability in fat, fatty acid, and tocopherol content, support the current practice of nutrient supplementation for LBW infants fed human milk.

Nutrient intakes of the donors was assessed using the 1983 computer database from Health and Welfare Canada. As expected, diets varied considerably. Some nutrient intakes differed significantly when diets of donors interviewed during summer and winter periods were compared. The database used for analysis was limited in its individual fatty acid and tocopherol data and therefore, estimated intakes of these nutrients must be viewed with caution. If a subsequent study were to be undertaken, laboratory analysis of diets would be, unquestionably, more accurate, although more time-consuming and expensive.

The effects of ad libitum maternal intake on milk composition was examined. This study confirmed many reports that maternal diet does not influence total milk lipid levels. Correlation analysis between dietary variables and fatty acid composition of the donor milks revealed some interesting results. Only when milk fatty acids were expressed as weight percents of total methyl esters, were significant relationships found. The fact that total fat was included in the conversion of percent fatty acids to absolute amounts (g/dL) probably led to the lack of associations.

Both positive and negative significant correlations were observed. Most associations, however, were not strong, probably due to small sample size and the physiological interactions with diet. Carbohydrate intake by the donors was found to influence the medium chain fatty acids, C12:0 and C14:0 of the milks, which is in agreement with several other reports. The majority of these reports, however, found associations when intake of carbohydrate was substantially greater than that of the donors in the present study. Total saturated fatty acids also correlated positively with dietary carbohydrate. On the other hand, milk C18:2 and total polyunsaturated fatty acids correlated negatively with dietary carbohydrate. Energy intake, which was highly correlated with dietary carbohyrate ($r = 0.81$, $p = 0.0001$), was found to influence percent C12:0.

Dietary linoleic acid and total polyunsaturated fatty acid intake negatively correlated with one milk saturated fatty acid, C16:0. This direct association has not been reported elsewhere. Vegetable fat intake was, however, correlated with C16:0 in one study.

Milk and dietary C18:2 were positively associated and this correlation was the strongest of all those found ($r = 0.61$). This finding was in agreement with many other publications. The results of this correlation analysis must be viewed with caution as dietary C18:2 represented a poor estimate. A view of the data points scatter and the regression line revealed one very noticeable outlier. Removal of this value from correlation analysis resulted in a non-significant relationship. However, correlation between milk C18:2 and total dietary polyunsaturated fatty acids, which represented a much better estimate of dietary intake, was very close to approaching significance ($r = 0.054$). Dietary and milk P/S ratios were found to correlate positively.

Relationships between nutrient intake and milk trans-fatty acids and α-tocopherol content were not found. To date, there have been no reports of significant correlations between diet and milk tocopherol.

In general, the nutrient content of the donor milks was found to be highly variable. There was association found between milk composition and ad libitum carbohydrate intake but not with total fat intake. Associations between fatty acid intake and milk composition were somewhat inconsistent, some of which could be related to inadequacies of diet analysis and probably sample size. The composition of milk is thought to be the result of a balance between diet, metabolism, transport and secretion. The physiology of diet and lactation interrelationships must not be over-simpified and warrants further investigation.

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Appendix A

LETTER OF STUDY EXPLANATION

THE UNIVERSITY OF MANITOBA

FACULTY OF HUMAN ECOLOGY
Department of Foods and Nutrition

Winnipeg, Manitoba
Canada R3T 2N2

(204) 474-9901

October, 1982

Dear Donor:

I am at present conducting a study on the composition of human milk fat, and its relationship to sources of fat in the diet. It is known that high dietary levels of polyunsaturated fatty acids will raise the levels of these fatty acids in human milk. When this milk is fed to very small premature infants this may affect their requirement for Vitamin E. We would therefore like to know the approximate levels of the unsaturated fatty acids in the milk of Manitoba mothers.

I would appreciate your help in this study. Information gathered will be kept confidential by the investigator, and individual donors will not be identified when the results of this study are reported.

If you volunteer to take part in the study, I will contact you to arrange two(2) interview times with you. These interviews will be used to obtain information on foods eaten for three consecutive days. The first interview will provide direction on how to use the 3-day dietary record forms. The dietary record will be reviewed and collected during the second interview along with a one-ounce(25mls) sample of milk. Participation in this study will thus involve two visits by the interviewer. When analysis of the milk has been completed, the results of the analysis of your own milk will be available to you on request.

If you are interested in taking part in this study, please return the enclosed consent form to the Breast Milk Programme at the Health Sciences Centre. Your name and address will be forwarded to me, and you will be contacted within the next few weeks.

Thank you for considering participation in this research project.

Yours truly,

Judith Britten

Judith Britten, B.Sc.H.Ec.
Graduate Student

Appendix B

CONSENT FORM

I, _____, do hereby freely consent to participate in the study conducted by the Department of Foods and Nutrition at the University of Manitoba entitled "Fatty Acids in Human Milk". The conditions of the study have been fully explained to me by the experimentors and I understand them completely. I also understand that I am free to withdraw from the study without penalty and that all information will remain strictly confidential.

Signature _____

Date _____

Address: _____

_____

Phone: _____

Appendix C

GENERAL INSTRUCTIONS FOR DIETARY RECORDS

1. Please record ALL foods and beverages taken on the dates specified. These three consecutive days are designed as part of the study so please record your intakes on those days.

2. Eat as you ordinarily would if no records were being kept. DO NOT change your normal eating pattern.

3. Be sure to record everything you eat or drink whether you are at home, away from home or in a resturant. Record items as soon as possible after eating.

SUGGESTED WAY OF MEASURING FOODS/BEVERAGES IN YOUR DIET:

FOOD	DESCRIPTION	MEASURE
milk, cream, etc.	- whole, 2%, skim chocolate, half 'n' half, evaporated - in tea, coffee, on cereal	ounces, tablespoons cups, teaspoons
cereals	- type: dry, cooked brandname	cups, individual pkgs. (size), tablespoon
potatoes	- mashed, boiled, fried, french fries	cups, dimensions, number
vegetables	- type: canned, fresh frozen	cups, number of pieces
fruit	- type: canned, fresh frozen	number, dimensions, tablespoons
sugar	- type: white, brown, icing - in tea, coffee, on cereal	teaspoons tablespoons
biscuits, buns, rolls	- type: dinner, crusty, whole wheat, baking powder, Danish	number, dimensions

bread	- type: white, rye, whole wheat (60%, 100%),etc.	number of slices
meat	- type and method of cooking - cooking fat used	ounces, slice, piece & dimensions
condiments	- jam, jelly, peanut butter, salad dressing (type), mustard, etc.	teaspoons or individual packages
sweets	- type: chocolate, candy marshmallows	number, weight or dimensions
beverages	- type: soda, coke, dry wine, rum & coke	ounces list alcohol & mix separately
desserts	- type & flavor ie. peanut butter cookie cherry pie	slice, dimensions or number
salads	- list all ingredients	cups, number of pieces
snack foods	- type: potato chips, peanuts, pretzels, crackers	number of pieces, weight of package
fats and oils	- type: butter, oil, margarines Include brandname, soft (tub), brick	teaspoons
gravies and sauces	- type: flavor & ingredients	tablespoons ounces, cups
mixed or combination dishes	- type: list ingedients -pizza, chili, perogies, sandwhiches, casseroles	cups, slices, dimensions number

Appendix D

CLINICAL DATA QUESTIONNAIRE

Thank you for completing your three-day dietary record. As discussed during my first visit with you, I would now appreciate it if you would answer the following questions.

CODE # _____

1. What is the date of your birth? _____

2. What is your infant's date of birth? _____

3. How long have you been breastfeeding your infant? _____

_____

4. Is this your first child? _____

5. If no, how many other children do you have? _____

6. Do you have your infant on a set feeding schedule? _____

7. What is your schedule for expressing milk for HSC? (how often? time of day?)

_____

8. Are you at present taking any medications? _____

9. If yes, please identify them. _____

_____

10. Are you at present taking any vitamin or mineral supplements? _____

11. If yes, please give the type (ie., vitamin E, iron) and brandname.

_____

_____

12. If you take vitamin or mineral supplements, how often do you take them?

_____

13. Have you made any major changes to your diet since you became pregant?

_____

If so, please list these changes. _____

_____

_____

14. Now that you are breastfeeding, are these changes to your diet "still in effect?

_____

Appendix E

CONVERSION FACTORS FOR FATTY ACIDS OF MILK LIPIDS

FATTY ACID	CONVERSION FACTOR
C4:0	0.867
C6:0	0.897
C8:0	0.916
C10:0	0.929
C12:0	0.939
C14:0	0.947
C16:0	0.953
C18:0	0.958
C14:1	0.946
C16:1	0.953
C18:1	0.958
C18:2	0.957
C18:3	0.957
Other (includes C15:0, C17:0)	0.952

adapted from Posati et al (1975)

For fatty acids not identified above, the following equation was used to determine the conversion factor:

F =grams fatty acid (M.W.)/grams FAME X 1.005

where FAME = fatty acid methyl ester

C16:2	0.952
C20:0	0.962
C20:1	0.962
C20:4	0.961

Appendix F

NUMBER OF AVAILABLE VALUES IN FOOD GROUPS FOR SELECTED NUTRIENTS

TABLE 26

Number and Percent of Available Nutrient Values in the 1983 Nutrient Database by Food Group

Food Group	# food items	C18:2	Saturates	Mono*	PUFA**	Tocopherol†
Milk(a)	29	29 (96.6%)	28 (96.6%)	28 (96.6%)	28 (96.6%)	7 (24.7%)
Meat(b)	46	25 (55.6%)	44 (95.6%)	44 (95.6%)	44 (95.6%)	17 (37.0%)
Bread(c)	56	0 (0%)	43 (76.8%)	43 (76.8%)	43 (76.8%)	27 (48.2%)
Oils(d)	23	20 (87.0%)	23 (100%)	21 (91.3%)	21 (91.3%)	21 (91.3%)
F & V(e)	94	2 (2.1%)	94 (100%)	94 (100%)	94 (100%)	45 (52.1%)
Snack(f)	64	1 (1.6%)	57 (89.1%)	57 (89.1%)	57 (89.1%)	15 (23.4%)
Misc(g)	33	9 (27.3%)	28 (84.8%)	28 (84.8%)	28 (84.8%)	4 (12.1%)
Total	345	85 (24.6%)	317 (91.9%)	315 (91.3%)	315 (91.3%)	136 (39.4%)

* Monounsaturated fatty acids
** Polyunsaturated fatty acids
† Total tocopherol
a) Milk and milk products
b) Meat, fish, poultry, eggs
c) Breads, cereals, nuts
d) Food fats and oils
e) Fruits and vegetables
f) Snacks and desserts
g) Miscellaneous

Appendix G

CLINICAL DATA COLLECTED FROM INDIVIDUAL DONORS

TABLE 27

Age, Length of Lactation, and Parity of Individual Donors

Donor	Age (years)	Length of Lactation weeks (months)	Parity†
1	29	62 (14)	2
2	29	33 (7)	1
3	30	40 (7)	3
4	29	17 (4)	1
5	21	43 (10)	1
6	27	41 (9)	1
7	28	36 (8)	1
8	24	19 (5)	1
9	25	16 (3)	2
10	21	20 (5)	1
11	26	61 (14)	1
12	26	71 (16)	1
13	34	16 (4)	1
14	30	27 (6)	1
15	22	11 (3)	1
16	30	12 (3)	1
17	29	30 (7)	1
18	21	16 (4)	2

† indicates total number of children including infant presently being breast-fed.

TABLE 28

Milk Expression Routine Followed by Individual Subjects

Donor	Milk Expression Schedule for HSC (# times/day)	Method of Expression (manual, pump)
1	2/day (a.m., p.m.)	manual, one breast
2	1/day (a.m.)	manual, one breast
3	2/day (a.m., p.m.)	manual, both breasts
4	2/day (a.m., p.m.)	manual, one breast
5	1/day (a.m.)	manual, one breast
6	1/day (early evening)	manual, one breast
7	1/day (a.m.)	manual, one breast
8	1/day (a.m.)	manual, one breast
9	1/day (a.m.)	manual, one breast
10	1/day (a.m.)	pump, both breasts
11	1/day (a.m.)	manual, one breast
12	2/day (a.m., p.m.)	manual, both breasts
13	1/day (a.m.)	manual, one breast
14	4/day (a.m., noon, early evening, late p.m.)	manual, both breasts
15	2/day (a.m., p.m.)	manual, one breast
16	1/day (a.m.)	pump, both breasts
17	2/day (a.m., p.m.)	manual, both breasts
18	1/day (a.m.)	manual, one breast

TABLE 29

Use of Vitamin/ Mineral Supplements by Donors

Donor	Type of Vitamin/ Mineral Supplement
1	None
2	None
3	None
4	None
5	None
6	None
7	None
8	Multivitamin supplement (1/day)
9	None
10	Prenatal supplement with iron (1/day)
11	None
12	Calcium supplement (3/week)
13	None
14	Iron supplement (1/day); Calcium supplement (2/day); Multivitamin supplement (1/day)
15	Vitamin E supplement (1/day); Calcium supplement (6/day)
16	Prenatal supplement (1/day)
17	None
18	Multivitamin supplement (prescription) (1/day)

TABLE 30

Dietary Changes Made by Donors During Pregnancy and Lactation

Donor	Dietary Change	In Effect During Lactation?
1	Increased milk, fruit intake	yes
2	Larger servings; increased carbohydrate	yes
3	None	---
4	Increased milk, fruit intake; Decreased alcohol consumption	yes
5	Increased milk, fruit, vegetable intake; decreased alcohol, caffeine consumption	yes
6	Increased milk intake; added eggs, fish to diet	yes
7	Increased milk intake; larger servings	yes
8	None	---
9	Decreased caffeine, "junk" food	yes
10	Increased milk intake	yes
11	Increased legume intake; decreased alcohol, meat consumption	yes
12	Increased fruit, vegetable intake; decreased "junk" food	yes
13	Decreased salt, fat, "junk" food intake	yes
14	Larger servings	yes
15	Increased milk intake; eating three meals/ day	yes
16	Increased milk, protein intake	yes
17	Increased fruit, vegetable intake; eating three meals/ day	yes
18	None	---

Appendix H

ALPHA-TOCOPHEROL EQUIVALENT DATA CALCULATED FOR 18 MATURE DONOR MILKS

TABLE 31

Alpha-Tocopherol Equivalents (T.E.)/ 100 Kcal in Donor Milks

Donor	Energy (Kcal/dL)	α-Tocopherol (μg/dL)	α-T.E./ 100 Kcal
1	83.36	732.50	0.879
2	52.74	350.00	0.664
3	70.30	287.50	0.409
4	46.88	145.00	0.309
5	50.04	192.50	0.385
6	88.36	529.50	0.671
7	70.48	460.00	0.653
8	58.66	242.50	0.413
9	50.42	165.00	0.327
10	50.52	117.50	0.233
11	57.49	245.25	0.427
12	85.95	190.00	0.221
13	61.28	347.50	0.567
14	74.20	362.50	0.489
15	63.78	363.75	0.570
16	66.61	417.75	0.627
17	72.58	417.50	0.575
18	44.53	85.00	0.191
Mean	63.79	317.24	0.497

TABLE 32

The Ratios of α-Tocopherol Equivalent/Total Lipid and α-Tocopherol/Linoleic Acid in Donor Milks

Donor	α-tocopherol equivalent/lipid†	α-tocopherol equivalent/C18:2‡
1	0.142	1.205
2	0.149	1.041
3	0.072	0.578
4	0.089	0.546
5	0.106	1.087
6	0.094	0.651
7	0.113	0.515
8	0.094	0.672
9	0.111	1.562
10	0.069	0.684
11	0.100	0.551
12	0.034	0.366
13	0.122	1.544
14	0.082	0.656
15	0.117	1.017
16	0.118	0.977
17	0.099	0.680
18	0.079	0.997
Mean	0.099	0.852
SEM	0.006	0.817
C.V.	27.270	40.686

† mg tocopherol equivalents/ g milk lipid
‡ mg tocopherol equivalents/ g linoleic acid

Appendix I

MACRONUTRIENT AND ENERGY INTAKES FROM FOOD GROUPS

TABLE 33

Three-day Mean Intake of Macronutrients and Energy from Seven Food Groups

Food Group		Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Energy (kcal/day)
Milk(a)	Mean	28.90	25.41	42.09	507.94
	Range	3.4 - 52.1	3.5 - 47.0	4.8 - 88.7	63.7 - 902.3
Meat(b)	Mean	32.62	23.69	3.16	366.10
	Range	14.4 - 55.1	9.8 - 35.6	0 - 18.9	160.7 - 563.4
Bread(c)	Mean	17.57	12.29	87.64	519.53
	Range	6.6 - 36.9	1.5 - 50.1	37.0 - 176.4	207.7 - 986.8
Oils(d)	Mean	0.44	19.29	1.88	179.54
	Range	0 - 1.8	4.0 - 36.4	0 - 9.3	42.9 - 364.3
F & V(e)	Mean	7.35	3.01	84.10	368.77
	Range	2.2 - 15.2	0.3 - 13.1	22.9 - 227.2	94.7 - 1042.2
Snack(f)	Mean	4.28	10.31	74.95	410.08
	Range	0.1 - 10.9	0 - 39.1	18.6 - 244.6	115.1 - 1317.5
Misc(g)	Mean	1.18	0.62	15.65	69.83
	Range	0 - 6.6	0 - 2.8	0.1 - 45.1	0.4 - 193.6

a) Milk and milk products
b) Meat, fish, poultry, eggs
c) Breads, cereals, nuts
d) Food fats and oils
e) Fruits and vegetables
f) Snacks and desserts
g) Miscellaneous

TABLE 34

Percent Macronutrient and Energy Contribution from Seven Food Groups

Food Group		Protein (%)	Fat (%)	Carbohydrate (%)	Energy (%)
Milk(a)	Mean	30.70	26.66	13.53	20.69
	Range	5.8 - 46.6	5.5 - 46.2	1.3 - 23.5	2.9 - 30.9
Meat(b)	Mean	34.85	25.91	1.08	15.58
	Range	16.7 - 58.4	10.1 - 42.5	0 - 7.3	5.7 - 28.1
Bread(c)	Mean	19.63	13.00	28.32	21.52
	Range	8.0 - 42.6	1.8 - 36.6	13.7 - 52.5	9.8 - 40.6
Oils(d)	Mean	0.52	20.46	0.62	7.32
	Range	0.5 - 2.7	6.2 - 34.0	0 - 3.3	2.1 - 10.7
F & V(e)	Mean	8.07	3.21	28.20	15.60
	Range	2.8 - 18.0	0.3 - 15.0	5.0 - 59.0	4.0 - 40.0
Snack(f)	Mean	4.55	9.90	23.04	16.32
	Range	0.1 - 9.5	0.1 - 26.8	8.3 - 47.0	5.2 - 35.2
Misc(g)	Mean	1.49	0.81	5.07	2.87
	Range	0 - 11.3	0 - 4.6	0.3 - 13.4	0 - 7.5

a) Milk and milk products
b) Meat, fish, poultry, eggs
c) Breads, cereals, nuts
d) Food fats and oils
e) Fruits and vegetables
f) Snacks and desserts
g) Miscellaneous

Appendix J

FATTY ACID INTAKES FROM FOOD GROUPS

TABLE 35

Three-day Mean Intake of Fatty Acids From Seven Food Groups

Food Group		Saturates (g/day)	Monounsaturates (g/day)	Polyunsaturates (g/day)
Milk(a)	Mean	15.87	7.24	0.91
	Range	2.16 - 29.50	1.00 - 13.52	0.13 - 2.30
Meat(b)	Mean	7.43	8.17	1.98
	Range	2.83 - 12.16	2.40 - 13.98	0.44 - 4.50
Bread(c)	Mean	2.36	5.29	2.50
	Range	0.21 - 7.69	0.51 - 27.30	0.21 - 9.24
Oils(d)	Mean	5.77	6.53	5.57
	Range	1.58 - 12.96	0.90 - 15.58	0.22 - 18.15
F & V(e)	Mean	0.67	0.48	0.49
	Range	0 - 3.63	0 - 2.85	0 - 2.78
Snack(f)	Mean	3.06	3.88	1.50
	Range	0 - 13.35	0 - 13.47	0 - 10.26
Misc(g)	Mean	0.15	0.29	0.09
	Range	0 - 1.13	0 - 1.14	0 - 0.40

a) Milk and milk products
b) Meat, fish, poultry, eggs
c) Bread, cereals, nuts
d) Food fats and oils
e) Fruits and vegetables
f) Snacks and desserts
g) Miscellaneous

TABLE 36

Percent Fatty Acid Contribution from Seven Food Groups

Food Group		Saturates (%)	Monounsaturates (%)	Polyunsaturates (%)
Milk(a)	Mean	43.14	23.10	8.34
	Range	7.90 - 64.55	4.51 - 40.30	1.70 - 21.74
Meat(b)	Mean	21.71	27.67	17.39
	Range	4.33 - 37.23	4.89 - 56.83	1.60 - 37.56
Bread(c)	Mean	6.91	15.79	22.51
	Range	0.54 - 20.39	1.97 - 46.60	2.07 - 58.36
Oils(d)	Mean	16.74	19.53	35.89
	Range	4.77 - 47.52	2.79 - 36.06	2.39 - 72.33
F & V(e)	Mean	1.82	1.49	3.74
	Range	0 - 10.66	0 - 10.94	0 - 28.17
Snack(f)	Mean	7.82	11.23	11.05
	Range	0 - 24.61	0 - 27.50	0 - 42.98
Misc(g)	Mean	0.53	1.18	1.01
	Range	0 - 4.14	0 - 5.13	0 - 6.09

a) Milk and milk products
b) Meat, fish, poultry, eggs
c) Breads, cereals, nuts
d) Food fats and oils
e) Fruits and vegetables
f) Snacks and desserts
g) Miscellaneous

Appendix K

DIETARY FATTY ACID INTAKE - PERCENT OF TOTAL FATTY ACIDS

TABLE 37

Dietary Fatty Acids Expressed as Percent of Total Fatty Acids

Donor	Total f.a.* intake(g/day)	Saturates (%)	Monounsaturates (%)	Polyunsaturates (%)
1	65.16	38.16	38.14	23.70
2	103.68	46.45	40.04	13.50
3	113.22	44.02	38.14	17.82
4	67.81	35.41	41.88	22.71
5	95.35	44.38	37.83	17.79
6	53.99	49.60	35.36	15.04
7	101.42	38.91	36.20	24.90
8	63.40	45.84	39.29	14.87
9	61.12	56.90	36.19	6.90
10	131.38	41.91	37.27	20.82
11	115.85	32.56	50.56	16.88
12	83.92	54.52	38.45	7.03
13	70.03	48.65	37.24	14.11
14	55.84	50.96	38.59	10.44
15	74.70	44.23	43.22	12.54
16	84.06	42.80	40.11	39.91
17	53.17	48.82	38.64	12.52
18	56.05	48.65	39.57	11.75
Mean	80.68	44.31	39.51	16.18

* Fatty Acids; Calculated from Table 20.