Postgraduate Symposium

Assessment of individual fatty acid intake

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Dietary assessment of individual fatty acid intake is difficult due to a number of limitations. Information regarding the type, quantity and brand-name of fat used in cooking and at the table is required. In addition, margarine manufacturers may change the component oils used for reasons of cost, which changes the fatty acid composition of their products from season-to-season. Independent markers of fatty acid intake are required, therefore, to compensate for these limitations. Adipose tissue concentrations have been used as a measure of habitual intake of fatty acid groups and individual fatty acids in numerous studies. Saturated (SFA) and monounsaturated fatty acids (MUFA) are generally poorly correlated with adipose tissue concentrations, which can be explained partly by endogenous synthesis. In general, adipose tissue concentrations of exogenously-produced fatty acids (*n*-3 and *n*-6 polyunsaturated fatty acids (PUFA)) are well correlated with estimates of habitual intake. Correlations between dietary *trans* unsaturated fatty acids (TUFA) and adipose tissue concentrations vary between countries, which may be due to differences in dietary sources. Correlations may be affected by differences in bioavailability or selective retention of fatty acids in certain tissue lipids.

Biomarker: Adipose tissue: Dietary fatty acid: Cardiovascular disease

Cardiovascular disease accounts for a major proportion of deaths in the Western world, and while fat intake is the dietary factor most often implicated, the effects of consumption of individual fatty acids on the development of cardiovascular disease has raised intense interest. In general, dietary studies have tended to examine the intake and effect of fat in terms of the three major classes of fatty acids (i.e. saturated (SFA), monosaturated (MUFA) and poly-unsaturated fatty acids (PUFA)). It is now agreed that individual fatty acids within the same class have very different effects on cardiovascular disease risk (Ulbricht & Southgate, 1991). With regard to SFA there is an agreement in the scientific community that lauric ($12:0$), myristic ($14:0$), and palmitic acids ($16:0$) raise LDL-cholesterol levels. In contrast, stearic acid ($18:0$) has a neutral or slightly lowering effect on LDL-cholesterol. In addition, MUFA, e.g. oleic acid ($18:1n$-9), lower LDL-cholesterol and have a neutral effect on HDL-cholesterol, while the *trans* isomer of elaidic acid ($18:1t9$) raises LDL-cholesterol and lowers HDL-cholesterol levels (Kris-Etherton & Yu,

1997). The triacylglycerol-lowering effect of PUFA of marine origin, e.g. eicosapentaenoic acid ($22:5n$-3; EPA) and docosahexaenoic acid ($22:6n$-3; DHA), is also generally accepted. In contrast, hydrogenated fish oils have been shown to increase LDL-cholesterol levels due to the presence of long-chain *trans* unsaturated fatty acids (TUFA) (Almendingen *et al.* 1995). Thus, each individual fatty acid should ideally be considered separately with regard to intake.

Dietary assessment methods

Dietary analysis of fat or fatty acid intake can be measured retrospectively using food-frequency questionnaires or diet histories, or prospectively using food records either weighed or estimated. Investigators are agreed, however, that there are shortcomings with all self-reported dietary intake measures (Bingham, 1987). Such shortcomings include under-reporting by individuals with a high BMI, or biased reporting of foods which are regarded by the individual to be

more socially acceptable, e.g. fruit and vegetables (Nelson & Bingham, 1997). In addition, difficulty with recall memory or reactive changes in usual eating pattern during the period of dietary assessment can also give false estimates of habitual intake (Nelson & Bingham, 1997). Dietary assessment of fat or fatty acid intake is particularly challenging for numerous reasons. Information regarding the type and quantity of fat used in the preparation of foods is difficult to acquire for those individuals who do not prepare their own food. In addition, brand-name information is required to translate food intake into fatty acid intake. Use of photographs of the different brands of foods which contribute to fat intake in the diet is a useful way of gathering this information. Moreover, an inconsistency in the composition of foods from season-to-season, e.g. the linoleic acid content of milk, may affect calculation of fatty acid intakes. In addition, margarine and cooking fat manufacturers may change the component oils used depending on production costs. For example, the species and content of fish oils used in margarine manufacture may change from season-to-season. The use of these fats in the production of cakes, biscuits and pastries will in turn alter the fatty acid composition of these foods from season-to-season. This continuing change in the fatty acid composition of foods makes dietary estimation of individual fatty acid intake, which is limited to the nutrient database used, extremely difficult. To analyse the individual fatty acid content of foods, standard samples of each fatty acid present are necessary for identification by GC. These standard samples are available for most SFA, MUFA and PUFA. However, industrial hydrogenation of vegetable and marine oils produce TUFA which are C_{16}-C_{24} and have a variable number of positional and geometric isomers. A greater number of these isomers are formed from fatty acids which are highly unsaturated before hydrogenation. Identification of these isomers formed as a result of hydrogenation of polyunsaturated marine oils is very difficult due to a lack of analytical standards for the numerous positional and geometric isomers formed. Calculation of the dietary intake for each individual fatty acid is therefore not feasible due to the limitations associated with food composition tables used to translate food intake into fatty acid intake.

Biomarkers of fatty acid intake

To compensate for these limitations, an independent marker of fatty acid intake must be used. These biomarkers serve as an independent measure of intake unbiased by self-reporting or changes in the fatty acid composition of the food supply from season-to-season. In addition, biomarkers of fatty acid intake can be used to validate measures of self-reported dietary intake. Depending on the body compartment used these biomarkers reflect different time periods of fatty acid intake. For example, serum triacylglycerol reflects fatty acid intake in the last meal eaten, cholesteryl esters and phospholipids are more likely to reflect intake over the past few days, while erythrocyte membranes and adipose tissue reflect longer periods of intake from several weeks to years (Bates et al. 1997). A suitable biomarker of fatty acid intake can therefore be chosen depending on the duration of the study and the time frame of interest. In general, the habitual

fatty acid intake is of interest to determine the relationship between dietary intake and disease outcome. Thus, a long-term measure of fatty acid intake, such as adipose tissue concentrations, is usually of more importance. The half-life of adipose tissue in human subjects in energy balance is approximately 600 d, and thus its composition should reflect dietary fatty acid intake over the preceding 2·5 years (Hirsch et al. 1960). The effect of sample site, age and sex on the fatty acid composition of adipose tissue has been shown to be negligible (Van Staveren et al. 1986; Plakké et al. 1983). Although there is no satisfactory marker of total fat intake, adipose tissue concentrations of fatty acids reflect the type of fat consumed by different populations in international comparisons (Beynen et al. 1980) and within individuals (Van Staveren et al. 1986).

Saturated and monounsaturated fatty acid intake v. adipose-tissue-biopsy concentrations

Correlation coefficients ranging from 0·14 to 0·46 have been obtained for estimates of dietary SFA intake (g/100 g fatty acids) v. adipose-tissue-biopsy concentrations (g/100 g fatty acids; Table 1). MUFA intake is also poorly correlated with adipose-tissue-biopsy concentrations with r −0·04–0·22. These low correlations indicate that the relative proportions of adipose tissue SFA and MUFA are not wholly dependent on the proportions of SFA and MUFA in the diet. Endogenous synthesis from carbohydrate, energy expenditure and conversion of SFA to MUFA, and vice versa, may all determine the final proportions of SFA and MUFA found in adipose tissue biopsies (Beynen et al. 1980). In vitro human studies have shown preferential formation of 16 : 0, 16 : 1n-7 and 18 : 1n-9 from radiolabelled glucose and acetyl-CoA. Compared with dietary intakes, MUFA were relatively over-represented in adipose tissue (Van Staveren et al. 1986; London et al. 1991; Hunter et al. 1992; Tjønneland et al. 1993), which may be explained partly by the conversion of SFA to MUFA endogenously.

Linoleic acid intake (g/100 g fatty acids) v. adipose-tissue-biopsy concentrations (g/100 g fatty acids)

The linoleic acid content of adipose tissue is generally accepted as a good indicator of intake, since its appearance in adipose tissue is due primarily to dietary intake. Correlations range from 0·28 to 0·70 (Van Staveren et al. 1986; London et al. 1991; Hunter et al. 1992; Feunekes et al. 1993). Van Staveren et al. (1986) reported the highest correlation (r 0·70) when analysing dietary intake nineteen times over a 2·5-year period. These multiple assessments of dietary intake have been shown to reduce the effect of a large day-to-day variation in dietary intake, improving the correlation between dietary assessment and adipose-tissue-biopsy concentration. Large fluctuations in body weight have been shown to disturb the relationship between the fatty acid profile of adipose tissue and the average fatty acid composition of the diet (Dayton et al. 1967). A change in weight of 3 kg weakened the correlation of linoleic acid intake with adipose tissue concentrations from 0·62 for those who experienced a weight change to 0·82 for those

Table 1. Correlation coefficients of dietary estimates of fatty acid intake (g/100 g fatty acids) *v.* adipose-tissue-biopsy concentrations (g/100 g fatty acids)

Reference	SFA	MUFA	PUFA	Linoleic acid	EPA	DHA	TUFA
Godley *et al.* (1996)*	–	–	–	–	0·41	0·43	–
Van Staveren *et al.* (1986)†	–	–	0·68	0·70	–	–	–
Hunter *et al.* (1992)*	0·18	0·14	0·50	0·48	0·47	–	0·29
Hunter *et al.* (1992)‡	0·16	0·22	0·49	–	–	–	–
London *et al.* (1991)†	0·16	0·07	0·37	0·35	0·48**	–	0·51
Feunekes *et al.* (1993)*	–	–	0·24	0·28	–	–	–
Garland *et al.* (1998)§	0·16	−0·04	0·40	0·37	–	–	0·40
Garland *et al.* (1998)ll	0·14	0·08	0·37	–	–	–	–
Marckmann *et al.* (1995)¶	0·34	–	0·38	–	0·40	0·66	–
Tjønneland *et al.* (1993)‡	0·46	0·19	0·57	0·51	0·44	0·55	–
Tjønneland *et al.* (1993)*	0·24	0·05	0·44	0·44	0·47	0·41	–
Lemaitre *et al.* (1998)*	–	–	–	–	–	–	0·55–0·67
L Hogan and M Cantwell (unpublished results)*	–	–	–	0·58	–	–	0·17
Plakké *et al.* (1983)‡	0·24	0·22	0·54	–	–	–	–
Van Staden *et al.* (1991)ll	–	–	–	0·37	–	–	–

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TUFA,
   trans unsaturated fatty acids.
* Food-frequency questionnaire.
† Average of nineteen 24 h recalls.
‡ Two 7 d diet records.
§ Average of two food-frequency questionnaires.
ll 1-week diet record.
¶ Three 7 d weighed food records over 8 months.
** Average of EPA and DHA.

individuals whose weight remained stable (Van Staveren *et al.* 1986).

n-3 Fatty acids of marine origin

Adipose tissue concentrations of *n*-3 fatty acids have been correlated with dietary intakes by several investigators (London *et al.* 1991; Hunter *et al.* 1992; Tjønneland *et al.* 1993; Marckmann *et al.* 1995), showing correlations of the order of 0·45 for EPA and 0·51 for DHA. A stronger association has been shown between dietary intakes and adipose tissue concentrations for DHA compared with that for EPA (Tjønneland *et al.* 1993; Marckmann *et al.* 1995). Adipose tissue concentrations of EPA have also been shown to be lower than those of docosapentaenoic acid and DHA concentrations, despite similar dietary intakes of EPA and DHA and a lower dietary intake of docosapentaenoic acid (Marckmann *et al.* 1995). It would appear, therefore, that individual marine *n*-3 PUFA are selectively metabolized, and adipose tissue measurements may reflect relative rather than absolute dietary intakes. Sinclair & Gale (1987) concluded that EPA intake does not alter EPA concentrations in adipose tissue following consumption of an 'Eskimo diet' by one individual for 74 d. Very low levels of EPA were found, and levels were undetectable after 100 d of consumption of the same diet. It could be possible, however, that these investigators did not wait for a sufficiently long enough time to assess accumulation of EPA in adipose tissue. Dayton *et al.* (1967) reported that a permanent change in dietary fat (e.g. an increased linoleic acid intake) is optimally reflected in the fatty acid composition of subcutaneous adipose tissue only after a period of at least 3 years.

Total *trans* unsaturated fatty acid intake (g/100 g fatty acids) *v.* adipose tissue concentrations (g/100 g fatty acids)

Five studies have examined the intake of total TUFA *v.* adipose tissue concentrations and shown correlations of the order of 0·17–0·67. Hydrogenated vegetable oils are the main source of TUFA in the USA and the UK, where the majority of these studies were carried out (London *et al.* 1991; Hunter *et al.* 1992; Garland *et al.* 1998; Lemaitre *et al.* 1998). In the Republic of Ireland, hydrogenated vegetable and also marine oils are used in the food industry. *Trans* isomers of 18 : 1 and 18 : 2 are the predominant TUFA formed by hydrogenation of vegetable oils. In contrast, hydrogenation of marine oils produces, in addition to these isomers, a variety of both positional and geometric isomers of longer-chain fatty acids (C_{20} and C_{22}). While elaidic acid (18 : 1*t*9) and other C_{18} TUFA are easily identified in foods using GC, the full spectrum of TUFA, in particular the longer-chain TUFA derived from hydrogenated marine oils, are not. This situation is due to the wide range of both positional and geometrical isomers present, and to the lack of pure analytical standards for these fatty acids. The total TUFA content of foods can be measured using i.r. spectroscopy, but this method cannot be reliably used for adipose tissue samples due to the small yields obtained by needle-biopsy aspiration. In a recent study (L Hogan and M Cantwell, unpublished results) GC analysis of adipose tissue revealed an almost complete absence of TUFA other than geometric and positional isomers of 16 : 1, 18 : 1 and 18 : 2. This study found that total TUFA in the diet was poorly correlated (*r* 0·17) with total TUFA in adipose tissue. The study group (*n* 84) was divided into quartiles on the basis of their *trans* C_{20} and C_{22} fatty acid intake. Table 2 shows that

Table 2. A comparison of total *trans* unsaturated fatty acid intake (g/100 g fatty acids) and total *trans* unsaturated fatty acid content of adipose tissue based on quartiles of *trans* C_{20} and C_{22} fatty acid intake

(Mean values and standard deviations)

Quartiles of *trans* C_{20} and C_{22} intake	Total *trans* unsaturated fatty acid intake		Adipose tissue total *trans* unsaturated fatty acid content	
	Mean	SD	Mean	SD
1	3·76	1·04	4·01	1·06
2	4·99[a]	1·46	4·10	0·93
3	6·23[ab]	1·72	4·35	0·73
4	8·18[abc]	2·38	4·42	0·58

* Data from L Hogan and M Cantwell, unpublished results.
Mean values were significantly different from that for quartile 1: [a] $P < 0.05$.
Mean values were significantly different from that for quartile 2: [b] $P < 0.05$.
Mean value was significantly different from that for quartile 3: [c] $P < 0.05$.

the total TUFA intake significantly differed between quartiles, with those subjects in the top quartile of *trans* C_{20} and C_{22} fatty acid intake having the greatest total TUFA intake. In addition, those subjects in the top quartile of *trans* C_{20} and C_{22} fatty acid intake also had the greatest total TUFA adipose tissue content. However, there was no significant difference in adipose tissue concentrations of total TUFA amongst the quartiles. Clearly the longer-chain TUFA are not incorporated into adipose tissue triacylglycerol to the same extent as *trans* isomers of C_{18}. A study carried out by Peters *et al*. (1991) has shown that the absorption of long-chain fatty acids in human subjects decreases with increasing chain length. The absorption of 20 : 0, 22 : 0 and 24 : 0 were calculated at 41·6, 28·8 and 14·8 % respectively. In addition, Webb *et al*. (1991) has shown that only 2·8 % of the total amount of behenic acid (22 : 0) fed to rats, or 14·7 % of the total amount absorbed, is found in the total carcass fat. It has also been noted that there was no marked retention of behenic acid in the carcass fat of rats when it was fed as a component of hydrogenated fish oil (Webb *et al*. 1991). This finding indicates that the long-chain TUFA are taken up into tissues in disproportionate low amounts compared with their level in dietary fat.

Conclusions

Since SFA and MUFA are supplied in the diet as well as being formed endogenously, their abundance in adipose tissue has limited diagnostic value for estimating dietary intakes. However, linoleic acid intake is in general well correlated with adipose tissue concentrations, as it is provided only from the diet. The results of studies comparing adipose tissue concentrations of *n*-3 PUFA with dietary estimates indicate that DHA is most strongly related to habitual dietary *n*-3 PUFA and fish intake when compared with EPA and docosapentaenoic acid. The relatively low incorporation of EPA into adipose tissue could be explained by selective retention in other tissue lipids, enhanced oxidation, or due to its conversion into the less-metabolically-active docosapentaenoic acid (Marckmann *et al*. 1995). The large range of values for correlation coefficients for the relationship between dietary estimates of

TUFA and adipose tissue concentrations could be due to inaccuracies in food composition tables used in different countries. A more likely explanation is the difference in food sources, i.e. hydrogenated vegetable or marine oils. It appears that adipose tissue concentrations do not reflect dietary intake in those countries where hydrogenated marine oils are used to produce a wide variety of foods. Adipose tissue concentrations only reflect relative intakes of fatty acids, and are not a measure of total fatty acid intake. Thus, a combination of a biological marker of intake with a dietary estimate would be optimal to validate dietary estimates.

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