

## Short Report

## Analysis of Fatty Acids in Early Mid-Life in Fertile Women: Implications for Reproductive Decline and Other Chronic Health Problems

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**ABSTRACT** The relationship between adipose fatty acid levels and age is examined in 635 Scottish fertile women aged 25–48. Fatty acids levels are highly significantly correlated with age. Factor analysis identifies four factors that account for 79.6% of variance in the data. Three Factors show significant regressions with age and patterns of involvement of specific fatty acids suggest that these Factors represent the activity of fatty acid delta-desaturase enzymes as follows: Factor 1—delta-9-desaturase, Factor 2—delta-5-desaturase, and Factor 4—delta-6-desaturase. Key changes, apparently reductions in enzyme activity, occur through the 30 and 40-year-old age groups. Such changes in enzyme function could account for decline in female fertility and increases in body fat and chronic disease common in early mid-life. *Am. J. Hum. Biol.* 00:000–000, 2009. © 2009 Wiley-Liss, Inc.

Body composition and function change throughout life, however, mid-life often heralds a range of chronic illnesses including diabetes, cardiovascular disorders (Young, 1997), changes in lean mass, and body fat distribution (Douchi et al., 2002). Furthermore, from the mid-thirties there is a dramatic increase in miscarriage and reduction in fertility (Ford et al., 1994). To date, a general physiological explanation for these negative changes in early mid-life has not been demonstrated.

Age-related changes in fatty acids, independent of diet, were observed in adipose tissue of women aged 40–59 years (Bolten-Smith et al., 1997). Data from younger women collected at the same time were not previously analyzed. This article revisits the original data set, includes omitted data and analyses data from women aged 25–48 who were fertile at the time of specimen collection. The results give insight into changes in fatty acid metabolism that occur over the same chronological age span that is associated with changes in a spectrum of health parameters.

## MATERIALS AND METHODS

Subjects were obtained from the Scottish Heart Health Study (SHHS): a cross-sectional study of men and women across 22 districts of Scotland. Details of subject recruitment, biopsy methods, and laboratory techniques were described by Tavendale et al. (1992). Fatty acid composition was analyzed by age and region for subjects aged between 40 and 59 years. Bolten-Smith et al. (1997) showed that age-related changes in essential fatty acids occurred independently of diet.

Samples collected included relatively small numbers of younger subjects whose data was previously excluded. Since we wished to focus on younger fertile women this study includes those data. The youngest women were aged 25 years and we chose 48 as our upper age limit. A questionnaire conducted in 1997 established the year of menopause (absence of menstruation for 6 months). This enabled us to determine retrospectively that at specimen collection, 635 women met our criteria of “fertile”: they experienced regular menstrual cycles and were more than 6 years before menopause.

## Statistical analysis

Data on 12 fatty acids from adipose tissue of 635 women were analyzed using SPSS. Initial analysis determined mean levels of fatty acids in each of four 6-year age groups. Individual fatty acids were examined for correlations with age and with other fatty acids. The fatty acid data was subjected to Factor Analysis with Principal Component Extraction to determine the underlying relationships between the different fatty acids. The four Factors obtained were then regressed against age.

## RESULTS

Table 1 shows mean fatty acid levels for 635 women in four age groups. A Correlation Matrix of individual fatty acids showed changes (data not shown in the table) that were correlated with age. Highly significant  $P = < 0.01$  positive correlations with age were found for saturated fatty acids 16:0 palmitic acid, 18:0 stearic acid and omega 3, 22:5 docosapentaenoic acid, and 22:6 docosahexaenoic acid. Myristic acid (14:0) showed a significant ( $P = 0.015$ ) positive change with aging, omega 6 18:3 gamma linoleic acid, and omega 9 18:1 oleic acid showed highly significant negative correlations. Most fatty acids were also significantly correlated, either positively or negatively with each other.

Factor Analysis with Varimax Rotation identified four major factors with Eigenvalues greater than 1 that together account for 79.6% of the variance (Table 2). Factor 1 (26.1%) involved positive correlations with 14:0 myristic acid, 16:0 palmitic acid, and 18:0 stearic acid and negative correlation with 18:1 oleic acid. Factor 2 (21.1%) involved positive correlations with both 22:5 docosapentaenoic acid and 22:6 docosahexaenoic acid and dihomo-gamma-linoleic acid. Factor 3 (18.8%) was positively associated with 18:3

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TABLE 1. Mean fatty acid levels by age group (mean  $\pm$  standard error)

Fatty acid	Age 25–30 (n = 43)	Age 31–36 (n = 36)	Age 37–42 (n = 229)	Age 43–48 (n = 327)
14:0 myristic acid	1.96 $\pm$ 0.10	2.11 $\pm$ 0.13	2.31 $\pm$ 0.05	2.25 $\pm$ 0.04
16:0 palmitic acid	18.13 $\pm$ 0.32	18.87 $\pm$ 0.33	19.41 $\pm$ 0.15	19.53 $\pm$ 0.13
16:1 palmitoleic acid	9.70 $\pm$ 0.26	9.12 $\pm$ 0.27	9.10 $\pm$ 0.12	9.17 $\pm$ 0.11
18:0 stearic acid	2.99 $\pm$ 0.15	3.57 $\pm$ 0.16	3.65 $\pm$ 0.08	3.58 $\pm$ 0.07
18:1 oleic acid	52.55 $\pm$ 0.44	52.17 $\pm$ 0.57	51.22 $\pm$ 0.19	51.42 $\pm$ 0.15
18:2 linoleic acid	9.98 $\pm$ 0.28	9.66 $\pm$ 0.44	9.74 $\pm$ 0.17	9.38 $\pm$ 0.13
18:3 gamma-linoleic acid	0.22 $\pm$ 0.02	0.27 $\pm$ 0.14	0.21 $\pm$ .01	0.19 $\pm$ 0.01
20:1 eicosenoic acid	3.28 $\pm$ 0.09	3.17 $\pm$ 0.06	3.14 $\pm$ 0.04	3.18 $\pm$ 0.03
20:3 dihomo-gamma-linoleic acid	0.14 $\pm$ 0.01	0.15 $\pm$ 0.01	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01
20:4 arachidonic acid	0.63 $\pm$ 0.03	0.60 $\pm$ 0.02	0.59 $\pm$ 0.01	0.60 $\pm$ 0.01
22:5 docosapentenoic acid	0.18 $\pm$ 0.01	0.20 $\pm$ 0.01	0.23 $\pm$ 0.01	0.24 $\pm$ 0.01
22:6 docosahexenoic acid	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	0.17 $\pm$ 0.01	0.18 $\pm$ 0.01

TABLE 2. Component scores for rotated component matrix (principal component analysis with Varimax rotation and Kaiser normalization)

Fatty acid	Factor 1	Factor 2	Factor 3	Factor 4
14:0 myristic acid	0.807	-0.185	-0.063	-0.215
16:0 palmitic acid	0.904	0.073	-0.185	-0.164
16:1 palmitoleic acid	-0.480	-0.130	-0.276	-0.644
18:0 stearic acid	0.777	-0.003	0.282	0.301
18:1 oleic acid	-0.809	-0.028	0.068	-0.256
18:2 linoleic acid	-0.111	0.084	-0.097	0.918
18:3 gamma-linoleic acid	0.309	-0.264	0.818	0.222
20:1 eicosenoic acid	-0.132	-0.212	0.881	-0.087
20:3 dihomo-gamma-linoleic acid	0.018	0.738	-0.094	0.263
20:4 arachidonic acid	-0.231	0.431	0.770	-0.057
22:5 docosapentenoic acid	-0.011	0.927	-0.038	-0.087
22:6 docosahexenoic acid	-0.027	0.876	-0.032	0.049

Key components of each factor with correlations greater than 0.7 are shaded.

gamma-linoleic acid, 20:1 eicosenoic acid, and 20:4 arachidonic acid. Factor 4 (13.6%) was positively associated with 18:2 linoleic acid and negatively with 16:1 palmitoleic acid.

Individual Factor scores were used in Linear Regression analysis with age. Factor 1 scores gave a  $t$  value of 4.31,  $P < 0.000$ ; Factor 2 gave a  $t$  value of 5.07,  $P < 0.000$ ; Factor 3 gave a  $t$  value of  $-0.763$ ,  $P = 0.446$  and Factor 4 gave a  $t$  value of  $-2.37$ ,  $P = 0.018$ . Component scores for the Rotated Component Matrix are shown in Table 2 and graphs of the mean Factor Scores by age groups in Figure 1.

## DISCUSSION

In an unselected population of 635 “fertile” women, factor and regression analyses identified three key factors significantly associated with aging.

Factor 1 identifies a pattern of high-positive correlations of saturated fatty acids and negative correlation with oleic acid. Stearic acid can be obtained by diet or through elongase of palmitic acid (itself the product of elongation of myristic acid). Stearic, the last product of saturated fatty acid elongation, increases if its conversion to oleic acid is suppressed. Palmitoleic acid, another desaturation product, is also reduced with aging but was not significant. Since the enzyme delta-9 desaturase (stearoyl-CoA desaturase-1) converts saturated fatty acids to oleic and palmitoleic acids, Factor 1 is consistent with reduced function of this enzyme.

Factor 2 identifies high correlations of 22:5 and 22:6 long chain Omega 3 fatty acids and 20:3 DGLA. Factor 2

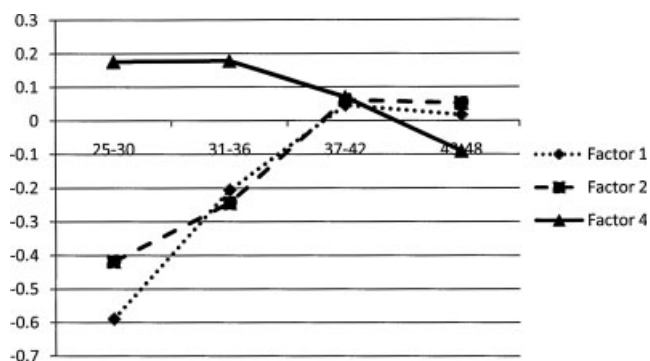


Fig. 1. Regression scores of Factors with age. The mean scores are depicted for each of the three factors (Factor 1, Factor 2, and Factor 4) that are significantly associated with aging. The x-axis depicts the age groups and the y-axis the Factor Scores.

is consistent with reduced function of delta-5-desaturase, which usually converts Omega 3 eicosatetraenoic acid to eicosapentaenoic acid and docosahexaenoic acid and Omega 6 DGLA into arachidonic acid. This reaction prefers the Omega 3 pathway and where its activity is limited, the enzyme preferentially desaturates omega 3 fats if they are present.

Factor 3 identifies high-positive correlations with 18:3 GLA, 20:1 eicosenoic acid, and 20:4 arachidonic acid and the factor shows a negative but not significant decline with age. Accumulation of GLA might occur if the activity of delta-6 elongase was suppressed but no literature was found to explain whether this could also cause accumulation of 20:1 eicosenoic acid.

Factor 4 is characterized by high-linoleic acid. This factor, which shows significant negative regression with age, might reflect suppression of delta-6-desaturase, which converts 18:2 linoleic acid to 18:3 gamma linoleic acid.

### Relevance of enzyme changes to age-related risks of obesity and diabetes

Wang et al. (2006) studied hepatic fatty acid elongase and desaturase expression in rat models of metabolic disease and found that all three delta-desaturase enzymes and elongase-5 and 6 were involved. Delta-9-desaturase was independently regulated by a liver receptor agonist. In humans, delta-9-desaturase plays an interactive role with leptin in weight gain and loss (Cohen et al., 2002) and delta-5 and delta-6 desaturases have been proposed to

play an important role in developing the insulin resistance syndrome (Das, 2005).

Our findings suggest that loss of function of delta-9 and delta-5 desaturase enzymes and to a lesser extent delta-6 desaturase occur in early mid-life in women. This may be critical to the onset of metabolic disease with aging in humans.

*Relevance of changes in fatty acids to deterioration of reproduction in mid-thirty age group*

Fatty acids can affect reproduction through many different and interdependent mechanisms including prostaglandin and steroid hormone synthesis and glucose metabolism. Mitochondrial changes might also play a key role in age-related decline in reproduction. Schon et al. (2000) found morphologically defective mitochondria in only 8.2% of cells of younger women but in 63.3% of cells of older women. Structurally defective mitochondrial were also dysfunctional (Volarcik et al., 1998).

The saturated fatty acids stearic, myristic acid, and palmitic acid are toxic to mitochondria (Belosludtsev et al., 2006; Martins de Lima et al., 2006; Schönfeld et al., 2004). Thus, a significant rise in saturated fatty acids with aging could account for the decline of mitochondrial function in reproductive cells and possibly also in other aging tissue.

*Significance of changes in fatty acids with early aging*

Fatty acid metabolism is critical to a diverse range of cellular functions and is extremely complex. Fatty acids play critical roles in immunity, inflammation, and endocrinology and considerable research is now focused on how improvements in diet might prevent many chronic diseases. This analysis is unusual in that it examines fatty acid levels in younger women and finds that changes commence before age 35. Factor analysis has detected patterns of changes between different fatty acids that imply underlying changes in the function of critical enzymes. The study is limited in that it analyses existing data and does not evaluate enzyme function. However, the analyses

suggest that critical changes occurring as part of normal aging can account for a plethora of negative health problems in early mid-life.

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