CORONARY HEART DISEASE AND HYDROGENATED FAT; AN INVESTIGATION OF THE LEVELS OF CHEMICALLY - MODIFIED FATS ON ADIPOSE TISSUE

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DECLARATION

This dissertation has not been nor is being currently submitted for the award of any other degree or similar qualification.

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LIST OF ABBREVIATIONS

T	Total trans unsaturated acids.
L	Lower acids - the sum of the acids: 14:1, 15:0, 15:0br., 15:1, 16:0br., 17:0 and 17:1.
H	Higher acids - the sum of the acids: 20:0, 20:1, 20:2, 20:3, 22:0 and 22:1.
T_L	Lower trans acids - the sum of the trans acids 16:1 and 18:1.
T_H	Higher trans acids - the sum of the trans acids of chain length C_{20} and C_{22}.
R	Ratio of the acids (16:0 + 16:1)/(18:0 + 18:1)
HF	Hydrogenated fat.
HMO	Hydrogenated marine oil.
HVO	Hydrogenated vegetable oil
RAF	Ruminant animal fat.

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ABSTRACT

A total of 231 samples of human adipose tissue have been analysed for (a) odd-number and branched - chain acids (L) which are characteristic of ruminant fat in the diet, and (b) trans unsaturated acids (T) which arise mostly from commercially - hydrogenated fats. A total of 136 specimens were taken at post mortem examination from persons dying of ischaemic heart disease (cases) and the remainder from persons dying from unrelated causes (controls). After due matching for area of residence, it is shown that the case specimens had a significantly lower proportion of L and a higher proportion of T than had the control specimens. The proportions of certain higher (C_{20} and C_{22}, mostly monoenoic) acids (H) were similar as also were proportions of polyunsaturated acids.

The ratio T/L was significantly higher in the cases which suggests that they had consumed more hydrogenated fat relative to ruminant fat than had the controls.

The ratio T/L increased linearly with H within both the case and control specimens which suggests in respect of the similarity in the mean levels of H that the difference in the trans content may be concentrated in the "lower" trans acids.

To justify such a conclusion later GLC work permitting direct measurement of the proportions of "lower" (16:1 plus 18:1) trans acids (T_L) together with the "higher" C_{20} and C_{22} trans acids (T_H) has been undertaken. Whereas mean levels of T_H are virtually identical for cases and controls the mean value of T_L was significantly higher for the case specimens. Although these "lower" trans acids are present in small amounts in ruminant animal fat they are more characteristic of commercially hydrogenated fats. It is concluded therefore that the cases consumed on average a higher proportion of those hydrogenated fats rich in 16:1 trans and 18:1 trans acids, and the lower proportion of ruminant - fat than did the controls.

Fatty - acid compositions are also reported for adipose tissue of still-born infants, superficial versus deep-site tissue, and for a variety of margarines and animal fats.

1. GENERAL INTRODUCTION

1.1. Trends in fat consumption in the UK, period 1960 to 1973

The national consumption of visible and invisible fat rose slightly after de-rationing in 1954, but since 1960 it has remained sensibly constant at around 145g (all figures in g/person per day, fat content). The same is true of butter (20g) and lard and compound cooking fat (16g). Consumption of margarine, however has steadily declined from about 19g before de-rationing to 16g during the five-year period up to 1960, to 12g from 1967 to 1973 inclusive. From such data (see below) average consumption of margarine for 1960-73 can then be put at 13g. Whereas use of "other oils and fats" has slowly increased since 1960, the average being 15g, upto 1973. Ascertaining the proportions of such an item as "lard and compound cooking fat" presented difficulties, however it appears that ca. 30% (4g) is hydrogenated "compound fat" or shortenings[1].

The percentage of energy derived from fat has increased smoothly from 38% in 1960 to 43% in 1973.

The above figures are taken from the statistics issued yearly by the Ministry of Agriculture, Fisheries and Food National Food Survey Committee (NFS) [2], and refer to "supplies moving into consumption". Comparison with consumption in the private household[2] shows that of the total of 145g, only about 23g was consumed in restaurants, canteens etc..

It is interesting to add that, in so far as precise statistics are available, regional and social-class preferences

in fat consumption patterns re-asserted themselves rapidly from the common pattern of 1939 to 1954 to pre-war habits.

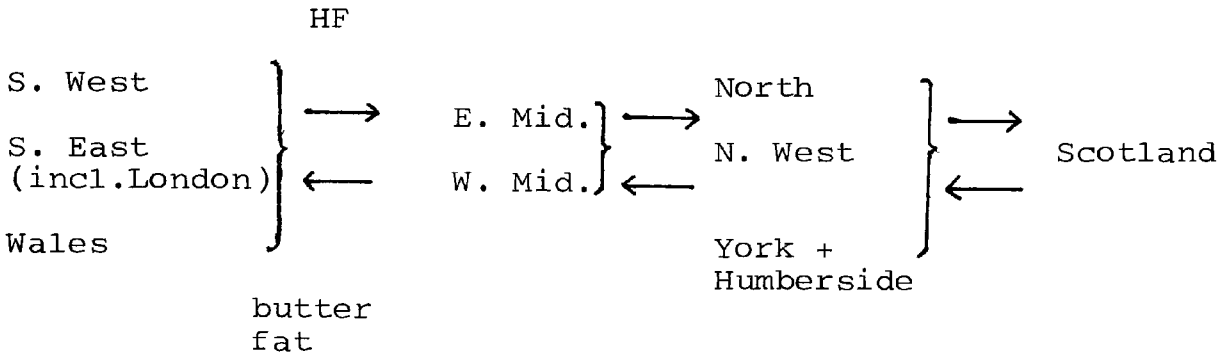
Regional differences in the period 1960-73 in total (household) fat intake were remarkably small and no region differed from the UK average by more than 2g with the exception of Scotland which showed a figure about 10g below average. For total butter fat (which includes dairy products in addition to butter) Wales, the South-West and South-East including London - were above average (43g) by about 4g, East and West Midlands had average consumption, and the remaining regions (North, North-West, Yorkshire and Humberside, Scotland) had 2 to 4g less than average. Regional differences in consumption of commercially hydrogenated fat (HF) - margarines, shortenings, branded cooking fats - are considered later, but generally areas having high intake of HF have low butter consumption.

Differences in total (household) fat consumption due to social-class preferences are more marked than regional differences. Using the classification of the NFS which rests purely on the income of the head of the household (highest income class A1), total fat consumption is 8g higher than average for A1 and falls smoothly through A2 and B to 4g lower than average for classes C + D1. There is also a gradient in butter consumption, being highest for A1 (26g) and lowest for C + D1 (18g). The gradient in respect of HF[3] is in inverse order - with A1 showing 4g less than average and C + D1, 2g higher than average.

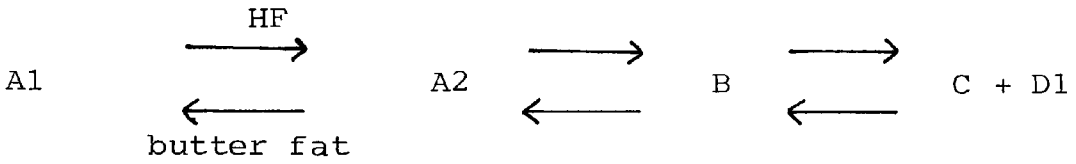
The broad picture which emerges therefore is that whereas differences in total fat are small and probably of little

significance - but with the "higher" social classes having highest consumption - the following gradients exist in respect of HF and butter-fat (arrow → indicates direction of increasing consumption):-

Standard regions



Social Classes



Detailed comparison of these patterns with mortality experience from arteriosclerotic disease is considered later, but it is obvious that by superimposing the above trends that highest consumption of butter fat (and lowest consumption of HF) will appertain for social class A1 in the South, South East and West and the lowest consumption of butter fat (and highest consumption of HF) in the "lower" classes in Northern England and Scotland.

The various fat-containing items of food together with their contributions to the above average total 145g are given in Table 1.1, overleaf. The last column shows fractional

TABLE 1.1.

Contributions of fat-containing items to total fat consumption; period 1960-73[+]

Item		g per person per day	Fractional contributions	
Beef, veal, suet	Ruminant animal fat	13	.092	.426
Mutton, lamb		6	.042	
Total milk fat, including butter		42	.292	
Pork, bacon, ham, lard		33	.233	.304
Poultry, rabbit		10	.071	
Margarine (household)*	Hydrogenated fat	11	.079	.149
Margarine (other)		2	.014	
Shortenings (household)*		2	.014	
Shortenings (other)		2	.014	
"Other oils and fats" (hardened)		4	.028	
"Other oils and fats" (unhydrogenated)		11	.078	
Offals		2	.014	
Eggs		4	.029	
Fish		1		
Cereal fats		2		
TOTAL		145	1.000	

*include fat content of cakes, biscuits etc. bought as such for home consumption.

[+]figures compiled by Thomas, L.H. (unpublished) from Government and other statistics.

contributions calculated on the basis of all component fatty acids C_{10} to C_{22} inclusive (In order to compare later with human depot fat, acids C_4, C_6 and C_8 of butter fat are omitted); the last two items in the list are omitted on the grounds that they make very little contribution to the total and their fatty-acid compositions are very uncertain.

Additional Government Statistics[4] include in addition to the materials used in the manufacture of margarine and "compound fat" (shortenings, cooking fats), total production of vegetable oils and fats by "all known hardeners and refiners" in the UK. Subtraction then gives the composition of the tabulated 15g of "other oils and fats" (34% palm, 15% coconut/palm kernel, 18% soya, 9% peanut, 8% rape, remainder mostly cottonseed and sunflower) which agrees well with the earlier statistics of Anderson and Williams[5].

The extent to which this 15g is hydrogenated for the production of "bulk" (non-retail) compound cooking fat and frying oils is problematical, but it has been calculated[3] that the fat content of such items as cakes, pastries, biscuits, potato crisps etc., purchased as such makes a large contribution (14g). Amounts of butter in such items are negligible (Quantities of butter consumed in the private household is virtually identical with amounts "moving into consumption"). Counting the tabulated 4g for non-household margarine/shortenings still leaves 10g for such items. In view of the fact that cakes and pastries have specific requirements in respect of fatty-acid composition (See composition of typical "cake" and "puff-pastry" hydrogenated fats as given by Anderson and

Williams[5], it seems very likely that this material is hydrogenated in some degree - say to an extent equivalent to about 4g of hard fat.

The following facts emerge:

(1) The fractional contribution of total ruminant fat (0.43) is much higher than that of HF (around 0.15), i.e. about 3 times higher.

(2) The contribution of margarine and shortenings, purchased as such by the private household accounts for only about 60% of the total consumed. The remaining 40% derives through the purchase of pastries, biscuits, buns, scones, potato crisps etc..

(3) Although hydrogenated in lesser degree - and in amount very difficult to estimate - there is a further contribution from oils intended for frying purposes, and there is no doubt that use of cheap, hydrogenated soya oil has increased over the last 10-15 years.

1.2. Dietary fat and ischaemic heart disease; epidemiological evidence

On health grounds medical opinion has tended towards the view that consumption of animal fats should be reduced and replaced in some measure by vegetable fats including margarine. More recently the accent has been on substitution by polyunsaturated fats which usually (but not invariably) are present in softer margarines in substantial amount. From a personal point of view owing to the lack of a medical background no competant opinion can be proffered on the "experimental" premises upon which such recommendations have been made; neither is any particular viewpoint taken regarding the pathological manifestations and aetiology of coronary heart disease other than it appears that medical opinion is manifestly far from unanimous. This is shown for example by the W.H.O. clinical trial of the cholesterol lowering drug Clofibrate[6] and its mortality follow up[7] which casts doubts on this conventional viewpoint.

The contrary view that there may be hazards associated with the altered fatty-acid components of industrially - hydrogenated oils and fats has from time to time been expressed in the literature. On purely epidemiological grounds, Thomas[3] reported in 1975 that for the UK, mortality from arteriosclerotic disease is highest in those regions/conurbations and social classes which consume highest amounts of HF. Since this work initiated the present case/control study, it will now be summarised in some detail.

Table 1.2 shows standardised mortality ratio (SMR) within

TABLE 1.2

Household consumption of hydrogenated fat, 1962-71, g per person per day; ischaemic heart disease SMR ages 25 to 64 y, 1968-71

Region as type	HF consumption		Ischaemic heart disease SMR		RANK ORDER		Differences in rank order HF v. IHD	
	g	RANK ORDER	F	M	F	M	F	M
Scotland	28.9	1	168	128	1	1	0	0
North	28.1	2	136	113	2	5	0	3
North West	27.9	3	131	119	3	3	0	0
Yorks and Humberside	27.8	4	120	109	6	6	2	2
Provincial conurbations	26.7	5	124	114	5	4	0	1
East Midlands	25.4	6	95	93	9	11½	3	5½
Rural districts	25.0	7	89	88	11	14	4	7
Large towns	24.8	8	108	106	7	7	1	1
Small towns	24.7	9	99	102	8	8	1	1
West Midlands	22.6	10	94	99	10	9	0	1
South West	22.5	11	85	96	12	10	1	1
South East plus East Anglia	21.6	12	79	89	14	13	2	1
Wales	20.9	13	127	121	4	2	9	11
Greater London	19.3	14	82	93	13	11½	1	2½
Wales (S.East)*			143	127				
Wales (Remainder)*			91	108				

*NFS statistics not available for Wales I and Wales II separately.

The intensity of the association between HF consumption and mortality rank order was assessed through Spearman's Rank Order Coefficient.

Women (all regions or type) 0.74**; women (excluding Wales) 0.94***

Men (all regions or type) 0.58*; men (excluding Wales) 0.79**

* $p < .05$; ** $p < .01$; *** $p < .001$.

standard regions and urban/rural aggregates for men and women aged 25 to 64 y. averaged over the 4 - year period 1968-71 and household consumption of HF arising from margarine and shortenings purchased as such plus the (hydrogenated) fat content of "cakes and pastries", "buns, scones and teacakes", biscuits, potato crisps etc., likewise bought for at-home consumption. (Absolute magnitudes are of course subject to error, but in as much as the same factors for fat-content were used throughout, relative magnitudes can be accepted with some confidence).

Quantities of HF consumed[3] through meals outside the private household (ca. 7g average) should be added to give totals consumed. Unfortunately precise statistics are unavailable, but it is obvious that (in the period in question) men consume on average substantially the higher fraction of this extra amount than women. Total consumption of HF - within and without the private household - will therefore reflect the tabulated "home" amounts more closely for women than men. In men the association is weak but still significant at $p < .05$; for women-as expected - the association is stronger. It must be admitted however that Wales is quite exceptional and its mortality experience is considerably higher than would have been expected as an area of low HF consumption. There is however one source of HF which has not been included in the above figures, namely fat consumed outside home in fish and chip shops. It was estimated from statistics supplied privately by the National Federation of Fish Friers that this extra amount was ca. 3g for the East and West Midlands and for the South-

East, 3½g for the North, North-West and London. For the other regions, the figures was much higher at around 6g (Wales 5.5, Scotland 6.5) thereby further increasing in a general way the disparity between the "good" and "bad" areas. The Welsh area now attained 6th position in the 10 ranks compounded from its 9 standard regions plus Greater London - which is somewhat more in keeping with its SMR.

There is no doubt however that mortality in Wales is exceptional. In this connection however it should be observed (Table 1.2) that the SMR is much higher in (industrial) South-East Wales than in the remainder of Wales, particularly for females; indeed for females in "rural" Wales, the SMR (91) is comparable with values in the best areas.

Table 1.2 also shows that mortality experience in rural districts is somewhat anomalous, with SMR some 10 units lower than expected from estimated HF consumption. In such areas however the number of meals consumed outside home (other than through packed lunches) can be expected to be lower than in urban aggregates, and fish and chip shops are likely to be less accessible. The discrepancy is therefore in the expected direction.

The use of SMR as the sole mean of relative mortalities fails to reveal one important feature, namely, that a bad area shows particularly high mortality at early age, whereas a good area shows low relative mortality in early life. For example for ischaemic heart disease (IHD) during the period 1968-71, the age specific mortality ratios (men) for the four age-groups 25 to 44, 45 to 54, 55 to 64 and 65 to 74 were 128, 116, 110 and 108 respectively for the North, and 85, 92, 94 and 97 for

Greater London. This greater risk at early age in a poor region could well be associated with high consumption of "instant" meals outside the private household.

Mortality from cerebrovascular disease (CVD) during the 1968-71 period is almost precisely parallel to the order in respect of IHD, highest mortality again appearing for Scotland, the North, North-West and Wales, and lowest for the South and South-East including London.

Secular changes in regional mortality from IHD over the period 1954 to the 1968/71 period were well accounted for in terms of changing consumption of HF. In the war years and up to de-rationing in 1954 (since which time HF consumption fell in the period under consideration), any differences in HF intake between one region and another were of course negligible. It is therefore obvious that the rate of fall-off in consumption in a given region from the earlier common level is determined simply by the consumption at any later date - including the 1962-71 period. In other words a region of low HF consumption in 1962-71 has a high rate of falling HF and should be showing some improvement in mortality rates relative to a region of high consumption.

That this is indeed true is shown by the death rates (per million living) from coronary heart disease in Table 1.3. for women aged 45 to 64 and men aged 25 to 44.

TABLE 1.3

Death rates from coronary disease; women (F) ages 45-64 and men (M) aged 25-44

Column Number	1	2	3	4	5	6	7	8	9
Region	Period 1958-1961 ICD 7th Rev. 420		Period 1968-1971 ICD 8th Rev. 410-414		Rate Increase		RANK ORDER Rate increase		RANK ORDER Household HF consumption
	F	M	F	M	F	M	F	M	
Scotland	1445	366	1954	451	509	85	1	7	1
North	1215	304	1585	439	370	135	4	1	2
North West	1110	332	1531	455	421	123	2	2	3
Yorks. and Humberside	1064	297	1397	405	333	108	5	3	4
East Midlands	816	231	1073	327	257	96	6	4	5
West Midlands	826	264	1057	353	231	89	7	5	6
South West	902	225	1002	287	100	62	10	8	7
South East and East Anglia	747	224	913	277	166	53	9	9	8
Wales	1077	358	1455	455	378	87	3	6	9
Greater London	746	249	950	290	204	41	8	10	10

The intensity of the association between rate increases and (falling) home consumption of HF was again assessed through Spearman's Rank Order Coefficient (R_s). Thus for women (columns 7 and 9), $R_s = 0.65$; $p < .05$. For men (columns 8 and 9), $R_s = 0.68$; $p < .05$.

Areas already having high mortality therefore continue to deteriorate relative to those of low mortality. The experience of Wales is again exceptional and the Principality continues to show marked deterioration.

The great (relative) improvement in Greater London is particularly noteworthy and follows a marked drop in margarine consumption from the common figures of 19g over the 1939 to 1954 period to 8g averaged over 1962-71. Over this period consumption of butter fat increased sharply in the London region with total fat-content of household diet remaining at about national average.

There is also a positive correlation of HF consumption and mortality within the social classes. The Registrar General's social classes are designed along broad lines to reflect a man's occupation, and to convey "some idea of his income, intelligence and education". The classification adopted by the NFS however rests purely on the income of the head of the household. In spite of obvious difficulties, Thomas was of the opinion that a meaningful comparison of the two definitions can be made as follows:

Social class 1 ≡ A1

Social class 2 ≡ A2

Social class 3 ≡ B

Social class 4 <u>plus</u> 5 ≡ C <u>plus</u> D1

The NFS data shows, as previously remarked that there is a class gradient in HF consumption in the order A1 → A2 → B → (C + D1). Thus for the major HF - containing foods (margarine, shortenings, "cake and pastries", "buns, scones and teacakes", biscuits) during the period 1959-63 inclusive, household consumptions for the above social classes were 19, 21, 22, 24g respectively. The gradient in respect of butter consumption was in reverse order, being highest for A1 (26g) and lowest for (C + D1) (18g), as also was the gradient for total fat consumption A1 8g higher than average to(C + D1) 4g lower than average. Table 1.4 below shows age specific mortality ratios (all classes England and Wales = 100) in respect of the Registrar General's "Group A" diseases (ICD, 7th Revision - which category consists essentially of vascular lesions of the central nervous system plus CHD; the nearest equivalent in the 8th Revision being CVD plus IHD) for men according to social class, and married women according to their husbands' occupation. The statistics cover the period 1959 to 63.

TABLE 1.4

Mortality statistics, cardiovascular disease, social classes; period 1959-63

Social class	Mortality ratios at ages 25-44	45-54	55-64	65-69
	Men			
1	65	90	94	93
2	84	86	93	94
3	99	102	102	106
4+5	117	107	102	97
	Women			
1	58	57	65	64
2	70	72	78	86
3	100	101	100	102
4+5	128	122	116	108

Subject to the above, equating of the RG's social classes and NFS income divisions, increasing HF consumption and decreasing butter consumption is paralleled by increasing mortality at all ages for married women. There is a similar but lesser gradient as expected - for men for the three age groups up to 64 y. The alarming pattern again emerges that differences are most marked at early age - particularly in the "lower" social classes.

In summary therefore, it may be stated that for the UK. (with the sole exception of South-East Wales which has only ca. 2% of the entire population of the UK.) mortality from arteriosclerotic disease is lowest in those areas having lowest consumption of HF and highest consumption of butter fat (South East including London, South West); it is highest in those areas having highest consumption of HF (Scotland, North, North West). The same pattern emerges for the social classes, highest mortality being experienced by those classes (4 + 5) consuming highest amounts of HF and lowest amounts of butter fat. In view of this pattern, it is difficult to understand how the idea that IHD is a "disease of affluence" has become widely accepted. Indeed, in spite of an earlier RG's clear statement to the contrary, a recent issue of The Lancet[8] has seen fit to remind its readership that (after allowance is made for differences in overweight, smoking, blood pressure, plasma cholesterol etc.) mortality from ICD for unskilled manual workers "was still 2.6 times greater than in the administrative grade" and that "indeed employment grade was a stronger predictor of a man's risk of dying from IHD than any of the more familiar risk factors". It must be added however that there was in fact some evidence

for higher mortality amongst classes 1 and 2 for older men (but not for younger men or women) at the earlier period 1949-53. This is readily explainable[3] by differential falling HF consumption after de-rationing in 1954.

It is realised that there are difficulties and dangers involved in international comparisons of death rates from coronary heart disease. However it has been pointed out[9] that in Europe for example, Southern Europeans like the Spaniards, Italians, Yugoslavs and Greeks, who consume little HF have considerably lower death rates than Northern Europeans like the British, Germans, Swedes and Finns who consume large amounts of HF. Eastern Eupopeans like the Bulgarians and Romanians who consume large amounts of saturated fat and cholesterol have low death rates from coronary heart disease; only trace amounts of trans acids were found[10] to be present in their red blood cells whereas levels in samples from Finland and America were reported to be 2.6% and 2.0% respectively.

In view of the above epidemiological evidence in the UK, it was decided to set up a further study of dietary fat and IHD on a more direct and "experimental" basis by examination of the composition of the body depot fat of persons dying of IHD compared with body fat of persons dying of unrelated causes. The (chemical) analytical problem is to ascertain whether there are present in the UK fat diet, particular fatty acids which could be taken as characteristic of some of the various fats making up that diet. Such a problem has to an extent at least been solved, and certain acids are found to

be present in relatively high concentration in ruminant animal fat whereas others are present in high amounts in industrially-hydrogenated fat.

1.3. Diagnostic features of natural versus hydrogenated fat

Three types of fatty-acid present in UK dietary fat (and also in human depot fat) serve to act as "markers" indicative of the presence of RAF and HF. These are:-

(1) Certain odd-number and branched chain acids 15:0, 15:0br., 15:1, 16:0br., 17:0, 17:1 plus 14:1 were grouped together and defined as L (Inclusion of 14:1 arose purely from experimental limitations in that GLC analysis failed to separate it from 15:0br it forms however only a minor fraction - ca. 15% - of total L)

(2) Marine oils are used extensively in the UK for the manufacture of margarine and cooking-fats/shortenings. They are of very diverse origin and include such fish-oils as "herring", menhaden, pilchards, anchovies etc.[5,11] as well as marine-animal oils obtained from the whale, seal etc. family. They contain large concentration of polyenoic C_{20} and C_{22} acids which by hydrogenation are converted mainly to monoenoic acids but with small amounts of saturated and di and trienoic components. Such hydrogenated marine oils (HMO) contain then high amounts of "higher acids" 20:0, 20:1, 20:2, 20:3, 22:0 and 22:1 which we collectively label H. According to source HMO contains from 25% to 50% of such C_{20} and C_{22} acids[12,13,14,15,16]. We have examined[17,18] 7 leading brands of UK margarine of high HMO content (from ca. 30 to 70% HMO) and it appears that in commercial practice a marine oil of mixed origin is used having a mean H content of about 35%[16]. More over the composition of such HMO content (i.e. relative amounts of 20:1, 22:1 etc) is reasonably constant and similar to those marine oils hardened to margarine

specification analysed by Ackman et al.[14] and by Lambertson et al.[16]. It should be added that it is very similar too to the composition of the H acids of our adipose tissue samples viz. 50% 20:1; 15% 22:1; 14% 20:0; 3% 22:0; remainder 20:2 plus 20:3 and indistinguishable between cases and controls.

(3) Total trans unsaturated acids were measured by infra-red spectroscopy and labelled T. Such acids arise during the commercial hydrogenation procedure whereby liquid oils are converted to solid or semi-solid fats. The percentages of trans acids thus formed vary greatly with the nature of the oil used and the use to which the product is to be put. An important point is that trans acids have a much higher melting point than their natural cis isomers, e.g.

Oleic acid (18:1* cis w 9+)	m.pt.°c	=	16
Elaidic acid (18:1 trans w 9)	"	=	44
Palmitoleic acid (16:1 cis w 7)	"	=	1
Palmitelaidic acid (16:1 trans w 7)	"	=	31

They may indeed have melting points approaching that of saturated acids of the same chain-length, e.g. stearic (18:0) 70°, and palmitic acids (16:0) 63°. The process of hardening can then be achieved not only through progressive removal of unsaturated components, but also (or instead) by conversion to isomeric trans material. In technical hydrogenation practice, addition of H_2 (progressive saturation) is unavoidably accompanied by geometrical isomerisation, but the operating conditions may be chosen to maximise trans acids if required.

*carbon chain length: number of double bonds.

+number of carbons in chain to nearest double bond from terminal methyl group.

In fact it is perfectly possible to produce a hard product with almost as much unsaturated components as the original oil. (indeed evidence is shown in section 4.2 that certain lower-priced "vegetable" margarines available in the UK are produced essentially by the isomerisation route, and these retain high levels of unsaturation including the poly-unsaturated 18:2. Such margarines would be regarded by majority medical opinion as beneficial and there is thus an incentive to increase production of such types. It should surely however be borne in mind that high levels of 18:2 in such materials is obtained only through concomitant formation of appreciable amounts of trans isomers - including isomers of 18:2 other than the natural (cis cis) linoleic acid. As will become clearer later it is considered safer to hydrogenate in greater degree and bring the level of linoleic acid up to the required level by subsequent blending with natural, unhydrogenated 18:2 - rich oil. Such materials have in fact appeared but retail at a substantially higher price).

Trans acids are present also in RAF as a result of bacterial hydrogenation in the rumen, but at levels substantially lower than in industrially - hydrogenated materials. It should be added however that the trans acids of the latter fat are widely spaced along the carbon chain whereas in RAF randomisation is far less and the (trans) bonds are more centrally situated[19]. This aspect of the problem is examined in more detail later.

The distribution of these "diagnostic" acids, T, H, L (and 18:2) in fats consumed in the U.K. are summarised below. The quantities given are based on laboratory work (in section 4.2).

using the same analytical techniques as were used for analysis of adipose tissue. The details for margarines are based on analysis of 7 leading brands of hard margarine and soft margarines purchased in 1976. Together they accounted for 95% of the retail market and weighting in proportion to market shares[20] permits evaluation of UK "average hard" and "average soft" retail margarines referred to below. The figures for butter are based on analysis of 3 popular brand blended materials purchased in 1976 and 6 similar brands purchased in 1980[21].

(1) RAF contains 6-8% L acids and ca. 1% H acids. Our figures for the T content of butter fat also lie between 6 and 8% and there is some evidence that these levels are higher than in earlier years an account of more intensive methods of milk production and winter supplementation of diet by 18:3 - rich seed-fat feed stocks[22]. Certainly our figures are higher than those of Hay and Morrison[23] (3 to 6% according to season) in their careful work on British milk fat. We believe the most probable average figure for RAF is 5 to 6%.

(2) Pig fat contains no trans acids. Amounts of H and L lie between 1 and 2% although it appears very likely that these acids are derived essentially from dietary supplementation by such as rape and fish oils.

(3) Other natural, unhydrogenated, oils and fats have virtually zero L and zero T. With the sole exception of rapeseed (and in much lesser extent peanut) which contains up to around 30% erucic acid 22:1 cis w 9, they contain very

little H acid. It is unlikely however that rapeseed oil is present in UK margarines in amount greater than about 5%.

(4) Hydrogenated vegetable oils (HVO) have virtually zero L and zero H. Their trans content depends on the commercial procedures used. A lower limit of about 12% is set to ensure that the product will keep, this being particularly true of soya with its high content of 18:3, and an upper limit seems to be about 30%. In the UK the average concentration appears to be around 16%.

(5) Hydrogenated marine oils have ca. 2% L and on average 50% T and 35% H.

British margarines and shortenings contain HVO and HMO in varying proportions blended with amounts of natural, unhydrogenated fat. Table 1.5 overleaf lists magnitudes of T, H and L in such materials as were available to the public around 1976 and can therefore be related to our experimental results for adipose tissue. Comparison with the statistics of Anderson and Williams[5] shows that there have been no significant changes in the overall composition of British margarines since 1960.

TABLE 1.5

COMPOSITION OF U.K. HYDROGENATED OILS AND FATS AND NATURAL ANIMAL FAT

Materials	% HMO	% T	% H	%L	% 18:2	Ratio T/L
Average hard margarine	48	30	12	2	7	15
Average soft margarine	5	12	4	0.2	19	60
Branded shortenings	70	34	18	3	6	11
Cooking oils (variable)	0	5	1	0.1	24	50
Butter fat		7	1-2	5	3	1.4
Beef fat		5	1-2	8	3	0.6
Mutton fat		6	3	5	3	1.2
Lard		0	2	1	11	0

1.4 Comparison of British and American margarine

American margarine/shortenings contain only indigenous oils such as soya and in lesser degree, cottonseed, maize, sunflower etc., hardened in varying degree. They do not contain HMO nor animal fats[24]. Although further details are given later (results section 4.2.). British margarines are extremely complex and variable. Whereas some of the more recent higher-priced soft margarines are very similar to American counterparts, the great majority available around 1976 were composites of -

(a) Hydrogenated soya and hydrogenated palm oils.

(b) HMO.

(c) Natural; unhydrogenated, vegetable oils such as palm, coconut/palm kernel, peanut.

(d) Generally smaller amounts of animal fat (mainly lard).

Relative amounts are largely determined by economic circumstances, and generally the cheaper products contained most HMO.

In the American context it is true that consumption of "vegetable" oils and polyunsaturated fatty acids (PUFA) can be boosted by substitution of animal fat by margarine. In the UK context however the result of such substitution may well be very different; there may in fact be an increase in saturation and a decrease in PUFA. To add to the confusion we may add that one particular brand of margarine proved on analysis to contain over 90% lard, whereas another more-recently available brand consisted entirely of beef-tallow.

Of more concern from our point of view is the fact that whereas those soft margarines purchased in 1976 contained little

or no HMO, cheaper brands of soft margarines purchased in 1980 have been found[21] to contain amounts of HMO as high as the 1976 hard margarines. If such a trend continues, the intake of trans acids in the UK will show increase in the near future.

In view of the above dissimilarities in UK and American margarines, it is considered that "extrapolation" of American claims in the "butter versus margarine" controvesy could be a dangerous manoeuvre. Although perhaps obvious, the view is taken that international epidemiological comparisons are meaningful only when comparing like with like.

1.5 Rationale of the case-control study; adipose tissue composition as a reflection of dietary fat

Many workers have observed that in a general kind of way human adipose fat "reflects" the fatty acid composition of the diet. The exact degree of correspondence is however difficult to establish mainly for three reasons: (a) it is difficult to ensure adherence to a fixed diet, (b) triglyceride equilibration may be a very slow process, (c) precise knowledge regarding the fatty acid spectrum of all fatty components of the diet is required, by no means an easy task.

The first serious attempt to solve the problem would appear to have been the work of Hirsch[25]. Thus a number of adult patients were kept on a constant caloric intake with 40% of total calories derived from corn oil. No change in the adipose spectrum was apparent after 10 weeks, but after 160 weeks the "composition of depot fat approached that" of the ingested oil, i.e. it contained 43% linoleic acid (corn oil, 54%). Under conditions of weight-gain, the approach of depot fat to dietary fat was much quicker. Thus the linoleic acid content of the adipose tissue of a one-year-old malnourished child fed on a diet containing 65% calories from corn oil had attained 50% after 140 days. The palmitic acid content dropped from 28% to that of corn oil (10%) in only 40 days.

A further significant observation was that a number of obese adults, subject to a low caloric diet virtually free from fat for "many months" showed no change in the levels of the acids 16:0, 18:0, 18:1 and 18:2, even though great losses in body weight had occurred. There was therefore no observable selective

release of the (at least) main components of adipose fat; adipose fat in adults therefore responds only to changes in the fatty components of their diet.

There can be no doubt too that a great variety of different acids can be so taken up (including isomers which do not occur naturally) with no greater difficulty than experienced with linoleic acid as above. Thus we get elevated levels of 12:0 and 14:0 (up to 16%) from coconut oil[25,26] and of linolenic acid up to 14% from linseed oil. The work of Shorland et al[26] is of particular interest in revealing high levels of branched-chain acids on the depot fat of New Zealanders (and Polynesians on a New Zealand diet) whereas such components were entirely absent in Polynesians on a native diet; such acids clearly derived from ruminant-animal fat which was lacking in the native Polynesian diet. There is also no "barrier" to the take-up of trans and monoenoic C_{20} and C_{22} acids - which are present in natural fats in only small amounts - and also of odd-numbered fatty acids of ruminant animal origin.

Corresponding work on (non-ruminant) animals has reached quite bizarre proportions. Thus in the work of Garton et al[27] pigs were fed from weaning on a diet very high in crude whale oil. By low-temperature acetone separation, the depot fat was shown to consist of up to 60% of glycerides identical with those of the ingested oil and there was no evidence even of (a) any acyl interchange having taken place between the whale oil glycerides and others, (b) any change in the mean unsaturation of the polyenoic C_{20} and C_{22} acids deposited.

A difficulty in holding the opinion that depot fat composition reflects only that of the fat content of the diet would appear to be in the fact that conversion of glucose into fatty acids in adipose tissue can undoubtedly occur. The view further appears to be that such conversion will be largely into palmitic acid, thereby resulting in depot fat high in C_{16} content.

The question arises however to what extent synthesis of fat occurs under circumstances which could be regarded as normal. The two following observations are relevant.

(a) In a comparative study of Korean monks, Korean farmers, Korean city dwellers and Korean soldiers - the last on American diet - Scott et al[28] found depot fat percentages of palmitic acid 25, 25, 24 and 25 respectively and of (16:0 + 16:1), 36, 36, 33 and 33. These percentages are strikingly constant in spite of the fact that corresponding dietary total fat - as percentages of total calories - were 7, 6, 17 and 42.

(b) According to Denton et al[29] "conversion of carbohydrate into fat becomes less important as the proportion of fat in the diet is increased", and "samples of human adipose tissue (taken from Western subjects with their high fat diet) synthesize little or no fatty acids from glucose". Work on laboratory rats shows that "if the proportion by weight lipid is increased to 30-40% the capacity to convert glucose is almost entirely lost".

Except possibly under conditions of severe fat-deprivation, it may be concluded that amounts of endogenous fat deposited

are negligible in normal healthy man compared with deposits of exogenous origin; the composition of depot fat may then reflect only that of the dietary fat.

It occurred to Thomas that the above difficulty of ensuring compliance of an individual with a given fatty diet, may be overcome by a comparison of a population-average depot fat composition with its average fatty diet. Using then (a) known amounts of the various fat containing items of food which contribute to total consumption of fat in the UK (Table 1.1.), (b) our analyses of the composition of the main items (particularly those of Table 1.5), (c) literature values for minor items, Thomas[17] calculated the fatty acid composition of the average UK dietary fat for acids C_{14} upwards. Acids 10:0 and 12:0 were not included since it is well known that lower acids are metabolised by a different route, and are deposited in depot fat in only small amounts. Although other details of the calculation are not included in this thesis, it is necessary to include one very relevant point, namely the relative amounts of hard and soft margarines. This is difficult to estimate with any degree of certainty, but it can be pointed out that in the UK the popularity of soft margarine is quite a recent trend. According to the British Consumers Magazine "Which", soft margarine accounted for a fraction 0.3 of the retail market in 1970; statistical information prepared by the Government Statistical Service[4] gave the share as 0.5 in 1975. Assuming from zero share in 1960 a growth rate rather more exponential than linear, a reasonable estimate for

the 1960-73 period would appear to be 0.20.

The results of such calculations are given in Table 1.6 along with the average composition of 95 (control) adipose tissue samples drawn widely from various regions in England and Wales.

The dietary estimate involving least reliability is that for linoleic acid. This arises mostly from uncertainty over the content of this acid in pork fat which is variously quoted at percentages from 7 to 14. The figure 8% was assumed as most probable for the UK at the period in question. It is of interest to add that in Britain lard plus poultry fat accounts for ca. 40% of the total intake, with RAF providing an additional 15%.

Dietary levels of 16:0 and particularly, of 18:0 are beyond doubt higher than depot fat percentages. The same conclusion may be reached from the figures of McOsker et al[30] who give the % 18:0 in the average American diet as 13 (when reduced to the same basis of comparison as our calculations) whereas the corresponding percentage in depot fat is ca. 5%. Combined quantities (16:0 + 16:1) and (18:0 + 18:1) however, agree satisfactorily - 29.2% and 48.3% respectively for average diet, and 29.3 and 50.4% for average depot fat.

Individual values of 18:2 in our samples fell between 4 and 16%, but there is good agreement between the average found and that estimated.

It is seen too that agreement extends to such details as amounts of minor, odd-numbered and branched-chain acids (L) and to components higher than C_{18} (H).

TABLE 1.6

Comparison of percentage fatty acid composition of the average UK dietary fat with that of average control adipose tissue*

Fatty acid	Dietary fat		Adipose tissue fat	
14:0	5.9		3.8	
15:0,15:0br.,14:1	1.4		1.3	
15:1,16:0br.	0.2		0.2	
16:0	25.3	29.2	22.3	29.3
16:1	3.9		7.0	
17:0br.	0.4		0.6	
17:0	0.8		0.5	
17:1	0.4		0.7	
18:0	12.3	48.3	5.1	50.4
18:1	36.0		45.3	
18:2	8.6		8.3	
18:3	1.0		0.7	
20:0	0.4		0.6	
20:1	1.2		2.0	
20:2, 20:3	0.9		0.7	
20:4	0.1		0.2	
22:0	0.3		0.1	
22:1	0.9		0.6	
Total	100.0		100.0	
T	5.4		5.2	
H	3.7		4.0	
L	2.8		2.7	

*Taken from reference 17.

The percentages of trans acids found in depot fat range over extreme values 2 to 12 with an average of 5.1; c.f. 5.4 estimated figure for the average diet. In only seven samples out of a total of 231 examined has the percentage been 3% or lower - values which could reasonably be expected to arise in the event of consumption of zero amount of margarine. On the reasonable assumption[3] that a person consumes twice the average amount of (average type) margarine with a corresponding reduction in butter consumption - the percentage trans would be 8%. This agrees well with our observation that only in 16 samples have we found amounts equal to or higher than 8%.

The author can see no way of reconciling individual variability in adult adipose composition on the one hand, with the above agreement in respect of average compositions on the other, except on the following premises:-

(a) the depot fat of an individual truly reflects the molecular weight distribution (as well as the trans content) of his dietary fat. In a reasonably homogeneous community, individual differences will then cancel.

(b) A measure of desaturation of 16:0 to 16:1 and 18:0 to 18:1 occurs to give a degree of unsaturation consistent with optimum cell-membrane fluidity and functional integrity.

The conditions must however be added that (a) and (b) will apply only when sufficient time has elapsed to permit equilibration; also that rapid body-weight gain or loss may upset the equilibrium in so far as it may arise from drastic dietary change or pathological condition.

In our study decedents showing evidence of rapid body-wasting have been excluded and have shown that over the 15 y period prior to commencement of the work there is no evidence of any significant changes in the nature of HF consumed in the U.K..

It is therefore assumed for such tissue samples which form the subject of this investigation - that a population - average depot fat composition truly reflects the fatty-acid composition of its dietary fat, subject only to the difference that a measure of desaturation of dietary 16:0 to 16:1 and of 18:0 to 18:1 occurs. In particular it is held that a group of persons consuming margarine at the expense of butter will show corresponding changes in T, H and L.

1.6 The ratio T/L as a measure of the relativity of HF to RAF

A fundamental difficulty in our work should now be fully appreciated, namely that allthough the concentration of trans acids is low in RAF compared with any hydrogenated material, yet the consumption of RAF in Britain is about 3 times greater than that of HF so its contribution to total acids is not negligible. Our estimate is-

Trans acids from RAF	2.1%
Trans acids from HF	3.3%
Total trans acids (T)	5.4%

The position with regard to H acids is similar:

Higher acids from HMO	2.0%
Higher acids from RAF	0.6%
Higher acids from non-ruminant animal fat	0.6%
Higher acids from non-hydrogenated vegetable oils	0.5%
Total higher acids (H)	3.7%

L is much more characteristic (of RAF) but there is some contribution from HMO and pig/poultry fat:

From RAF	2.2%
From pig/poultry fat	0.3%
From HF	0.3%
Total (L)	2.8%

Clearly then, neither L nor particularly, T (or H) in isolation will be likely to yield unambiguous information - although much elevated T levels would be difficult to ascribe to RAF.

The ratio T/L however is much more discriminating and this ratio is central to our study[31]. Reference to Table 1.5 shows that T/L for RAF is around unity, but around 11-15 for hard margarines/shortenings; for HVO (with its virtually zero L content), the ratio is very high, say 50.

At our UK average consumption of RAF and pig/poultry fat, the value of T/L in dietary fat will be 2.1/(2.2 + 0.3) = 0.8 in the event of zero HF consumption. With inclusion of HF the value will rise sharply to 5.4/2.8 = 1.9 for average consumption to even higher figures at consumption above average. In agreement with these dietary estimates, we have found that of the 231 specimens analysed, only 5 (the "all butter" eaters!) have values lying between 0.9 and 1.0 the rest falling between 1.1 and a maximum 5.3 - with an average (control) value 1.9.

Use of ratio T/L has two further advantages:

(1) Values of T are percentage values=$\frac{\text{Trans fatty acids in g}}{\text{All fatty acids in g}} \times 100$

They are therefore affected by inclusion in the diet of items of fat which contain no trans components e.g. a given intake in g/day from RAF plus HF will be "diluted" by intake of say unhydrogenated vegetable oils.

Similarly for L.

Being a ratio, T/L however is not so affected and its value is uniquely determined by the relative amounts of only those items containing finite content of T and L. Specifically, if we assume that amounts of pig/poultry fat are reasonably constant (its contribution to L is

in any case very small, ca. 0.4%, and even this might arise from dietary supplementation), T/L is determined only by the ratio HF/RAF.

(2) Use of T/L is more-immediately related to the subject under consideration, i.e. to the hard animal fat (RAF) versus margarine controversy (mentioned previously in section 1.2).

1.7 Formal hypothesis

Based on epidemiological evidence produced by Thomas for the UK, it is expected that the depot fat of cases will tend to have higher levels of T and lower values of L than that of controls. Statistical evaluation of the results in the form of the ratio T to L should be more profitable than levels of T and L separately. In as much as (a) suspicion has been cast by some authorities on longer-chain acids, (b) polyunsaturated acids are widely held to be beneficial, comparisons of depot fat levels of H and 18:2 are also included in the project.

1.8 Some methodological limitations

Our work is not a case/control study in the proper sense i.e. with controls set to have zero consumption of HF and cases consuming appreciable quantities HF. Indeed, except for the occasional individual, differences in consumption are not likely to be large on account of the fact that on average about 40% of HF is "hidden" in the sense that it derives from such widely consumed items as cakes, biscuits etc., bought as such.

We can then do no other than take what differences may exist. Again, arising from the very nature of coronary heart disease (a large proportion suffering fatal attack with no previous overt symptoms) an unknown fraction of these decedents whose deaths were accidental and therefore qualified as controls could well have experienced fatal heart attack a short time later.

One other point - although obvious - should be stressed. Whereas control specimens will be expected to randomly reflect the various component fatty acids of the British diet, the (selected) case specimens will show elevated levels of:

(a) those particular fatty acids carrying a hazard (if any) purely in virtue of their chemical structure as such.

(b) any fatty-acid types which may be correlated with a toxic material (if any) purely because they may occur together in the diet.

In case that the latter point is obscure we mention at this stage that margarine with its high T content also contains for example a variety of artificial anti-oxidants which are

absent in "natural" fats; a toxic anti-oxidant in that case will of necessity be accompanied by trans acids which may not of themselves carry any hazard.

2. EXPERIMENTAL

2.1 Tissue material

Postmortem samples of adipose tissue about ½" cube from the anterior wall near to the umbilicus were supplied to the laboratory through the courtesy of Dr. P.C. Elwood (Director) and the MRC Epidemiology Unit (South Wales), between January 1976 and June 1978. Cases consisted of male decedents dying from ischaemic heart disease (ICD 8th revision, categories 410 to 414) from 10 areas in the UK; deaths in the same areas and of the same sex and similar age dying from unrelated causes were used as controls. All decedents showed no evidence of wasting, and deaths from cerebrovascular disease or malignant neoplasms were excluded. The specimens of adipose tissue obtained by pathologists co-operating with the study at post-mortem examination, were transported in dry ice and stored in our laboratory in sealed containers at $-20^{o}c$.

2.2 General Methods

2.2.1 Glassware

It is essential that all sources of contamination with extraneous oils, greases, plastics and plasticisers be avoided. Cleaning of glassware was accomplished by overnight soaking in a 2% w/v aqueous solution of Quadralene detergent (supplied by Fisons Scientific Apparatus Ltd., Loughborough) contained in a stainless steel bucket, followed by thorough rinsing with tap and then distilled water. The glassware was routinely dried at ca. 80^{o}c in a clean specially reserved oven, and stored either in dessicators or otherwise suitably prepared contamination free cabinets. Convential glassware with grease free joints and groundglass stoppers was used for some chemical manipulations including simple solvent distallations. Extensive use was also made of a particular type of glassware system marketed under the trademark of SVL (made by Sovirel, France, supplied by V.A. Howe and Co. Ltd., London). It utilizes a unique grease free connecting system of butt joints held together by screw threaded thermally resistant plastic flanges, protected by PTFE coated 'O' rings. In particular, the use of SVL type test tubes with PTFE protected screw caps providing air tight seals, was found advantageous as separatory or reaction vessels.

For the purification of two particular solvents namely chloroform and methanol the use of an efficient fractionating column was considered essential. This consisted of a glass column (50 x 2.5 cm diameter) packed with stainless steel gauze around which was a heat insulating vacuum sealed jacket.

The column was adapted to accommodate SVL type fittings. These included a solvent reservoir at the base (minimum capacity 500ml) and at the top a reflux head fitted with a N.P.L. short stem thermometer graduated in 0.1^{o}, a double walled condenser, and a SVL Torion valve to control the distillate take-off. Guard tubes containing silica gel were also fitted.

One particular technique frequently used in lipid chemistry is that of solvent removal by evaporation, normally by jet of inert N_2 gas. In such circumstances and in accordance with the recommendations of Christie 1973[32] use of rubber and plasticized tubing has been avoided and short lengths of neoprene tubing were found to be satisfactory with no artifacts encountered.

2.2.2 The problem of lipid oxidation

When unsaturated lipids are exposed to atmospheric O_2, there is first an induction period during which any antioxidants present - including the naturally occuring tocopherols (vitamin E) - are consumed, and free radicals being to accumulate. This period is then followed by rapid O_2 absorption and an autocatalytic radical reaction (autoxidation) sets in. The induction period is shortened and the subsequent rate of autoxidation increased by (a) increase in temperature, (b) irradiation, (c) use of non-polar solvents, (d) increasing surface: volume ratio, (e) catalysts such as Cu, Fe, Mn and other transition elements, (f) increasing number of double bonds[33].

The mechanism and pathways of oxidation are complex but it is now widely accepted that the first step is radical attack at a methylene group next to a double bond:

$$R - CH = CH - CH_2 - \; + \; R^{\cdot} \rightarrow R - CH = CH - \dot{C}H -$$

This is followed by allylic rearrangement of the double bond and formation of a hydroperoxide:

$$R - CH = CH - \dot{C}H - \rightarrow R - \underset{\cdot}{C}H - CH = CH - \rightarrow R - \underset{\underset{O - OH}{|}}{CH} - CH = \dot{C}H -$$

Linoleic acid with two double bonds is more prone to such an attack and according to Holman[34], the rate of autoxidation for linoleiate is approximately twenty times that of oleate and each further addition of double bond increases the rate still further by a factor of at least two. The position regarding linoleic acid is further complicated by the fact that double-bond migration would tend to the formation of conjugated acids. Thus it has long been known[35] that autoxidation of methyl linoleate at low temperature in the absence of catalysts gives mainly conjugated cis trans octadecadienoate hydroperoxides. At room temperature in the presence of visible or UV light, significant amounts of trans trans conjugated isomers are formed.

Because of the particular nature of this case-control study in which trans acid levels are used as one of the differentiating parameters, it was realised that all possible precautions against lipid oxidation had to be taken. Although natural tissue antioxidants such as tocopherols may afford some protection, it was thought essential to add an additional synthetic antioxidant. We have found that addition of the widely-used antioxidant 2.6. di-tert butyl p-cresol (BHT) to

methyl esters resulted in spurious GLC peaks which could be taken as 16:1 or 14:0 according to the stationary phase (EGSS-Y and EGSS-X respectively) used. Use of tert-butyl hydroquinone (BHQ) however[36] was shown to give no artifacts and, under the column conditions used, no perceptible elevation of base-line.

As an added protection against autoxidation all lipid samples were handled in an atmosphere of N_2. Thus evaporation of lipid solutions was achieved via a N_2 gas jet directed onto the surface of the solution kept in a warm water bath (ca. 30°c). Again it became standard practice never to leave (or allow) any lipid extract or derivative to reach the dry state, and they were immediately taken up and stored in inert and purified polar solvents (e.g. chloroform and carbon disulphide). For long term storage they were kept at -20°c under N_2 in sealed glass screw capped bottles.

2.2.3 Solvents and Reagents

Some details are given below regarding the preparation/ purification of the various solvents and reagents utilised.

(i) Benzene (anhydrous) - AnalaR grade, free from olefines, was dried by fractionation in the previously described apparatus and the distillate collected at constant still head temperature (80°c/760mm).

(ii) Pentane - Olefine free AnalaR grade was further purified by simple distillation. The fraction boiling at constant temperature (ca. 35°c/760mm) was collected.

(iii) Carbon disulphide (anhydrous) - AnalaR grade was found suitable. It was dried over anhydrous Mg SO_4 and shown to yield no artifacts or elevation of base line of the chromatograms when used as the solvent in GLC analysis of fatty acid esters.

(iv) Hexane (anhydrous) - BDH technical grade (67-70°c fraction from petroleum spirit) was employed. As this solvent was the main eluant in a programme utilizing argentation column chromatography, where exceptionally large volumes (upto 250ml) were evaporated to dryness (thereby possibly resulting in impurity concentration) the material was purified by washing with successive amounts of concentrated sulphuric acids (ca. 10% v/v) until the acid layer became almost colourless. Residual acid was removed by washing with water and 2% w/v sodium bicarbonate solution. The wet hexane was dried over anhydrous calcium chloride prior to simple distillation. The distillate boiling between 67-68°c/760mm, was collected.

(v) Ether (anhydrous) - The AnalaR grade used was invariably found to contain peroxides. These were removed by vigorously shaking with 0.5% w/v ferrous sulphate in 1 N sulphuric acid until the starch iodide test proved negative. It was then washed with water, dried over calcium chloride and distilled. The fraction being collected at 35°c/760mm was stored in a brown winchester and protected from atmospheric moisture by a calcium chloride trap.

(vi) Methanol (anhydrous) - A technical grade was freed from aldehyde impurities by refluxing over KOH pellets for an hour. Anhydrous methanol was then obtained by fractionating at a high reflux ratio ca. 10:1 through the column previously

described, (section 2.2.1); collecting only the middle third of distillate at a still head temperature of 64.4°c/760mm. The usual precautions were taken regarding protection from atmospheric moisture. (Since unlike ethanol and higher alcohols, methanol does not form an azeotrope with water, preparation of water-free material is possible by high efficiency fractionation[37]).

(vii) Chloroform (ethanol free) - BDH technical grade chloroform (containing ca. 2% v/v of ethanol as preservative) was purified by washing three times with an equal volume of water, and allowed to stand over calcium chloride for two days. In order to avoid the possibility that the presence of ethanol could lead to the formation of ethyl esters - thereby resulting in incorrect GLC identification, the material was then fractionated through the same column as used above for methanol purification. Slow distillation at a reflux ratio of ca. 10:1 then permitted the removal of the remaining ethanol as an azeotrope with the chloroform of b.pt.59.7°c/760mm. The pure chloroform was collected when the column head temperature had risen to that of the pure material (61.2°c/760mm). It was then restabilized by the addition of purified methanol at a concentration of 2% v/v.

(viii) Acetyl chloride - Fresh AnalaR grade material was simply distilled as required using Clearfit glass apparatus protected from atmospheric moisture with a P_2O_5 guard tube. The first 20% of distillate was discarded leaving a similar volume of residual material. The collected distillate boiled at 59°c/760mm.

(viiii) 5% Methanolic HCl (anhydrous) - This was prepared by the method recommended by Christie[38] i.e. by slow addition of freshly distilled acetyl chloride (5ml) to 50ml of cooled (0^{o}c) anhydrous methanol. We have found this procedure very satisfactory provided, 1) the temperature is not allowed to rise above 0^{o}c and 2) the reagent is prepared fresh immediately before use.

2.2.4 Extraction of tissue

The adipose tissue employed consists of almost pure (99%) triglyceride[39] so that simple extraction with chloroform sufficed. An exact amount ca. 1.7g was cut from the centre of each tissue sample and weighed on aluminium foil whilst the tissue was still thawing. The sample was homogenized and extracted with 25ml purified chloroform (stabilized with 2% v/v methanol) using a high speed homogenizer (MSE fitted with 100ml vortex beaker). The chloroform extract was removed by means of a pipette from the compacted fibrous (wet) residue, dried over anhydrous $MgSO_4$, transferred to a screw capped glass bottle and finally stabilised by the addition of 1.5ml 0.1% w/v solution of the antioxidant BHQ in pentane. The glass bottle was flushed with N_2 prior to storage at -20^{o}c.

2.2.5 Conversion to methyl esters

Methyl ester derivatives were prepared directly (transesterification) from the tissue extract using anhydrous methanolic HCl. This method is considered by Christie[38] to be less

likely to lead to side reactions than use of other reagents such as BF_3 or H_2SO_4 in methanol.

A 2ml portion of the chloroform extract (ca. 130mg lipid) was transferred to a weighed 30ml SVL test tube. After complete removal of the solvent in a N_2 jet at 35°c, 4ml of the methanolic HCl reagent and 2ml purified dry benzene was added. The dead volume was purged with N_2 and the sealed tube heated for 2 hours at 60°c. After cooling Na Cl solution (10ml of 5% w/v) was added, the methyl ester extracted with 3 x 6ml portions of purified pentane, washed with $NaHCO_3$ (6ml of 2% w/v) solution, and dried over anhydrous $MgSO_4$. At this stage 1ml of a solution of 0.01% BHQ in pentane was added so as to give a concentration of 0.1% w/w of the antioxidant in the final pure ester[36]. The solution was then evaporated in N_2, weighed and quickly taken up in dry AnalaR grade carbon disulphide so as to give ca. 13mg ester per ml CS_2. It was finally stored at -20°c until required.

In the preliminary work, a lengthier procedure for conversion to methyl esters than that described above which permitted removal of possible nonsaponifiables, was used as follows.

2.2.6 Saponification procedure

Tissue extract (100mg of lipid) was treated with 10ml 0.6M methanolic KOH (Aristar grade in purified methanol). Purified benzene (3ml) was added to increase lipid solubility so as to give a single phase and the mixture heated in a sealed SVL tube

to 60°c for 2 hours. After cooling, water (7ml) was added and nonsaponifiables extracted with 3 portions (6ml) of distilled 40-60°c petroleum ether. The residue was treated dropwise with 6M HCl (ca. 1ml) until strongly acidic to phenolphthalein. The free acids were extracted with 3 portions (ml) of the petroleum ether, the solvent evaporated in N_2 and finally treated with methanolic HCl in the manner described previously.

Experience showed that these two methods of preparation of methyl esters from adipose tissue gave identical results within the limits of experimental error. The saponification method was however used in the analysis of margarines, butter and (natural) animal fats (section 4.2).

3. ANALYTICAL METHODS

3.1 Infra-red spectroscopy and measurement of total trans unsaturated acids

A Perkin Elmer Model 457 IR spectrophotometer with matched KBr cells (1mm path length) was used to record the IR spectrum: the reference cell was filled with AnalaR grade CS_2 and the sample cell with the methyl ester solution (ca. 13mg per ml CS_2).

The percentages of trans acids were calculated - using a base-line from 944cm^{-1} (10.59μ) to 998cm^{-1} (10.02μ) in the usual manner for AOCS Standard method Cd 14-61 - from the trans absorption at 965cm^{-1} (10.36μ). The concentration of ester was determined from the C—O groups (stretching vibration) absorption band at 1169cm^{-1} (8.55μ) as recommended by Allen[40]. The equation (as measured from a transmittance spectrum, for example see Figure 3.1 overleaf) for % trans acids is then:

$$\% T = \theta \left\{ \frac{\log (100/x) - \log (100/(x + x_A))}{\log (100/y)} \right\} + K\theta \qquad \ldots\ldots\ldots(1)$$

in which log (100/x) and log (100/y) are the absorbances at 965cm^{-1} and 1169cm^{-1} respectively, and log (100/x + x_A) is the absorbance at 965cm^{-1} measured from the (AOCS) base-line. It has been found that whereas θ is a constant independent of ester composition, K has a finite value dependent to some extent on factors other than the presence of trans bonds as such. (As demonstrated later, see below, the AOCS base-line

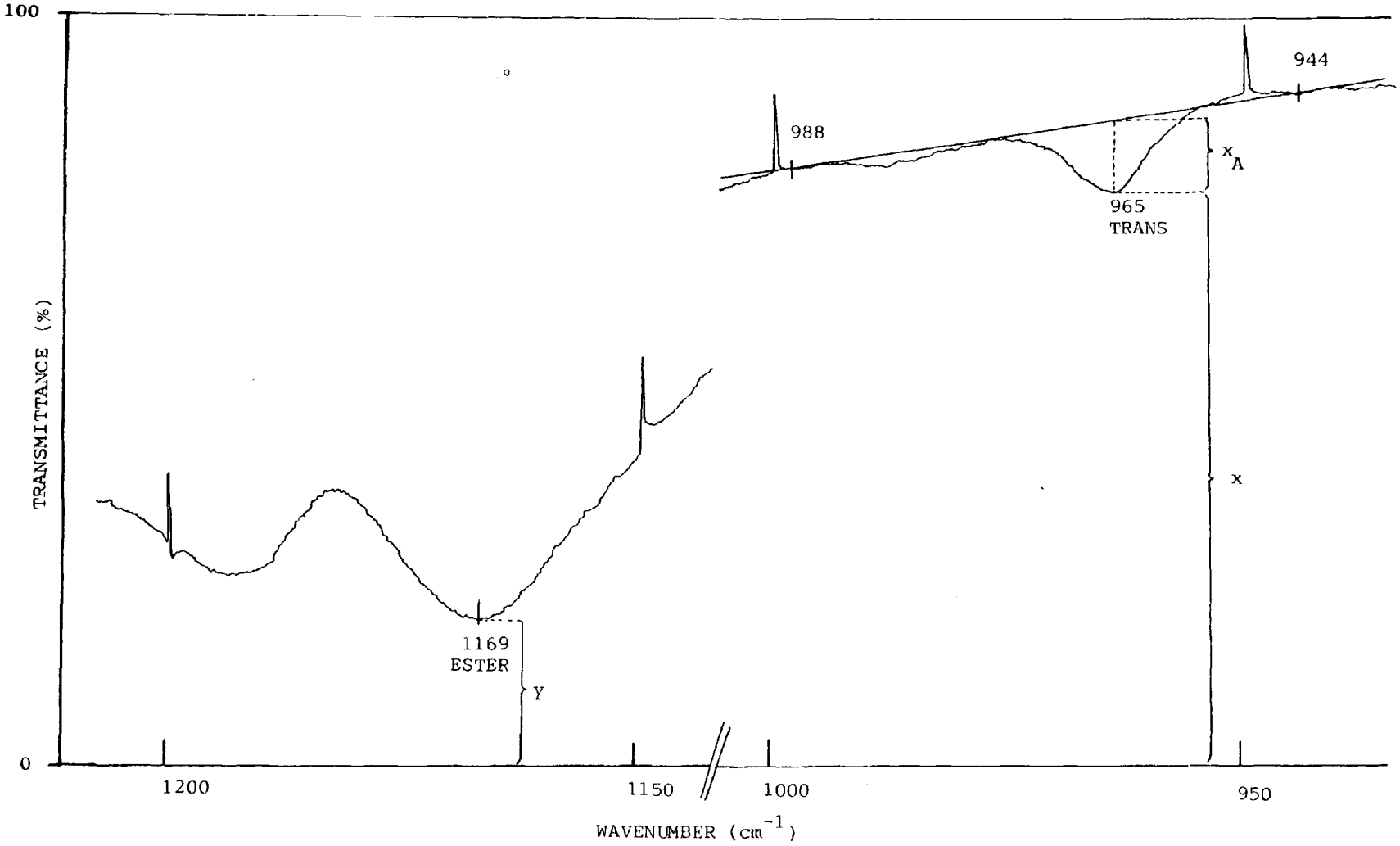


FIGURE 3.1. I.R. TRANSMITTANCE SPECTRUM OF ADIPOSE TISSUE METHYL ESTERS IN CARBON DISULPHIDE FOR MEASURING TOTAL TRANS - UNSATURATION WITH ESTER (C-O) ABSORPTION AS MEASURE OF CONCENTRATION.

method whereas undoubtedly a good compromise for oils containing relatively high trans content, does not make full allowance for variation in background absorption at 965cm^{-1} due to factors other than the presence of trans bonds. In effect the AOCS method assumes a zero value for K).

In order to evaluate θ and K for our instrument, it was necessary to prepare pure cis-unsaturated esters completely free of trans isomers. This was accomplished by the use of argentation column chromatography. Thus Florisil (60-80 mesh, made by Floridin Co., Pittsburg U.S.A. - supplied by Kernick and Co., Ltd., Cardiff) was acid washed and prepared in the manner described by Christie[41] and mixed with 20% of its weight of silver nitrate. A column of dimensions 50 x 2.0cm. diam. fitted with a Teflon stopcock was packed in the conventional manner and protected from light by aluminium foil. Samples of palmitoleic and oleic methyl esters (supplied in 99% purity from Sigma London Chemical Co., Ltd.) were then further purified by elution with dry hexane (acid washed, olefine free) containing 1.75% peroxide free dry ether (purified as in section 2.2.3, but in addition, prior to immediate use, passed through an alumina column). All trans (and saturated) impurities consistently migrated ahead of cis material and purity of the cis products was demonstrated by IR and GLC. Samples of methyl linoleate were similarly purified by eluting first with 1.75% ether solution to remove saturated impurities, monoenoic and conjugated dienoic esters and then with 6% ether in hexane solution. It was thus found that authentic samples of (cis)

palmitoleic and oleic esters were always contaminated with between 0.5 and 1% trans components. Authentic methyl linoleate likewise usually contained ca. 1% trans content, (and some conjugated isomer).

Using such standard esters (and also proven authentic saturated acids) in CS_2 it was found that K is dependent on amounts of (cis) unsaturation. Putting T = zero in (1) gives:

$$- K = \frac{\log (100/x) - \log (100/(x + x_A))}{\log (100/y)}$$

and a series of saturated esters 14:0, 16:0, 18:0 and 20:0 gave K (= K_s) constant at 0.021, within the limits of experimental error. Its value for pure cis 16:1 and 18:1 (K_u) was similarly constant at 0.016, and its value for pure ("double unsaturated") methyl linoleate (K_{du}) was zero.

It was further found that K is an additive function i.e.

$$(K)_{mixture} = f_s K_s + f_u K_u + f_{du} K_{du}$$

in which f_s, f_u and f_{du} are the corresponding weight fractions. Thus (a) a mixture of equal parts of 18:0 and cis 18:1 gave duplicate experimental K values of 0.016 and 0.023 (calc. value 0.0185), (b) a similar mixture of 18:0 and cis cis 18:2 gave duplicate values 0.009 and 0.011 (calc. value 0.0105), (c) similarly with cis 18:1 and cis cis 18:2 gave 0.007 and 0.009 (calc. 0.008).

To evaluate θ a standard mixture was prepared of (purified) methyl esters in relative concentrations approximating closely to the amounts in adipose tissue. This was achieved by first

making up three solutions (labelled A, B and C) in volumetric flasks with pure hexane at 0°c, with BHQ added at 0.1% w/w to ester concentrations. The esters were initially freed from any solvent and moisture in a vacuum dessicator connected to a high speed pump until the pressure had dropped to 0.1mm (Vacustat manometer). They were then accurately weighed ± 0.02mg..

Solution A. - 50ml		WEIGHT mg	%
methyl myristate	(14:0)	35.40	9.04
methyl palmitate	(16:0)	191.48	48.90
methyl palmitoleiate	(16:1c)	59.12	15.10
methyl stearate	(18:0)	47.98	12.25
methyl linoleiate	(18:2cc)	57.56	14.70
	TOTAL	391.54	99.99
Solution B. - 25ml			
methyl oleate	(18:1c)	163.00	6.52mg per ml
Solution C. - 50ml			
methyl elaidate	(18:1t)	219.64	4.39mg per ml

Amounts of solution B and/or C were pipetted (0°c) into solution A such as to give 5 standards of composition:

ACID	STD. NO. 1	2	3	4	5
	WEIGHT PERCENT				
14:0	4.51	composition in all standards			
16:0	24.45				
16:1c	7.54				
18:0	6.12				
18:2cc	7.35				
18:1c	50.02	44.81	39.50	25.0	0.00
18:1t	0.00	5.21	10.52	25.02	50.02
	99.99	99.99	99.99	99.99	99.99

For example the second standard of 5.21% trans content was prepared by pipetting 5.38ml of solution B (35.10mg of 18:1c) and 0.93ml of solution C (4.08mg of 18:1t) into 5ml of solution A (39.15mg of esters). The remaining standards were similarly made up by adding solutions B and/or C as necessary to provide a total 50.02% content by weight of 18:1 isomer.

Each standard (in hexane) was evaporated to "dryness" under a N_2 jet until constant weight, and quickly taken up in CS_2, so as to give 13mg ester per ml.

As a result the percentage amounts of trans acids (as 18:1t) were therefore zero, 5.21, 10.52, 25.02 and 50.02%. Each of these solutions was analysed on four separate occasions ca. weekly intervals. Subsequently a best mean square fit with equation (1) for this standard mixture gave θ = 113.40 and K = 0.016 (compare calculated $K_{mixture}$ = 0.352 x 0.016 + 0.576 x 0.021 + 0.73 x 0.0 = 0.0166). The measure of experimental accuracy achieved can be judged by the transmittance measurements and the calculated % T values recorded in Table 3.1 overleaf.

Unlike K, the value of θ had the same value for all mixtures examined. Thus taking K_u = 0.016 as above, a series of nine mixtures of 18:1 cis and 18:1 trans (0 to 45% trans) gave a mean value of θ=113.2 (S.D. 0.10); 100% 18:1 trans also gave 113.0. A mixture of 50% 18:0 and 50% 18:1 trans making K_m = (0.5 x .021)+(0.5 x 0.016) = 0.0185 led to θ = 113 and similar proportions of 18:1t and 18:2cc with K_m = 0.08 gave θ = 112.2.

It must be emphasized that use of a given (constant) value of K for all mixtures will lead to incorrect evaluation

TABLE 3.1

IR ANALYSIS OF STANDARD MIXTURE (0 - 50% trans)

% TRANS (BY WEIGHT)	% TRANSMITTANCE			% TRANS		
	x	$x+x_A$	y	CALC.	MEAN	DIFFERENCE*
0.00	90.0	87.6	20.0	-0.13	- 0.04	-0.04
	90.3	88.2	20.2	0.06		
	91.0	89.1	23.2	0.05		
	91.0	89.0	24.3	-0.12		
5.21	83.8	88.0	20.1	5.09	5.23	+ .02
	85.1	89.2	22.0	5.21		
	85.2	89.4	22.0	5.30		
	84.8	88.9	22.8	5.31		
10.52	78.4	88.4	21.2	10.50	10.57	+ .05
	77.2	87.3	20.4	10.49		
	78.9	88.6	21.3	10.88		
	78.4	88.1	21.8	10.41		
25.02	63.0	87.6	20.1	25.06	25.03	+0.01
	64.2	88.0	21.8	25.29		
	65.9	88.3	23.2	24.49		
	64.1	87.3	22.3	25.29		
50.02	46.8	88.7	22.2	50.16	49.99	-0.03
	47.0	88.0	22.9	50.16		
	47.4	87.6	23.2	49.59		
	51.7	89.0	27.8	50.05		

*Difference in % T (experimental mean minus prepared weight %)

of T: a difference in T values between two samples of say differing degrees of unsaturation could then arise purely by experimental artefact.

The extent of the errors introduced is demonstrated by the application of the standard AOCS method (K = zero) for methyl stearate which would give an apparent trans content of: T = 113 x (-0.021) = -2.4%, and similarly for 100% authentic cis 18:1 the apparent values would be - 1.8%.

Analysis of trans content of adipose tissue was conducted in batches and calibration against the standard mixtures (similar to adipose composition) was repeated for each such batch. The samples were submitted to us "blind" by the Epidemiology Unit and the IR analyses were therefore conducted with no knowledge as to which were cases and which controls. T values were calculated from the equation:-

$$\% T = 113 \left\{ \frac{\log (100/x) - \log (100/(x + x_A))}{\log (100/y)} \right\} + (113 \times 0.016)$$

- for return of the results to the Epidemiology Unit. Later on completion of GLC analysis, the T values were "corrected" for varying ester-composition using the appropriate K values above. Except for the occasional sample having unusually low or high amounts of 18:2, the "corrections" were around $\pm$ 0.05 to 0.1%. For adipose tissue samples, such "corrections" were mostly within the limits of experimental error (as demonstrated in section 4.1). Neglect of the 2nd term (Kθ) in equation (1) when dealing with margarines of highly varying compositions however would introduce quite serious error.

As an added precaution in the IR measurements, ester concentrations were arranged so that absorbance at 1169cm^{-1} (8.55μ) was kept within quite narrow limits, y = 20 to 25.

3.2 Gas - liquid chromatography and determination of fatty acid spectra

This was accomplished using a Perkin Elmer Model F17 gas chromatograph fitted with dual flame ionization detectors (FID). All columns were of stainless steel 6ft by ¼in.(o.d.) and 100 to 120 mesh acid washed and silanised chromosorb W was used as solid support. By using pairs of matched columns containing liquid phases of differing polarity[42], each pair temperature - programmed (and in differential-mode operation to eliminate base-line drift) it was found possible to identify main and most minor components up to the elution of the acid 22:1.

Operating conditions were as follows: 1) "X column" - packed with 8% EGSS-X; isothermal at 190°c for 16 minutes, thereafter linearly programmed to 200°c at 1°c per min, N_2 carrier gas at 35ml per min. 2) "Y column" - packed with 15% EGSS-Y; initial temperature 165°c linearly programmed to reach 200°c at 1°c per min: N_2 as carrier gas at 51ml per min. The injector/detector ports were constantly set at 225°c.

FID amplifier attenuation changes to greater sensitivity were made on the X chromatograms (Figure 3.2) after elution of acid 18:1 (4 times) and on the Y column (Figure 3.3) after elution of 18:2 (8 times). The chromatographic spectra were traced at 1cm per min., on a Servoscribe Model 1S flat-bed recorded fitted with a "ball and disk" integrator.

The optimum ester loadings were found to be 7 µl of the CS_2 solution (ca. 13mg per ml) on the Y column and 5 µl on the

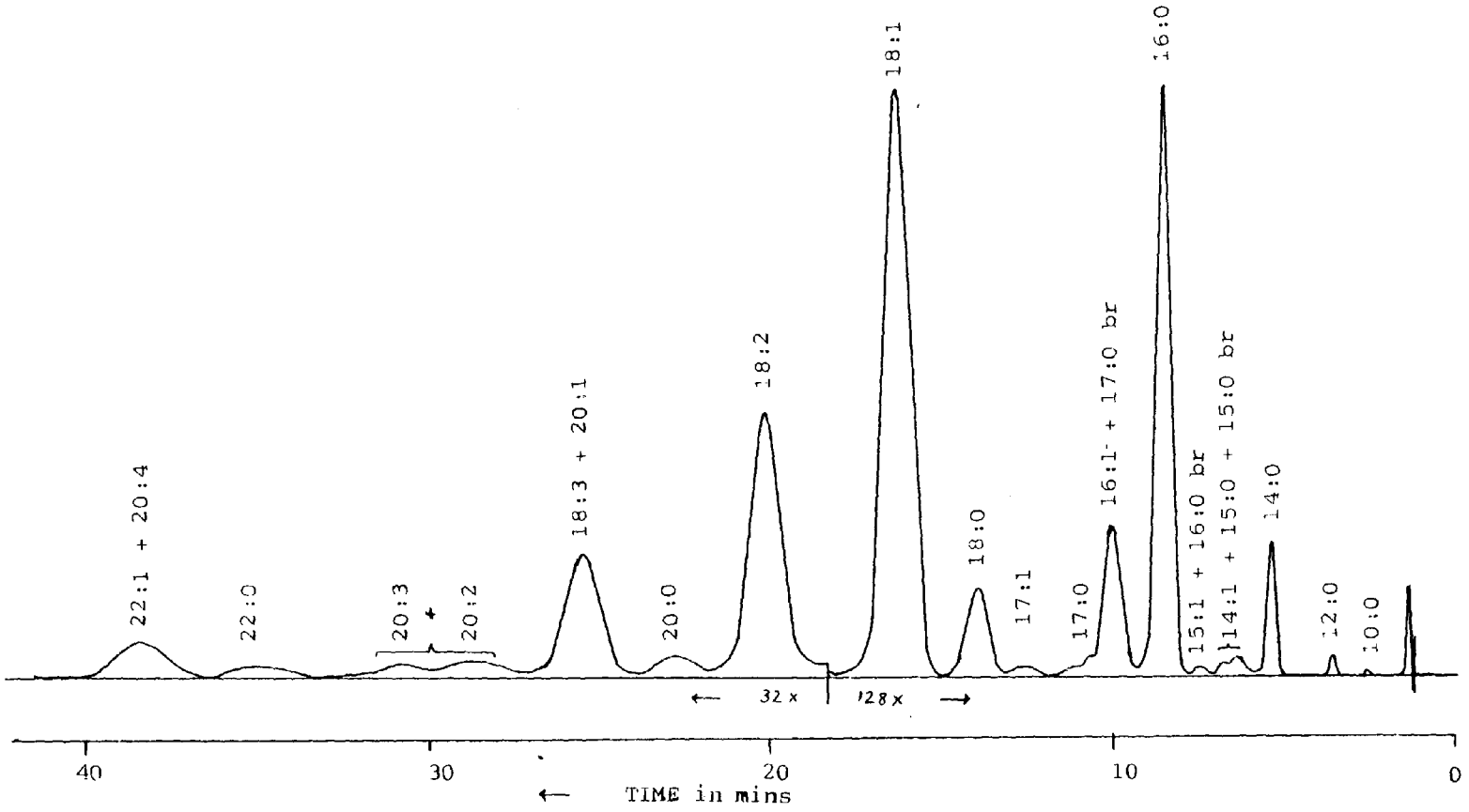


FIGURE 3.2. EGSS-X CHROMATOGRAM OF ADIPOSE FATTY ACIDS (AS THE METHYL ESTERS) - TEMPERATURE PROGRAMMED - SEE TEXT.

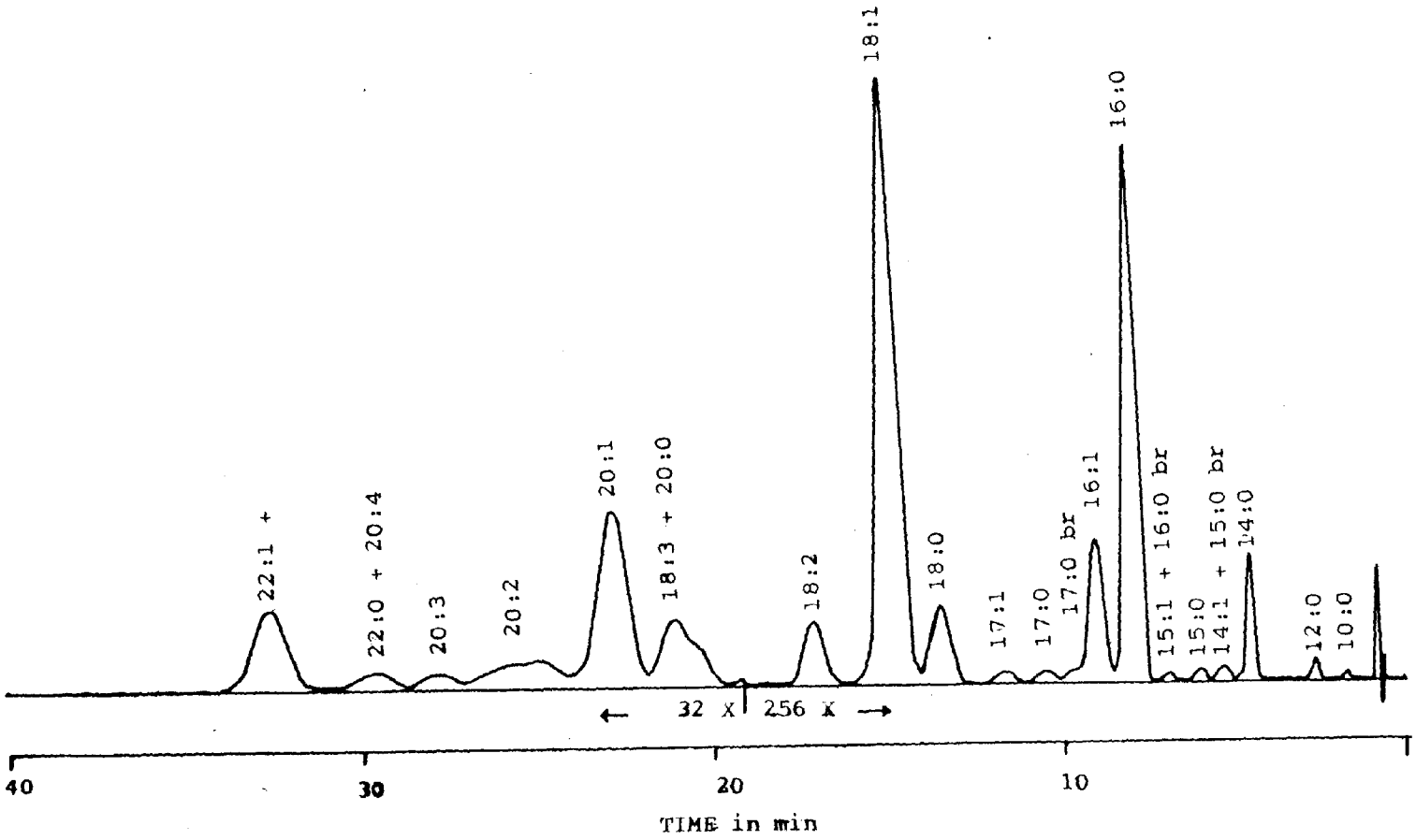


FIGURE 3.3. EGSS-Y CHROMATOGRAM OF ADIPOSE FATTY ACIDS (AS THE METHYL ESTERS) TEMPERATURE PROGRAMMED - SEE TEXT.

X column. The use of CS_2 resulted in substantially better base - line performance than when using hexane or other similar ionisable solvents, particularly for the earlier eluted components.

A total of 18 well defined peaks (plus one double shoulder) five of which were composite - were discernable on all the samples examined on the X column, the last being a composite peak (22:1 plus 20:4). On the Y chromatograms 19 well defined peaks (plus two shoulders) were observed - five being composite - the last being due to the acid 22:1. For ease of reference the recorded peaks and shoulders were numerically designated in order of elution.

Later peaks have also been observed in small quantities but being undoubtedly due to highly unsaturated C_{20} and C_{22} acids, they were judged of no relevance to the present work and were not quantified.

Identification of the peaks was achieved through the following:

1) Isothermal runs on both X and Y columns (at 200^{o}c otherwise conditions as previously given) by using equivalent chain lengths (ECL), as tabulated by Jamieson[43]. These reported values were useful as a guide only[44] as ECL values were also determined in the laboratory from analysing suitable fatty acid methyl esters (FAME) commercially obtained (Sigma London Chem. Co., Ltd., Supelco, Inc., supplied by Bioscan Canvey Island). The values obtained are tabulated in Appendix A.

2) Temperature programmed runs using a) a wide spectrum standard mixture similar in composition to adipose tissue

(given in Table 3.2).

3) "Doping" experiments, i.e. authentic methyl esters were added in small quantity to a portion of an adipose sample and rechromatographed; that peak whose area increased relative to others being taken as the identity of the standard added. In this technique care was taken that the amount of added component was such that the increased area did not overlap adjacent peaks.

4) The fatty acid profiles of the adipose tissue materials were found to be remarkably similar (although relative quantities of the components vary between small limits) and were soon seen to reflect faithfully the characteristic features of such major dietary fats as butter and hydrogenated marine oil, as previously described (1.5). The detailed compositions of both these fats have been previously well studied[45,46,12] and further details have been obtained during the course of the present investigation; thus in effect can be used as secondary standards. The measured ECL values (up to erucic acid) obtained for these various component methyl esters are included in Appendix A.

5) Use of preliminary techniques prior to analysis was found very useful for confirming the identity of acids particularly for those of identical or very similar ECL values. The technique utilised was: 5a) Argentation column chromatography principally by the method of Thomas [17] using ether in hexane at percentage levels (v/v) of 0.5, 1.75, 6 and 10 to separate by elution an adipose methyl ester mixture into respective fractions of saturated mono, di and trienoic esters. The "Y"

chromatograms (Figure 3.4) of these fractions together with the "master" analysis serves to illustrate the complex nature of such 99% pure triglyceride material[25]. 5b) Catalytic hydrogenation[48] was employed to confirm the presence of odd-numbered and branched chain acids.

Quantitative measurement of peak areas was effected using the integrator of the Servoscribe's recorder referred to previously. Partially overlapping peaks each with distinct maxima were quantified by using the perpendicular drop method[49]. The total area under all the peaks was equated to 100% and the percentage of each peak was then calculated. The peaks identified as 20:2 and 20:3 (see Figures 3.2 and 3.3) were rather ill-defined, but for the purpose of this study this was of little consequence and the corresponding areas were combined.

For GLC calibration a carefully prepared standard mixture was used (made up in a manner similar to that used for preparation of IR standards - see Table 3.2 overleaf) of all the even-numbered straight - chain saturated acids from 12:0 to 24:0 plus the acids 16:1, 18:1, 18:2, 18:3, 20:1 and 22:1 at relative concentrations approximating closely to the amounts in adipose tissue. All such acids were from Sigma London Chemical Company Limited.

Evaluation of GLC experimental accuracy was initially determined by analysing this standard "adipose" mixture six times at ca. monthly intervals with both pairs of columns. The percentage means for each component ester are recorded in Table 3.3 (columns 3 and 6) obtained from the X and Y

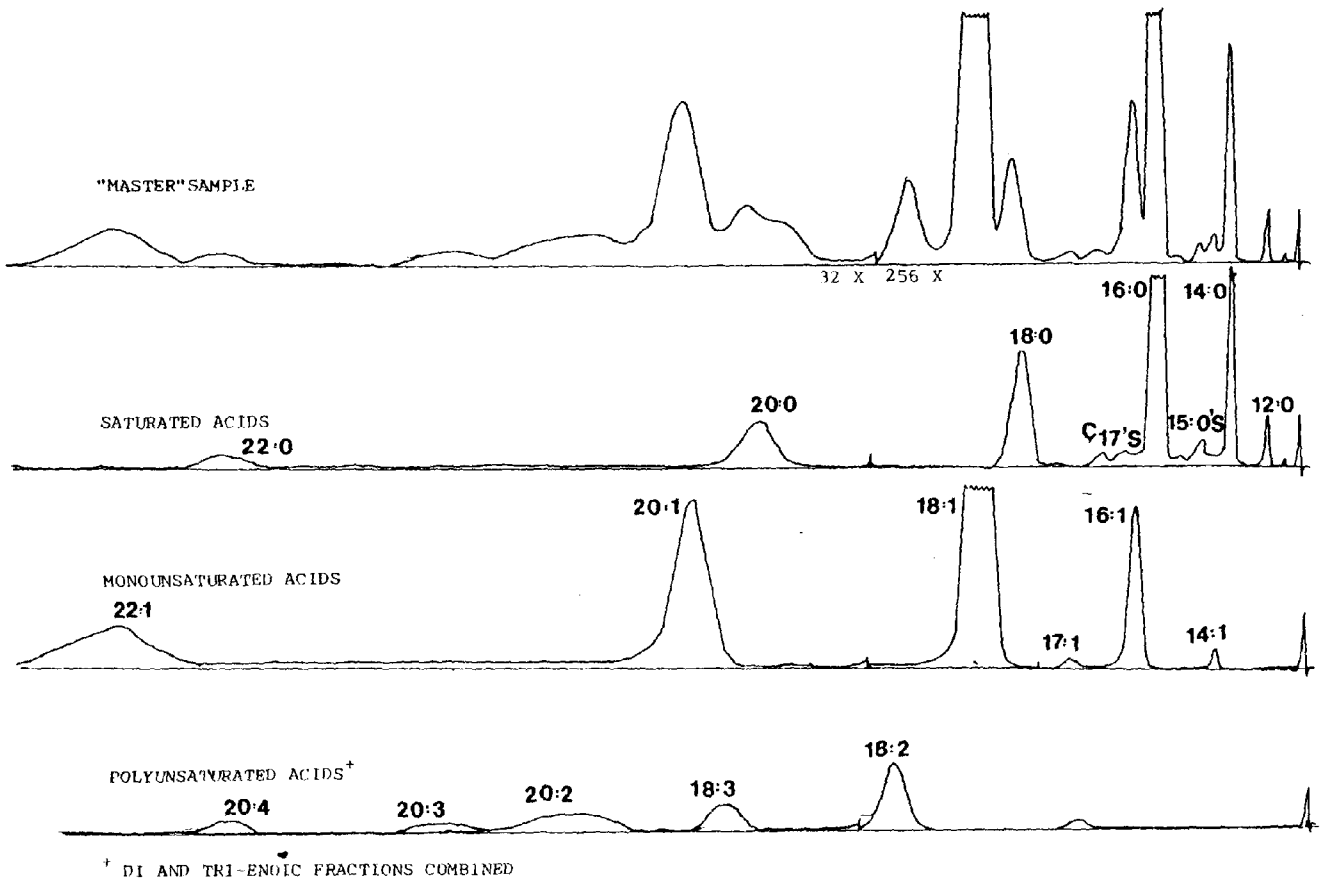


FIGURE 3.4. EGSS-Y CHROMATOGRAMS OF AN ADIPOSE FATTY ACID MIXTURE (AS THE METHYL ESTERS) AND ITS COMPOSITE FRACTIONS - SEPARATED ON A $AgNO_3$/FLORISIL COLUMN[47] - ISOTHERMAL, SEE TEXT.

TABLE 3.2

PREPARATION OF STANDARD MIXTURE (SIMILAR TO ADIPOSE COMPOSITION) FOR GLC CALIBRATION

FAME[+]	SOLUTION COMPOSITION		18:1c WEIGHT (mg)	STANDARD COMPOSITION WEIGHT %
	A WEIGHT (mg)	B WEIGHT (mg)		
12:0		15.06		0.64
14:0	18.87			3.66
16:0	113.11			21.95
16:1	39.35			7.64
18:0	26.97			5.23
18:1			48.86	47.41
18:2	44.56			8.65
18:3		12.95		0.55
20:0		14.61		0.62
20:1		40.05		1.73
22:0		13.25		0.56
22:1		21.20		0.90
24:0		10.80		0.46
TOTAL	242.86	128.32		100.00
HEPTANE ADDED TO ml ($0^{o}c$)	25	25		
ESTER CONC. mg.per.ml	9.714	5.13		

The standard mixtures composition (weight percent - given in last column of table) was prepared by adding (via pipette at $0^{o}c$) 5ml solution A and 1.10ml solution B to the weighed amount of 18:1c (48.86mg). Heptane replaced by carbon disulphide after evaporation of heptane under N_2 to give ca. 13mg ester per ml CS_2.

TABLE 3.3

GLC CALIBRATION ANALYSIS ON "ADIPOSE" STANDARD MIXTURE

ON BOTH EGSS-X AND EGSS-Y PACKED COLUMNS

FATTY ACID	WEIGHT %	EGSS-X			EGSS-Y		
		AREA % MEAN	DEVIATION FROM MEAN VALUE	DIFFERENCE WGT%-AREA%	AREA % MEAN	DEVIATION FROM MEAN VALUE	DIFFERENCE WGT%-AREA%
12:0	0.64	0.67	0.02	-0.03	0.64	0.01	0.00
14:0	3.66	3.62	0.02	+0.04	3.73	0.02	-0.07
16:0	21.95	21.65	0.12	+0.30	22.10	0.10	-0.15
16:1	7.64	7.90	0.11	-0.26	7.75	0.04	-0.09
18:0	5.23	5.48	0.10	-0.25	5.30	0.05	-0.07
18:1	47.42	46.84	0.23	+0.58	46.88	0.18	+0.54
18:2	8.65	8.71	0.08	-0.15	8.79	0.02	-0.14
18:3	0.55	0.72	0.07	-0.17	0.46	0.03	+0.09
20:0	0.62	0.72	0.05	-0.10	0.72	0.02	-0.10
20:1	1.73	1.69	0.02	+0.04	1.69	0.02	+0.04
22:0	0.56	0.67	0.05	-0.11	0.58	0.02	-0.02
22:1	0.90	0.89	0.02	+0.01	0.85	0.01	+0.05
24:0	0.46	0.44	0.02	+0.02	0.50	0.01	-0.04

chromatograms. The standard deviations of the mean values are shown in the fourth and seventh columns. The small random % differences obtained by subtraction, (columns 5 and 8) of each component peak area % from the corresponding made-up weight %, indicates that peak area (from FID response) is proportional to ester component by weight. The differences also appear satisfactorily random. However, perusal of both sets of differences tends to show that overall the Y column chromatograms provided the more accurate (i.e. lower differences) analysis.

Throughout the case-control study quantitative analysis was checked by periodical analysis of the "adipose" standard mixture (approximately once per 10 adipose tissue specimens). Such an exercise was not only undertaken to ensure freedom from instrumental secular drift but also to monitor the resolution performance of the packed columns which can vary[43] as a result of loss (due to bleeding) and/or chemical modification of the stationary phase due to column ageing. Finally on a daily basis - prior to analyses - the sample columns background bleed rate at 200°c was utilized to ensure optimum FID sensitivity. Simply achieved by obtaining a maximum deflection of the recording trace (on adjusting the H_2 flow rate) at an even higher attenuation (8 times) than the most sensitive used (see above). Only then was the recording pen adjusted as necessary to track the (zero integrating) baseline.

The relevance of the amounts of H and L the diagnostic parameters - in relationship to T - was not at first fully

appreciated and except for the first 43 specimens (referred to later in subsequent sections 4.1 and 4.5.1) restriction regarding blind submission to the laboratory was relaxed in respect of GLC analysis. For determination of H and L levels (and hence also of 18:2); the specimens were however still submitted by my supervisor "blind" for analysis. Consequently all chromatograms were measured with no knowledge of which cases and which controls.

In order to evaluate the higher acids the percentage 18:3 was calculated by subtraction of the % 20:0 obtained from the X column from the composite peak number 15 (18:3 + 20:0) of the Y column. The percentage 20:4 was similarly evaluated using the composite peak (22:0 + 20:4) of the Y column, and the 22:0 peak of the X column. The parameter percentage H was then obtained by subtraction of the percentage (18:3 + 20:4) from the total eluted from the Y column later than 18:2 and is therefore the total of the acids 20:0, 20:1, 20:2, 20:3, 22:0 and 22:1.

Evaluation of L was achieved by area summation of the Y column peaks 4 (14:1 + 15:0br), 5 (15:0), 6 (15:1 + 16:0br), 10 (17:0), and 11 (17:1).

3.3 Determination of "lower trans acids" (T_L), 16:1 trans and 18:1 trans by gas-liquid chromatography

At a later stage in the project, as a consequence of case versus control statistical evaluation of total trans-unsaturated fatty acids in relationship to higher acids in the adipose specimens it was concluded (see section 6) that risk was attached preferentially to the "lower trans acids" (T_L) i.e. the sum of 16:1 trans and 18:1 trans.

The earlier IR spectroscopic method of measuring "total" trans acids in the adipose specimens (see section 3.1) did not permit separate quantification of T_L. The availability of a more recently developed highly polar GLC column which can resolve cis/trans geometric isomers directly was therefore considered of sufficient import to rechromatograph all the 231 study specimens.

The methyl esters were those used previously (collected over the period 1976-1978). The methyl esters (ca. 100mg per sample) had been kept stored in CS_2 solution in sealed containers at $-20^{o}c$ in the presence of BHQ antioxidant. Re-measurement (in 1980) of properties (T by IR, fatty acid patterns, including L and H via GLC on EGSS-Y polyester) of a number of samples showed no evidence of chemical change over this period. As an added precaution the new GLC analyses were conducted in batches, each area at a time, in the order in which the specimens were originally received. The chromatograms were read and reported (to my supervisor) with no knowledge of which samples were cases and which controls.

Fatty acid chromatograms (for example, see Figure 3.5) were determined with the previously used P.E. F17 gas-chromatograph. The columns were of stainless steel 40ft by 1/8in (od), packed with 20% OV-275 (Supelco Inc., supplied by Jones Chromatography Ltd., South Wales) coated on 100 to 120 mesh acid washed and silanised chromosorb P.[50]. To elliminate base-line drift all measurements were conducted isothermally using pairs of matched columns. The column temperature was 220°c; carrier gas, N_2, flow rate 30ml per min; optimum ester loading 2µl of CS_2 solution (13mg per ml). Under such conditions, the retention time of 18:1 cis was about 50 min from the solvent peak.

Slight adjustments in carrier-gas flow were periodically made in order to keep the retention time of this major peak within narrow limits. Column performance was monitored by periodic analysis of an "adipose like" standard mixture, prepared as previously (in section 3.2), but in addition included the trans acids palmiteladic (16:1t) and elaidic (18:1t) at weight percentages of 1.4 and 4.5 respectively - which approximated to the amounts found in the adipose samples.

Identification of the peaks was achieved essentially as previously described (see section 3.2) by:-

(a) determination of ECL[43,51] values using also such published values as are available. The values obtained in the laboratory are tabulated in Appendix A and were obtained through running authentic methyl ester mixtures (components and mixtures obtained from Sigma London Chemical Co., Ltd.). Also included are wide spectrum

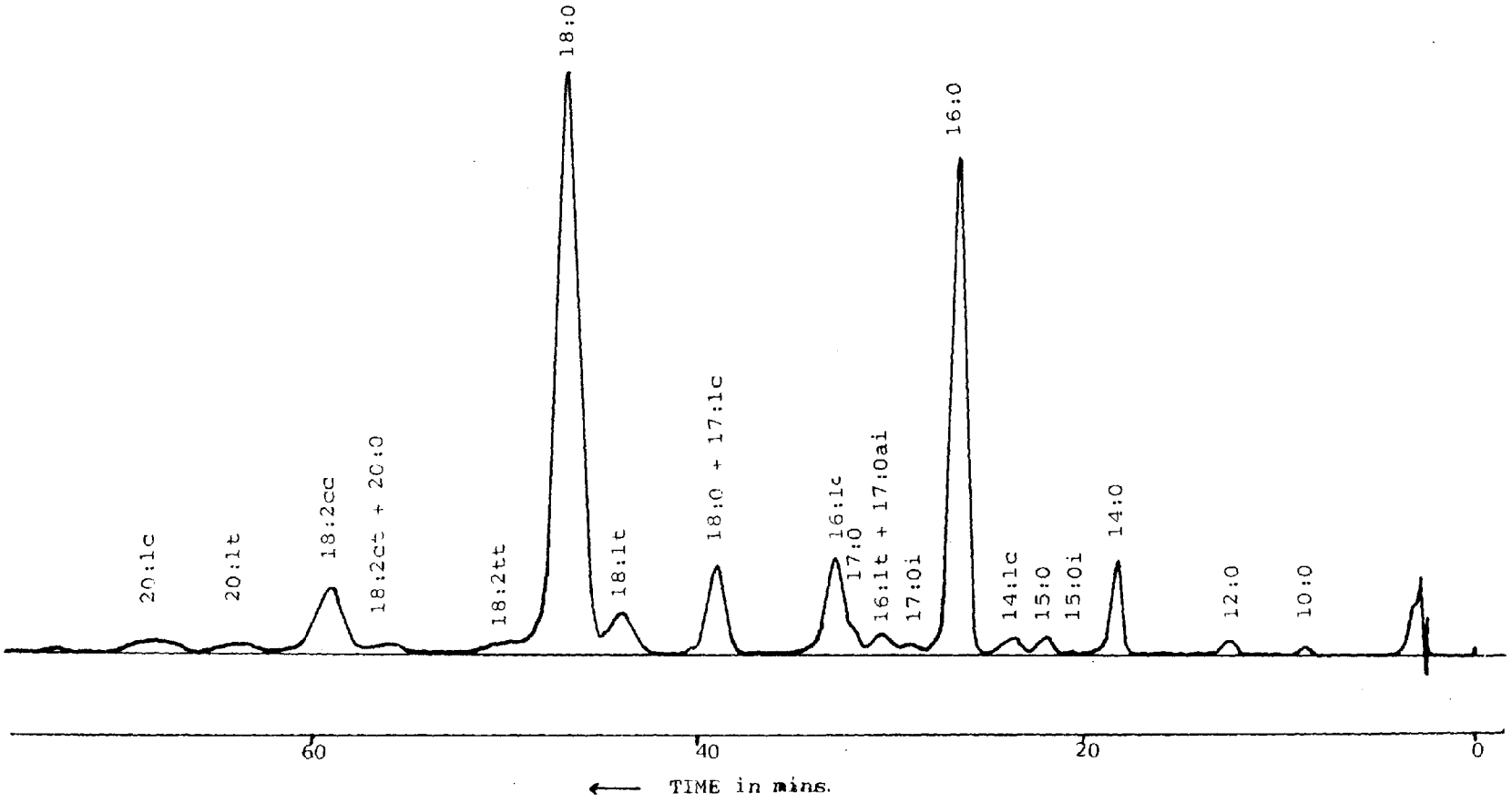


FIGURE 3.5. OV-275 CHROMATOGRAM OF ADIPOSE TISSUE FATTY ACIDS (AS THE METHYL ESTERS) AT 220°C.

(-not commercially available) fatty acids - up to to elution of erucic acid - obtained from analysing well studied[13,14,46] dietary fats (used in effect as secondary standards). It is important to point out that these later analyses also demonstrated the distribution of "lower" trans acids i.e. 16:1 trans and 18:1 trans. Further confirmation was achieved by:

(b) doping techniques with authentic methyl esters.

(c) catalytic hydrogenation[48].

From Figure 3.5 it can be seen that whereas 18:1 trans is directly quantifiable 16:1 trans is composite with 17:0 anteiso - the latter being derived mainly from RAF consumption and as such merited particular attention.

Seven leading brands of butter were analysed[21] and each chromatogram exhibited five distinct peaks between 16:0 and 18:0 as shown in Figure 3.6 which were identified as:-

Peak number	Fatty acid	ECL
1	17:0 iso	16.44
2	16:1 trans	16.74
	17:0 anteiso	16.74
3	17:0	16.97
4	16:1 cis	17.18
5	17:1 isomer*	17.44

*identity not established

Catalytic hydrogenation[48] resulted (see inset of Figure 3.6) in the complete disappearance of peaks 4 and 5 and reduction in area of peak 2 by a factor on average of about 2/3.

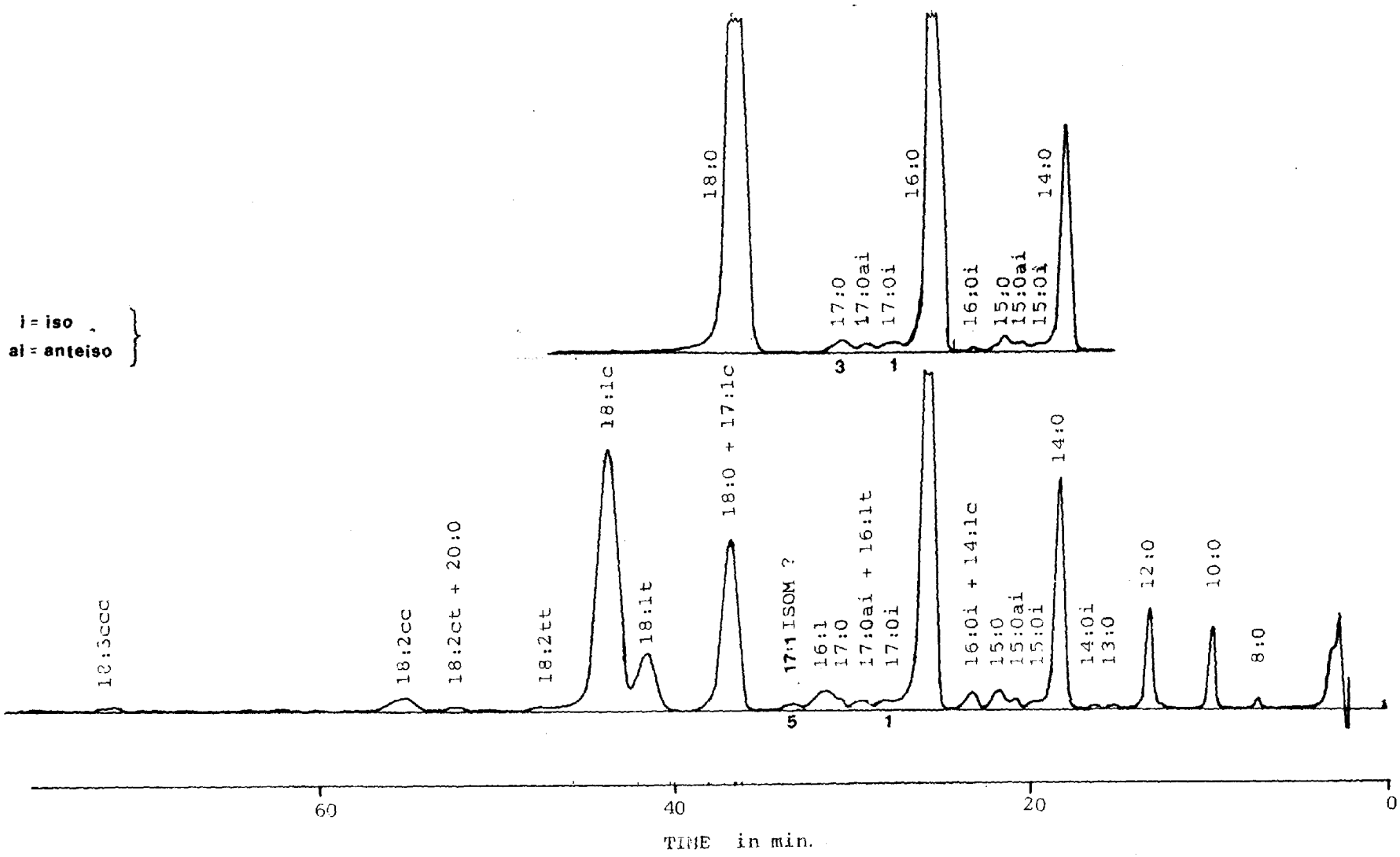


FIGURE 3.6. OV-275 CHROMATOGRAM OF BUTTER FATTY ACIDS (AS THE METHYL ESTERS) THE INSET SHOWS OV-275 CHROMATOGRAM (IN PART) OF HYDROGENATED SAMPLE.

In those margarines shown to contain HMO (from section 4.2) and subsequently rechromatographed[21] on OV-275 column, (see Figure 3.7 as an example) and also in the adipose tissue specimens, amounts of 16:1 cis substantially exceed the proportion present in butter fat. As a result the 16:1 cis peak is now broadened (see respective figures) and includes 17:0 and peak 5, an unsaturated acid (probably a 17:1 isomer) as respective leading and tailing "shoulders". The ECL values of these components remain however such that the height of the peak is determined by 16:1 cis only. In specimens having values of 16:1 trans, base-line separation between peak 2 and the adjacent large composite 16:1 cis peak was not always achieved. For this reason - and also for strict comparability with the "earlier" IR derived values of total trans acids (in section 4.5) - percentages 16:1 trans plus 17:0 anteiso were evaluated by measuring appropriate peak heights (h) and retention times (t) as described by Christie[52].

Then labelling the product of peak height and retention time for 16:1 cis as 'x' and that for the composite peak (16:1 trans plus 17:0 anteiso) as 'y', we may write:-

(% 16:1 trans + % 17:0 anteiso) / % 16:1 cis = y / x.

and by rearrangement then:-

% 16:1t = R(% 16:1c + % 16:1t) - (1-R).(% 17:0 ai)

= R(% 16:1) - (1-R).(% 17:0 ai) .....(1)

in which R = y/(x+y), t = trans, c = cis. ai = anteiso and i = iso. Then to evaluate the percentage 16:1t from

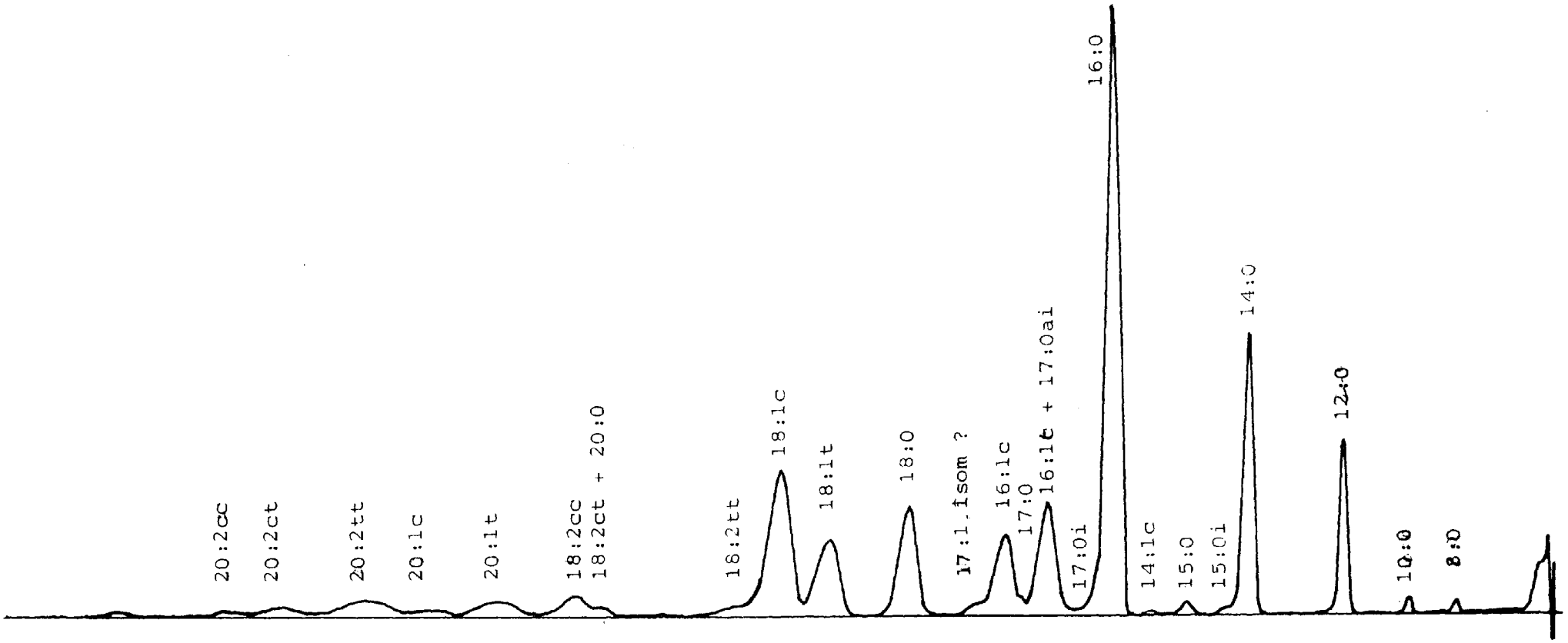


FIGURE 3.7. OV-275 CHROMATOGRAM OF A HMO BASED MARGARINE (AS THE METHYL ESTERS) AT 220°c.

this equation the percentage 16:1 values were used - combined cis and trans isomers as previously obtained (see section 3.2 and reported in Appendix C) from the EGSS-Y chromatograms for 124 specimens (75 cases and 49 controls).

For the remaining 107 specimens (61 cases, 46 controls) the EGSS-Y column had failed to separate completely 16:1 from the 17:0 branched-chain acids (br = iso plus anteiso) the column only giving the value of 16:1 and 17:0br., combined. However, it is easy to show that:-

$$\% \text{ 16:1t} = R (\% \text{ 16:1} + \% \text{ 17:0br}) - \% \text{ 17:0 anteiso} - R (\% \text{ 17:0 iso}) \quad \ldots\ldots\ldots(2)$$

in which combined percentages of 16:1 and 17:0br were those previously obtained (section 3.2; Appendix C).

Like the other odd-numbered and branched-chain components of L, the branched-chain C_{17} acids in adipose samples derive essentially from RAF, and it was found accordingly that in the 124 specimens (75 cases and 49 controls) the ratio 17:0br/L was sensibly constant at 0.22 for both cases and control samples. Furthermore, analysis of the butters (seven in total) previously mentioned showed that 60% of 17:0br is 17:0 iso a proportion which agrees well with that reported by Jensen et al[46] (61%). Equations 1 and 2 therefore finally become:-

$$\% \text{ 16:1t} = R (\% \text{ 16:1}) - 0.09 (1-R)L \quad \ldots\ldots\ldots(3)$$

and

$$\% \text{ 16:1t} = R (\% \text{ 16:1} + \% \text{ 17:0br}) - (0.09 + 0.13R)L \quad \ldots\ldots\ldots(4)$$

Percentages 16:1 trans were then evaluated using (a) values of R obtained from the OV-275 chromatograms and (b)

values of 16:1, L and 16:1 + 17:0br. from EGSS-Y chromatograms as previously described. It should be added that the magnitude of the term 0.09 (1-R)L in equation (3) is small (0.2 on average) compared with that of R (% 16:1) 0.9 on average. Differences in % 16:1 trans arise therefore mainly from differences in R and 16:1 values. The same applies for equation (4).

The 18:1 trans peak showed good but not quite base-line separation from the much larger 18:1 cis peak. However, in view of the well known[49] negative bias when quantifying by area in such circumstances i.e. small peak followed by a large peak, the percentages of 18:1 trans were evaluated from the heights and retention times of the 18:1 trans and 18:1 cis peaks using the combined 18:1 percentages previously measured (in section 3.2, and reported in Appendix C) on the EGSS-Y column.

The corresponding proportions of 16:1 trans and 18:1 trans acids were described as "lower trans" acids the sum of which are labelled T_L. The remaining trans acids distributed on the acids C_{20} and C_{22} (see section 6) can be described as "higher trans" acids and labelled T_H. So that the two groups together make up the total trans acid content (T) as measured by IR spectroscopy i.e. $T = T_L + T_H$.

4. RESULTS

4.1 Preliminary pilot programme and estimation of analytical reproducability

Fourteen specimens were initially submitted to our laboratories blind which subsequently proved to have been seven different samples, each in duplicate. All 14 specimens were analysed for percentage trans (T) on four separate occasions at approximately weekly intervals. The mean values for each sample are recorded in Table 4.1 (column 2), duplicate samples being indicated by braces. The root mean square deviations are given in the third column and duplicate differences are given in the fourth column. It can be seen that duplicate differences are no greater than errors in analysis and it may be concluded that specimens are sufficiently homogenous for purposeful measurements.

Summation of the percentage trans for all 14 samples on each of the four occasions gave virtually identical totals, showing that over this period at least there was no secular drift. Periodic returns of duplicate analyses from the MRC Unit have later confirmed this conclusion; one such return of the results of thirteen "blind" duplicates is reproduced as Table 4.2 (coefficient of variation based on "within sample" variance = 4%). The pilot programme together with such duplicates showed T to be reproducable with a SD of 0.25. This agreement is reassuring too in the sense that the specimens have shown no discernable change in the interval

TABLE 4.1

PERCENTAGES T, L, H AND ACID 18:2 IN 14 ORIGINAL SAMPLES*

SAMPLE	MEAN % T	ROOT MEAN SQUARE DEVIATIONS	DUPLICATE DIFFERENCES	% H	DUPLICATE DIFFERENCES	% L	DUPLICATE DIFFERENCES	% 18:2	DUPLICATE DIFFERENCES
1	3.8	0.1		2.75		2.45		5.5	
2	3.75	0.1	0.05	2.75	0.0	2.75	0.3	5.8	0.3
3	4.6	0.05		2.75		3.15		6.15	
4	4.6	0.1	0.0	2.85	0.1	2.9	0.25	6.2	0.05
5	4.85	0.3		4.3		3.4		6.8	
6	4.75	0.15	0.1	4.05	0.25	3.2	0.2	6.65	0.15
7	5.45	0.2		3.0		3.25		6.8	
8	5.5	0.2	0.05	3.6	0.6	3.5	0.25	6.8	0.0
9	6.15	0.1		3.4		2.1		8.7	
10	6.25	0.15	0.1	3.05	0.35	1.85	0.25	8.75	0.05
11	6.95	0.3		3.65		2.75		6.5	
12	6.75	0.2	0.2	3.9	0.25	2.65	0.1	6.35	0.15
13	9.15	0.05		4.6		1.85		6.7	
14	9.05	0.1	0.1	4.2	0.4	2.0	0.15	6.8	0.1

*DUPLICATES ARE SHOWN BRACKETED; ALL FIGURES TO NEAREST $\pm$ 0.05

TABLE 4.2

ESTIMATIONS OF 'TRANS' ON DUPLICATE SPECIMENS SUBMITTED 'BLIND' TO THE LABORATORY

	DUPLICATE SAMPLES		DIFFERENCES
	A	B	
	5.35	4.95	0.04
	9.45	9.35	0.10
	4.65	5.05	0.40
	5.45	5.55	0.10
	4.15	4.35	0.25
	3.50	3.75	0.15
	3.80	3.95	0.15
	3.10	3.25	0.15
	5.15	4.85	0.30
	3.85	4.20	0.35
	5.05	4.50	0.55
	6.20	6.00	0.20
	2.45	2.45	0.00
TOTAL	62.25	62.20	

Analysis of variance of the above data:-

SOURCE	D.F.	S. of S.	M.S.	
Between samples	12	69.38	5.78	
within duplicates	13	0.50	0.04	N.S
TOTAL	25	69.89		

Coefficient of variation (based on 'within sample' variance) = 4.1%

between collection and analysis.

The above 14 specimens were also analysed by GLC using both X and Y columns which permitted evaluation of H and L in the manner previously described (section 3.2). The values so obtained (together with 18:2 values; Y column only) are shown in Table 4.1. It is clear that duplicate differences do not exceed experimental error in GLC analysis.

Table 4.3 gives the full analytical figures for each of the 7 samples together with duplicate analyses, and the last column gives the average duplicate differences in respect of all fatty-acid components. Such differences are seen to be within the accuracy usually considered to be obtainable by GLC analysis (Thus for main component 18:1, the mean duplicate difference is only 0.78% thereby implying an accuracy of $\pm$ 0.4%).

Comparison of column performance - X versus Y - is made in Table 4.4 in respect of main components for 21 samples (7 in duplicate). The first column lists values of the function (Area of 18:1 peak plus area of 18:0 peak)/(Area of 16:0 peak) as obtained from the Y chromatograms, and the second column the corresponding ratios obtained from use of the X chromatograms. Differences (Y minus X) are shown in the third column. Of the 28 differences 14 are positive, 12 negative and 2 zero. The differences are therefore satisfactorily random and the mean difference is 0.022. The overall mean value of the ratio is 2.256.

Taking the mean percentage (18:1 + 18:0) - 50.5 - the mean Y minus X ratio difference 0.022 implies a mean difference

TABLE 4.3

FATTY ACID COMPOSITION* OF 14 ORIGINAL ADIPOSE SPECIMENS; DUPLICATE PAIRS ARE SHOWN IN BRACES FOR EASE OF COMPARISON

SAMPLE NO.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	AVERAGE DUPLICATE DIFFERENCE
PEAK NO.	ACID															
1	10:0	.05	.06	.04	.04	.11	.11	.15	.22	.0	.0	.02	.01	.0	.02	.01
2	12:0	.50	.63	.25	.22	.69	.61	.97	.88	.34	.33	1.32	1.16	.20	.25	.08
3	14:0	4.38	4.55	3.75	3.75	4.33	4.21	5.36	5.12	3.0	2.84	5.46	5.24	3.29	3.41	.15
4	14:1+15:0br.	.84	.72	.84	.70	1.14	1.10	.99	1.07	.44	.47	1.05	1.08	.36	.37	.06
5	15:0	.54	.61	.56	.62	.48	.45	.65	.73	.42	.39	.58	.51	.36	.26	.06
6	15:1+16:0br.	.21	.25	.29	.22	.26	.18	.29	.26	.08	.12	.13	.08	.06	.09	.05
7	16:0	24.46	25.13	23.09	23.28	20.87	19.92	23.40	22.08	21.20	20.69	22.59	22.10	23.15	22.37	.70
8	16:1	7.51	7.26	7.06	6.83	8.43	8.90	7.73	7.99	7.35	7.58	6.73	6.64	7.09	7.56	.28
9	17:0br.	.32	.30	.65	.59	.45	.32	.48	.49	.40	.37	.39	.51	.44	.44	.05
10	17:0	.44	.51	.59	.57	.65	.68	.71	.81	.46	.40	.49	.58	.62	.61	.05
11	17:1	.44	.64	.85	.81	.86	.77	.63	.63	.69	.49	.49	.40	.46	.66	.12
12	18:0	4.93	4.85	4.37	4.73	5.41	5.58	5.22	5.16	3.36	3.28	4.94	4.83	4.19	3.98	.15
13	18:1	46.18	44.93	47.90	47.78	44.20	45.42	42.49	43.10	49.31	50.40	44.67	45.57	47.52	47.88	.78
14	18:2	5.48	5.80	6.14	6.20	6.80	6.64	6.81	6.78	8.70	8.74	6.50	6.37	6.68	6.79	.12
15	18:3	.86	.83	.72	.72	.80	.80	.91	.94	.74	.72	.86	.89	.72	.80	.03
	20:0	.31	.31	.44	.47	.66	.61	.48	.54	.33	.33	.56	.57	.32	.31	.02
16	20:1	1.34	1.45	1.35	1.39	1.90	1.84	1.48	1.68	1.78	1.53	1.82	1.88	2.45	2.26	.13
17/18	20:2's	.40	.37	.49	.45	.43	.38	.43	.54	.46	.41	.58	.61	1.02	.94	.05
19	20:3	.13	.16	.15	.16	.11	.12	.06	.11	.18	.17	.12	.16	.19	.19	.02
20	20:4	.16	.16	.13	.11	.22	.24	.19	.16	.13	.13	.11	.11	.27	.29	.01
	22:0	.20	.18	.09	.11	.22	.20	.09	.13	.21	.21	.06	.07	.15	.10	.02
21	22:1	.32	.30	.25	.25	.98	.92	.48	.50	.42	.40	.53	.63	.46	.42	.05

*VALUES ARE EXPRESSED AS WEIGHT PERCENT OF THE TOTAL PEAK AREA

TABLE 4.4

COMPARISON OF PERFORMANCE OF EGSS-Y AND EGSS-X COLUMNS;

RATIO (18:0 + 18:1) TO 16:0 ACIDS FOR 28 SPECIMENS

SAMPLE NO.	AREA (18:0 PLUS 18:1) / AREA 16:0		DIFFERENCE Y MINUS X
	Y	X	
1	2.267	2.352	- .085
2	2.260	2.271	- .011
3	2.240	2.245	- .005
4	2.318	2.311	+ .007
5	2.005	1.991	+ .014
6	2.080	2.102	- .022
7	2.334	2.349	- .015
8	2.192	2.205	- .013
9	2.450	2.418	+ .032
10	2.594	2.515	+ .079
11	2.392	2.353	+ .039
12	2.555	2.555	± .000
13	2.040	2.043	- .003
14	2.189	2.162	+ .027
15	2.288	2.285	+ .003
16	2.328	2.347	- .019
17	2.501	2.493	+ .008
18	2.318	2.257	+ .061
19	2.008	1.996	+ .012
20	1.785	1.782	+ .003
21	2.197	2.180	+ .017
22	2.418	2.433	- .015
23	2.685	2.680	+ .005
24	1.930	1.930	± .000
25	1.991	1.947	+ .044
26	1.961	1.989	- .028
27	2.380	2.423	- .043
28	2.638	2.701	- .063
Mean value of difference			0.022
Positive differences = 14			
Negative differences = 12			
Zero differences = 2			

in determination of 16:0 by the 2 columns of (50.5/2.256) - (50.5/(2.256 + .022)) = 0.21% only - a figure which is very satisfactory.

Similar examination of the 28 ratios (Area 18:2 peak)/(Area 16:0 peak) for the Y and X columns gave a similar result, the plus and negative differences being quite random. The ratio $\left(\frac{\text{Area of peaks eluting later than 18:2}}{\text{Area all peaks}}\right)$ were likewise identical for the 2 columns.

There was therefore no evidence of any systematic differences in performance Y versus X column. The similarity in evaluation of 18:2 and total acids eluting later than 18:2 was reassuring in the sense that it seemed very unlikely that there were any "on column" losses of polyunsaturated acids. In consequence, evaluation of H in the manner described above can be accepted with confidence.

A further demonstration of analytical reproducability is given in Table 4.5 in respect also of minor components. The data are based on 35 authentic case/control specimens supplied in the earlier stages of the project; the 3rd column shows mean differences (averaged over all 35 samples) between X and Y runs.

TABLE 4.5

AVERAGE WEIGHT % COMPOSITIONS OF 35 ADIPOSE TISSUE SAMPLES AND ESTIMATION OF EXPERIMENTAL ERRORS; ALL FIGURES TO NEAREST ± 0.05

ACIDS	WEIGHT PERCENT	AVERAGE % DIFFERENCES BETWEEN X AND Y ANALYSES	ACIDS	WEIGHT PERCENT	AVERAGE % DIFFERENCES BETWEEN X AND Y ANALYSES
10:0	.0		18:0	5.05	.25
12:0	.5	.05	18:1	45.0	.7
14:0	4.15	.15	18:2	8.1	.1
14:1+15:0br	.75	.2	18:3	.8	.2
15:0	.55		20:4	.15	
15:1+16:0br	.2		20:0	.3	
16:0	22.3	.35	20:1	1.65	
16:1	7.4	.35	20:2+20:3	.6	
17:0br	.6		22:0	.1	
17:0	.55	.15	22:1	.55	
17:1	.75				

4.2 Composition of British Margarines

A total of seven leading retail brands of hard margarine, labelled A to G (and one cooking fat, H), and eight soft margarines, labelled a to h, were purchased in late 1976. Together they provided a reliable overall picture of British margarines then available to the private household, contribution from remaining brands was negligible. For comparison purposes, analyses were also carried out on three (1976) samples of butter, two lards and one each of mutton and beef fat from popular domestic cuts.

At a later date a further batch of seven soft margarines and six butters purchased in 1980 were analysed[53].

General methods and reagents used were those as described previously for preparation of adipose tissue methyl esters except that the samples were first saponified (see experimental section 2.2.6) to permit the removal of nonsaponifiables.

Fatty acid compositions were determined via GLC on EGSS-Y and/or EGSS-X columns in exactly the same manner used previously, except that for the 1980 butter samples a EGSS-Y column of narrower diameter (1/8 in) was used. This latter column permitted resolution of 17:0br. from 16:1 and 18:3 from 20:0. The total trans acid contents were determined using the IR spectroscopic method previously developed.

The analytical results for the 16 (1976) margarines are given in Table 4.6. By weighting the brands studied according to market shares, we have been able to estimate the compositions of the "average" UK hard and soft margarines available to the private household at that period. These are shown in the last

TABLE 4.6 FATTY-ACID PERCENTAGE COMPOSITIONS OF (1976) UK MARGARINES

FATTY ACIDS	Hard Materials								Soft margarines								Weighted Average	
	A	B	C	D	E	F+	G	H	a	b	c	d	e	f	g	h	Hard	Soft
10:0	.6	.1	.1	1.3	4	1.2	.1	.1	.1	1.1	.2	.3	.3	1.4	.1	.0	.5	.7
12:0	8.1	1.5	.7	9.6	4.4	8.2	.9	1.5	.3	10.6	1.2	1.1	2.5	10.4	.4	.0	4.0	5.5
14:0	5.3	6.0	9.5	5.7	9.2	8.2	2.1	13.4	.7	4.7	2.6	2.6	3.2	5.0	.4	.2	7.4	3.4
15:0 15:0br. 14:1	.5	.9	1.0	.4	1.0	.7	.6	1.4	.0	.2	.6	.0	.0	.0	.0	.0	.8	.1
15:1, 16:0br.	.0	.2	.0	.0	.2	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.1	.0
16:0	31.2	22.2	22.3	21.5	21.8	21.9	22.2	20.3	18.3	18.0	21.2	22.2	21.7	18.9	13.6	11.9	22.8	19.4
16:1	2.6	6.2	14.2	2.3	12.4	5.1	2.3	10.0	.7	1.5	2.9	3.8	4.0	1.5	.0	.6	8.7	2.1
17:0br.	.3			.1	.7		.7		.0	.0	.0	.0	.0	.0	.0	.0	.3	.0
17:0	.8	.6	1.9	.5	1.7	.7	.8	1.3	.0	.4	.6	.0	.0	.0	.0	.0	1.1	.1
17:1	.0	.2	.0	.3	.7	.3	.6	1.0	.0	.2	.4	.0	.0	.0	.0	.0	.4	.0
18:0	5.8	6.2	6.6	5.6	5.4	6.0	12.2	4.5	7.6	4.4	8.5	11.2	9.4	4.8	4.1	5.9	6.7	6.9
18:1	28.2	22.2	20.5	21.2	18.6	25.5	40.8	19.7	40.7	34.8	35.6	40.2	39.2	35.2	29.0	31.4	23.9	36.9
18:2	7.5	20.4	4.9	16.1	5.2	5.1	10.7	6.2	24.4	17.6	15.3	11.0	13.2	17.6	48.7	47.2	8.1	18.7
18:3	.0	-	.0	2.0	.2	.0	1.8	.0	1.6	1.7	1.7	1.1	1.3	1.8	1.4	1.0	.4	1.6
20:0	1.2	1.4	.7	1.5	.6	1.3	.6	2.0	.5	.4	.9	.5	.4	.2	.2	.5	.9	.4
20:1	2.6	5.4*	4.1	3.0	3.7	4.8	1.2	6.2	2.1	1.3	3.3	1.8	1.7	1.0	.5	.4	3.7	1.5
20:2 20:3	1.7	2.2	6.7	1.6	5.9	5.8	.6	3.2	1.3	1.0	.7	1.7	1.2	.6	.1	.3	4.4	.9
22:0	.6	1.3	2.0	1.9	2.1	1.6	.4	2.4	.3	.3	.5	.6	.3	.2	.5	.3	1.6	.3
22:1	1.6	2.3	1.5	3.4	1.4	2.5	1.4	4.4	.4	.8	2.7	.9	.6	.4	1.0	.3	1.7	.6
22:2 22:3	1.4	.7	3.3	2.0	4.4	.8	.0	2.4	∓1.0	∓1.0	∓1.0	∓1.0	∓1.0	∓1.0	.0	.0	2.4	.9
% trans	9	26	39	13	41	30	4	34	26	10	9	6	9	10	7	10	29	12

* Includes 18:3 +Contains 10% butter fat. ∓Based on analysis of a mixture of equal parts materials a to f.

two columns of the table.

The presence of elevated amounts of C_{20} and C_{22} acids is indicative of the presence of hydrogenated marine oils (HMO) - and is considered further below. The hard materials contained considerable amounts of HMO, ca. 45% on average; the soft margarines on the other hand contained little HMO. Overall, those brands containing a higher marine oil content retailed at a considerably lower price than the more vegetable oil based margarines. The (weighted) average contents of 18:2 were 8% for hard and 19% for soft margarines, the corresponding percentage levels of trans acids (T) being 20 and 12.

The compositions of the seven (1980) soft margarines are given in Table 4.7 the higher priced materials 1, 2 and 3 being broadly similar in composition to the earlier vegetable oil based materials f, g and h but with a substantially higher trans acid content. It was surprising however to find that the compositions of margarines 4, 5, 6 and 7 closely resembled the lowest-priced <u>hard</u> margarines, B, C, E and F, both types containing on average ca. 50% HMO and 30% T, the only significant difference being that in the 1980 materials 18:2 levels have been increased to about 15%. The increased popularity of soft margarines in the UK over the last decade has not therefore resulted in any decrease in consumption of HMO or of total trans acids; indeed it is clear that intake of the latter (following a slow decline in consumption of margarine in the previous 1960-1970[3]) may well have increased since around 1975.

TABLE 4.7 FATTY-ACID WEIGHT PERCENTAGE COMPOSITIONS OF (1980) UK SOFT MARGARINES AND ANIMAL FATS

	Soft margarines							Butter					Mutton	Beef
	1	2	3	4	5	6	7	1976*	S D	1980+	S D	Lard	fat	fat
10:0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	2.8	0.4	2.4	0.2	0.1	0.9	0.3
12:0	2.0	2.3	0.2	0.2	0.1	0.4	0.3	3.8	0.4	3.0	0.2	0.1	1.2	0.2
14:0	1.1	1.1	0.4	4.7	3.5	5.1	3.5	11.2	0.9	9.8	0.4	1.7	8.3	5.3
15:0,15:0br.,14:1	0.0	0.0	0.0	} 0.8	} 0.2	} 0.9	} 0.7	3.1	0.5	3.7	0.3	0.1	2.2	4.1
15:1,16:0br.	0.0	0.0	0.0					0.3	0.0	0.5	0.1	0.0	0.6	0.4
16:0	13.2	12.6	11.5	15.8	19.1	18.5	21.4	26.8	0.4	22.9	0.6	24.0	23.3	24.2
16:1	} 0.7	} 0.2	} 0.2	} 4.7	} 4.1	} 9.6	} 4.3	2.2	0.9	2.7	0.3	3.4	3.6	6.1
17:0br.								0.6	0.0	0.6	0.1	0.0	0.6	0.3
17:0	0.2	0.0	0.1	1.3	0.1	1.5	0.7	1.2	0.2	1.4	0.1	0.6	1.6	1.3
17:1	0.0	0.0	0.0	0.4	0.0	0.2	0.4	0.4	0.2	0.8	0.3	0.3	0.9	1.5
18:0	7.5	7.4	8.7	6.4	4.3	5.1	8.0	13.0	0.9	12.8	0.2	14.1	17.4	9.8
18:1	36.4	44.0	44.6	31.5	29.1	28.9	27.8	26.4	3.3	32.4	0.4	40.9	32.4	41.0
18:2	32.5	27.5	29.3	10.1	22.0	11.5	18.4	3.0	0.7	2.3	0.4	11.2	3.5	3.0
18:3	} 5.2	} 4.2	} 4.8	} 4.0	} 3.7	} 4.6	} 3.5	1.4	0.0	1.4	0.1	1.0	} 2.2	} 1.6
20:0								0.4	0.1	0.9	0.1	0.5		
20:1	1.1	0.5	0.2	8.9	5.9	5.7	4.6	0.6	0.2	0.2	0.0	0.7	0.6	0.5
20:2,20:3	0.0	0.0	0.0	3.6	0.1	3.3	1.4	0.6	0.0	0.2	0.0	1.1	0.5	0.2
22:0	0.0	0.0	0.0	0.8	1.1	0.8	0.8	0.2	0.1	0.1	0.0	0.1	0.1	0.1
22:1	0.0	0.0	0.0	6.8	6.7	3.9	4.2	0.2	0.1	0.2	0.0	0.1	0.1	0.1
% trans	16	19	23	40	22	34	22	7.4	0.6	8.6	0.5	zero	6	5

*also 0.3% 10:1/11:0, 0.4% 12:1/13:0 and 1.1% of a component assumed to be C_{19}

+also 0.4% 10:1/11:0, 0.8% 12:1/13:0 and 0.5% of a component assumed to be C_{19}

It may be relevant to add that in America where soft margarines based on such as soya/maize have long been popular and where hydrogenated marine oils (with their much higher T content) are not in use, the intake of trans acids seems certain to be substantially lower than in Britain.

Analytical results in respect of the butter samples are also given in Table 4.7. The compositions of the three earlier samples were very similar to one another, as were the six later purchased materials, and the table shows mean (1976 and 1980) values only. The table also includes results (mean values) for the two lards and one sample each of mutton and beef fats from popular domestic cuts.

Our results for butter closely resemble values reported in the literature in respect of main components, and also for odd-numbered and branched-chain acids, compare e.g. Herb et al[32] and Jensen et al[46]. Our results for C_{20} and C_{22} acids however are higher than previously reported, the same being the case for the lard, mutton and beef fats. This could possibly be due to animal diet supplementation by rapeseed oil[54] or fish meal[35].

The figures for percentage trans-acid content of the three earlier samples of butter are 6.9, 7.4 and 8.0 (mean 7.4); those for the six later brands are uniformaly higher at around a mean value of 8.6. These levels are considerably higher than those reported by Hay et al[23] (3 - 6% according to season) as previously indicated (in section 1.3). It is well known that the trans acids arise by bacterial hydrogenation in the rumen, of polyunsaturated fat, particularly of linolenic

acid 18:3, levels of which are higher in summer on fresh pasture than in winter[22]. It seems very likely therefore that our high trans contents arise from more intensive production methods and winter dietary supplementation by oils such as rape, linseed and soya bean.

The trans content of our mutton and beef fat samples are 5 to 6%. It is of interest that no trans acids have been detected in non-ruminant animal (pig, chicken) fat.

An unexpected feature of the 1980 butters compared with the earlier materials (1976), was a change in relative amounts of major components. Thus the later materials had higher contents of 18:1, essentially at the expense of lower amounts of 16:0 and 14:0. The mean value of the ratio R = (16:0 + 16:1)/(18:0 + 18:1) in the earlier materials is 0.74 (SD, 0.071) which is in fair agreement with the value (0.69) of Jensen et al[46] but the mean value for the 1980 samples is 0.567 (SD, 0.022). The difference is statistically highly significant; Student's "t" = 5.7, $p < .001$. This may be due to source change, in imports, but it seems more likely (compare change in trans content above) that it arises from winter - feed supplementation by unsaturated vegetable oils. That bovine fat is sensitive to such change in diet has been demonstrated by Hilditch et al[54].

It is possible to reach some conclusions regarding the make-up or formulation of UK margarines by taking into account the fact that most oils used in its manufacture have certain distinguishing features. This has previously been mentioned in section 1.3 above but these features are now considered

in more detail. These are:-

(a) Hydrogenated marine oils (HMO) contain high quantities of H, ca. 25 to 50% according to source[13,14,15,55] whereas the percentages of such acids in other oils and fats is low ca. 0.2% in vegetable oils to 2% in lard (an exception is rapeseed oil which may contain up to 40% of the characteristic erucic acid, 22:1 cis w 9). The trans-acid content varies from about 30 to 60%. (According to Anderson and Williams[5] about 60% of the total marine oil used in the UK in 1960 was whale oil but use of a wide variety of oily fish has increased sharply since then[56]. Even though of variable composition it is clear that the maintenance of a saleable commodity will have required use of materials of mixed origin; the above variations in H and T have therefore tended to average out to give reasonably homogeneous products. An important feature of hydrogenated marine oils from our particular standpoint is that in contrast to variations in C_{20} and C_{22} acids, the ratio R is fairly constant, varying between 1.1 and 1.4 irrespective of type (The 14:0 content is also constant at 7-8%).

Consideration in particular of those (1976) margarines of highest H content leads to a ratio $Q = C_{22}/C_{20}$ of ca. 0.7 which indicates use of HMO similar to the commercial materials studied by Lambertson et al[16], and Ackman et al[15] but containing somewhat more fish oils such as sardines, pilchards, etc.. It will contain therefore about 33% H, 50% T and have a value of R ca. 1.3. The later HMO appears to have a somewhat higher value of Q in line with the steadily increased use of hydrogenated "herring" oils. It will contain ca. 40% H,

45% T and have R = 1.2.

Use of H (and 14:0) percentages leads to estimates of HMO contents of the margarines studied reliable, probably to within ± 5%.

(b) In the absence of HMO, any 16:1 acid indicates the presence of lard; this acid being virtually absent in all vegetable oils.

(c) The R values of the C_{18} - rich vegetable oils - whether hydrogenated or not - are very much lower (ca. 0.2 to 0.4) than that value characteristic of HMO, the sole exception being palm oil (ca. 1.0). Its value in lard is 0.5 and in ruminant-animal fat about 0.6; the latter however is not normally present unless actually stipulated.

(d) Coconut and palm kernel oils are readily diagnosed by their high content of 14:0 (18%) and 12:0 (53%).

(e) An amount of T in excess of that calculated from the HMO content is indicative of the presence of hydrogenated vegetable oil (HVO), and the conclusion is readily reached that the only HVO widely used in Britain is palm and soya. The former would appear to be only lightly hydrogenated to a trans content of about 12% and 18:2 content about 7%. Soya on the other hand (with its high 18:3 content) requires a greater degree of hydrogenation to give an acceptable product [57,58], and examination of the quantities of 18:2 in relation to trans content remaining in the majority of the margarines studied shows further that UK hardened soya closely resembles the American (soft margarine) product, the composition of which has been well documented[12,55,57,58]. Its trans content

will be about 12% and its 18:2 content about 48%. A few of the later (1980) margarines however clearly contained soya less-selectively hydrogenated to a product containing ca. 25% T and 25% 18:2.

Using the above considerations we have estimated the percentages of the main components; these are recorded in Table 4.8. By way of example, details of the type of calculation involved are given in Table 4.9 in respect of material B - a hard margarine. Brand G "margarine" is something of an innovation in being essentially lard with probably a few percent rapeseed oil; another brand-for which analysis is not recorded - was virtually identical, but contained 10% added butter.

In the calculations it is obvious that there is a likelihood that relatively minor components such as rapeseed oil will have been underestimated. However it is our opinion that quantities higher than 5% on average are unlikely in UK margarine.

Examination of Table 4.8 shows that British margarines are of a very diverse nature and quite often substantial compositional differences exist between even two similarly branded products. It is clear therefore that from a dietary viewpoint, description of British margarines as hard/soft or animal/vegetable can be totally misleading; a more meaningful division would reflect contents of HMO, HVO and natural unhardened materials.

It is considered misleading to the consumer to describe for example a given brand as "containing only pure vegetable

TABLE 4.8. FORMULATION OF UK MARGARINES

Materials	Hydrogenated Components %				Natural Oils %						Total Natural Oils %	T	L	18:2 %	H	Ratio T/L
	Marine oil	Soya	Palm	Others+	Soya	Palm	Coconut/ Palm Kernel	Peanut	Lard	Others+						
A	20					55	15	10			80	9	1.3	8	7.7	7
B	40	20	25				5			10	15	26	1.9	20	11.6	14
C	60		40								0	39	2.9	5	15	13
D	30	20	30				20				20	13	1.2	16	11.4	11
E	60		30				10				10	41	3.6	5	13.7	11
F	50	5	20				15			10	25	30	1.7	5	16	18
G									93	7	100	4	2.0	11	4.2	2
Weighted average hard*	45	5	25	0	0	5	10	0	10	0	25	20	2.4	8		12
H ACIDS	70	10				20					20	34	3.7	6	20.6	9
a	10	40‡				20		30			50	26	0.0	24	4.6	26
b	10	30	20				20	20			40	10	0.8	18	3.8	12.5
c	20				10	10		20	40		80	9	1.6	15	8.1	5.6
d	10					25	5	20	35	5	90	6	0.0	11	5.5	
e	10	20	10				5	20	35		60	9	0.0	13	4.2	
f		10	30				20	40			60	10	0.0	18	2.4	
g		40				10		20		30	60	7	0.0	49	2.3	
h				100							0	10	0.0	47	1.8	
Weighted average soft*	10	20	5	5	5	10	5	20	20	0	60	12	0.2	19		60
1		95‡					5				5	16	0.2	32	6.2	80
2		95‡					5				5	19	0.0	28	4.7	
3		100‡									0	23	0.1	29	5.0	230
4	65	35‡									0	40	2.5	10	24.1	16
5	50						25		25		50	22	0.3	22	17.5	73
6	50	30‡	20								0	34	2.6	11	18.3	13
7	40	30				15			15		30	22	1.8	18	14.5	12
Average	30	55	3								12	25		22		
Butter fat												7	5.0	2.9	2.0	1.4
Lard												0	1.0	11.2	2.5	0

*Components to nearest 5% +Butter, rapeseed, sunflower, maize oil

‡Non-selectively hydrogenated to trans content of ca. 30 to 50%

TABLE 4.9

FORMULATION OF MARGARINE B

The percentage composition of the 5 component oils - a to e - are as follows (to nearest %; minor components omitted):-

FATTY ACIDS	a	b	c	d	e
10:0	0	0	0	5	0
12:0	0	0	0	53	0
14:0	8	0	1	18	0
16:0 + 16:1	32	12	45	8	7
18:0 + 18:1	25	39	47	14	20
18:2	2	49	7	2	73
C_{20} + C_{22} acids	33	0	0	0	0
	100	100	100	100	100
T	50	12	12	0	0

a Mixed HMO used in 1976 margarines

b Selectively - hydrogenated soya

c Hydrogenated palm oil

d Coconut/palm kernel (unhydrogenated)

e Material of high 18:2 such as sunflower/safflower (unhydrogenated)

Estimated formulation continued overleaf -

TABLE 4.9. continued

Estimated formulation of the margarine:-

FATTY ACIDS	.40 x a	.25 x c	.05 x d	.20 x b	.10 x e	Composition of the margarine		
						Estim-ated	Act-ual	Diff-erence to ± 1%
10:0	.0	.0	.25	.0	.0	.2	.1	0
12:0	.0	.0	2.65	.0	.0	2.7	1.5	1
14:0	3.2	.25	.9	.0	.0	4.4	6.1	2
16:0 + 16:1	12.8	11.25	.4	2.4	.7	27.5	29.3	2
18:0 + 18:1	10.0	11.75	.7	7.8	2.0	32.2	28.8	3
18:2	.8	1.75	.1	9.8	7.3	19.8	20.7	1
C_{20} + C_{22} acids	13.2	.0	.0	.0	.0	13.2	13.5	0
	40.0	25.00	5.0	20.0	10.0	100.0	100.0	
T	20.0	3.0	0.0	2.4	0.0	25.4	26.0	

It is believed that agreement is within the variability of composition of the components.

oils" when that oil has been chemically modified to a point where there may be present high amounts of acids which are entirely absent in the original (unhardened) material.

Comparison of hydrogenated and other fats with respect to parameters T, L, H and T/L

Table 4.8 gives the percentages of trans acids (T) in the hydrogenated components of British margarines and shortenings, ca. 15 to 50, which are always greater than amounts in RAF, ca. 5 to 8. In those margarines containing much HMO there are also elevated levels of higher acids (H). Percentages of L acids on the other hand are around 5 to 7 in RAF, about 2 in HMO, 1 in lard and virtually zero in vegetable oils whether hydrogenated or not. High values of T coupled with low values of L will therefore indicate the presence of industrially - hydrogenated fat. The ratio T/L in RAF is around unity, whereas in for example 1976 average hard margarine the ratio is 12, and in vegetable - based margarines ~150.

A further distinguishing feature is the ratio R. Its value for RAF, around 0.6, is near to the value for lard, 0.5. The majority of vegetable fats have values between 0.2 and 0.4, whereas hydrogenated marine oils have much higher values lying between 1.1 and 1.4.

The distribution of the total trans acids over the various unsaturated components of margarine is at present under detailed study[21]. Preliminary information is given in

section 3.3 on such distribution, but it already appears certain (compare Hay et al[23]) that butter unlike - HMO which contains about 60 to 90% of its total trans within the C_{20} and C_{22} acids - that over 90% resides within 18:1 with only small amounts in 16:1 (see Figure 3.6 in section 3.3). It is relevant to add[23] that the cis components butter are essentially the natural 18:1 w7 and 16:1 w 7 acids, whereas HMO and HVO contain substantial amounts of cis isomer with the (cis) double bond in a variety of other positions[12,13,14,55]. The presence of trans acids in a commercially-hydrogenated fat is therefore accompanied by quite large amounts of positional 18:1 and (from HMO) 16:1 isomers - a fact which is discussed later (in section 7) when assessing the relative risks of natural versus hydrogenated fat in the context of the case versus control study.

4.3 Comparison of fatty acid composition of human adipose tissue in superficial and deep site

There have been many studies on the effect of sample site on human adipose fatty acid composition, and when conducted on thinly insulated extremities, the results would appear to be unambiguous. Thus material from abdominal wall (or mesentery) was shown by Imaichi et al[59] to be persistently higher in saturated acids 14:0, 16:0 and 18:0 than samples taken from the arm, hand or foot of the same individual; this increase is mainly at the expense of a diminution in 14:1 and, in particular, 16:1. Comparisons of subcutaneous samples with material from deeper site, however, are not often in agreement and are somewhat fragmental. Thus Pietropaolo et al[60] for example found no significant differences between abdominal, omental, perinephric and mesenteric tissue, whereas Hirsch et al[39] reported slight differences, particularly in respect of pericardial site. Later, however, Hirsch[25] regarded the differences as "questionable and probably insignificant".

In the general rationale of our case versus control study of adipose composition (see section 1.5) it has been thought prudent to re-examine the question with some degree of thoroughness, and choice of pericardial site would appear to be appropriate.

Samples of pericardial fat from 18 male subjects have been analysed as previously discussed in section 3 by using GLC to determine fatty acid compositions (using EGSS-Y column only) and IR for total trans acid content. These results were

compared with the compositions of abdominal samples from the same individuals. The mean compositions from the two sites are given in columns 2 and 3 of Table 4.10.

The most obvious difference between the two averages was in respect of acids 16:0 and 18:0, both of which were present - as may have been expected for deeper site tissue - in higher percentage on the pericardium samples (and conversly higher unsaturation at superficial and exposed sites is generally believed to be due to the necessity of maintaining proper melting point and cell-membrane fluidity). The mean values of the 18 differences (column 4; pericardial minus abdominal) were 2.35% for 16:0 and 1.46% for 18:0, and the corresponding standard deviations (S.D.) of the differences were 1.48 and 1.001. Student's "t" values were therefore 6.73 and 6.18 respectively showing that both differences were highly significant ($p << 0.001$).

With the exception of 18:1 which was a little higher on pericardium (not significant) the differences for all other component acids (pericardial minus abdominal) were negative as shown in column 4. However, some decreases must occur to make up for the percentage increase in 16:0 plus 18:0. To make allowance for this, the figures in column 5 were evaluated on the basis that the decreases are spread proportionately. Thus for one sample having 16:0 plus 18:0 = 27.56% and 16:1 = 6.89% on abdominal tissue, and 16:0 plus 18:0 = 31.82% on pericardial tissue, the "expected" pericardial value for 16:1 (i.e. 6.48%) will be 6.89 multiplied by the factor (100-31.82)/(100-27.56). The actual 16:1 pericardial percentage

TABLE 4.10

MEAN FATTY ACID COMPOSITION OF 18 PAIRED SAMPLES ABDOMINAL VERSUS PERICARDIAL SITE

ACID	MEAN % COMPOSITIONS		MEAN DIFFERENCE PERICARDIAL - ABDOMINAL	MEAN "ADJUSTED" DIFFERENCE	STANDARD DEVIATIONS OF "ADJUSTED" DIFFERENCES	p.
	ABDOMINAL	PERICARDIAL				
10:0,12:0	.57	.46	- .11	- .07	.182	N.S.*
14:0	3.92	3.44	- .48	- .27	.428	< .01
14:1,15:0br	.77	.58	- .18	- .15	.164	< .001
15:0	.54	.35	- .18	- .15	.128	≪.001
15:1,16:0br	.24	.18	- .06	-.04	.112	N.S.
16:0	22.09 } 30.09	24.44 } 31.23	2.35		1.48	
16:1	8.00 }	6.79 }	-1.21	- .82	1.220	~ .01
17:0br	.58	.53	- .05	- .02	.216	N.S.
17:0	.76	.61	- .15	- .09	.237	N.S.
17:1	.78	.59	- .18	- .15	.232	~ .01
18:0	4.53 } 49.42	5.99 } 50.98	1.46		1.001	
18:1	44.89 }	44.99 }	.10	2.42	1.700	<<.001
18:2	7.27	6.62	- .65	- .25	.838	N.S.
18:3,20:0	1.19	.98				
20:1	1.91	1.91				
20:2,20:3	.79 } 5.02	.63 } 4.41	- .61	- .34	.982	N.S.
22:0,20:4	.39	.38				
22:1	.74	.51				
TRANS ACIDS	5.36	5.04	- .32		.838	N.S.

*NOT SIGNIFICANT.

for this sample was 4.86% and the "adjusted" decrease is then 6.48% minus 4.86% - and so on for all the 18 paired samples. The "adjusted" mean sample differences for the various fatty acids are recorded in the 5th column, their S.D.'s in column 6 and the corresponding p values in the last column. It can be seen that whereas of course "adjusted" differences are smaller than before, they remain significant for the majority of the acids concerned.

The picture which emerges from such "adjusted" percentages can now be summarized as below:-

a) To an extent the higher percentages of 16:0 and 18:0 on the pericardium are compensated by a (real) decrease in 16:1 - a result which may have been expected to accompany passage to a deeper site. Peculiarly however, main component 18:1 which would have been expected to decrease, is actually higher on pericardium by an amount 2.42% which is highly significant. This result supports the view of Goto et al[65] that the solidity of depot fat is determined by amounts of 16:0, 18:0 and 16:1 and not of 18:1.

b) A further compensation, however, was a noted decrease in the levels of the odd-numbered and branched acids 14:1, 15:0br., 15:0, 17:1, and also (in contrast to the findings of Imaichi et al[59]) of the even-numbered acid C_{14}. These acids are precisely those which are characteristic of dairy and ruminant-animal fat (RAF) and which are virtually absent in most commercial vegetable fats. It should be added, however, that they

may also be present in some British margarines analysed previously (section 4.2) those being derived essentially from hydrogenated marine oil: smaller amounts of the lower saturated acids may also derive from coconut oil.

We do not know that physiological conclusion can be attached to this observation; it may be however, that heart muscle is able to mobilise such acids more readily than the higher even-numbered C_{16} and C_{18} straight - chain acids.

c) The percentages of 18:2 and of acids higher than C_{18} are lower on the pericardium but not significantly so. The trans acid content is also a little lower but by an amount which is significant only at the 13% level ($p = .13$).

d) Table 4.11 records the parameter percentages obtained at both sites in the 18 samples with trans acids (T) ranging from 2.3 to 10.5%, "higher acids" (H plus 18:3 and 20:4) from 3.51 to 8.57% and "lower acids" (L plus 17:0br.) from 2.08 to 5.1%.

Correlation between trans acid content at the two sites is very strong (Spearman rank coefficient, $r_s = 0.914$, $p << .001$) as can be clearly demonstrated by comparing columns 2 (reported in increasing order) and 5.

Correlation in respect of "higher acids" is less strong ($r_s = 0.476$) but still significant ($p \sim .025$).

Any differences in trans and H acids between our case and control samples will not therefore be due to

TABLE 4.11

PERCENTAGE LEVELS OF DIAGNOSTIC PARAMETERS T, L AND H OF 18 PAIRED SAMPLES* AT ABDOMINAL AND PERICARDIAL SITES

MATCHED PAIR NO.	ABDOMINAL SITE			PERICARDIAL SITE		
	T	L^{+}	H^{++}	T	L^{+}	H^{++}
1	2.45	3.53	3.71	2.30	3.42	3.59
2	3.10	2.08	4.63	2.70	2.08	4.64
3	3.30	5.10	3.94	3.30	4.55	3.51
4	3.40	2.50	4.91	3.00	2.40	4.72
5	4.00	3.02	4.54	4.00	2.93	4.40
6	4.55	3.78	4.10	4.70	3.51	3.81
7	4.80	3.45	4.34	4.50	3.26	4.10
8	4.85	4.12	4.49	3.50	3.89	4.24
9	4.85	5.08	4.13	5.50	4.81	3.91
10	4.90	3.64	4.88	3.50	3.54	4.74
11	5.00	4.37	4.86	4.00	4.20	4.67
12	5.65	2.98	5.65	5.90	2.86	5.43
13	5.75	4.02	5.05	5.05	3.66	4.60
14	6.20	4.58	4.25	8.08	4.27	3.96
15	6.50	2.91	5.97	5.50	2.78	5.70
16	7.20	2.78	5.03	6.40	2.61	4.73
17	9.90	4.33	8.57	8.40	4.09	8.10
18	10.05	3.32	7.45	10.50	3.08	6.91

$^{+}$includes 17:0br. $^{++}$includes 18:3 and 20:4.

fortuitous choice of sample site.

In respect of L (i.e. sum of 14:1, 15:0br., 15:0, 15:1, 16:0br., 17:0 and 17:1) plus in this case 17:0br. correlation is weak, with $r_s = 0.291$ $p \sim .1$. Undoubtedly however, this is due to the relatively high standard deviation of the C_{17} acid measurements (see column 6 Table 4.10); thus for 14:1, 15:0br., 15:0, 15:1 plus 16:0br., $r_s = 0.622$, $p < .01$.

4.4 Adipose composition of full term stillborn infants

Eight postmortem samples of adipose tissue from the abdominal wall near to the umbilicus of stillborn infants were analysed using the materials and methods described previously. The proportions of component fatty acids were measured by GLC, with the EGSS-Y polyester column only. As at this stage in the project this column was capable of resolving the composite peaks (18:3 + 20:0) and (20:0 + 20:4) directly for quantifying the individual acids. Total trans acids were determined by IR spectroscopy.

The fatty acid chromatograms were found to be remarkably consistent for both major and minor components as demonstrated by the mean composition and S.D.'s shown in column 2 and 3 of Table 4.12 overleaf.

Percentage trans acid levels in the stillborn (0.45 to 1.1; mean 0.70) are substantially lower than in the adult specimens (3.75 to 9.15%; mean 5.83); for comparison purposes figures from the pilot programme are shown in the last column (Unfortunately it was not possible to examine levels in the mothers). Later GLC analysis of the stillborn samples with the OV-275 column (resolving geometric isomers - see section 3.3) confirmed such levels and identified the sole trans contributor as elaidic acid as shown by Figure 4.1. This is in contrast with a "range" of trans acids found in the adult tissue (shown later in section 6).

Combined amounts of odd-numbered and branched chain acids (characteristic of RAF) are also much lower (mean 0.26%) compared to adult average of ca. 4%. Interestingly no finite

TABLE 4.12

AVERAGE WEIGHT PERCENTAGE COMPOSITIONS OF ANTERIOR ABDOMINAL ADIPOSE TISSUE; STILL-BORN INFANTS

FATTY ACID	FULL TERM INFANTS					ADULTS*
	STILLBORN	S.D.	+	LITERATURE VALUES (39)	(63)	
10:0	0.05	0.02	0.05			0.10
12:0	0.17	0.04	0.18			0.78
14:0	3.85	0.39	4.35	3.0	3.8	5.01
14:1	0.47	0.11				0.84
15:0br.	0.00					0.14
15:0	0.07	0.01	0.07			0.57
16:0br.	0.00					0.12
15:1	0.04	0.01				0.01
16:0	44.36	2.78	56.13	40.2	48.9	21.29
16:1	12.26	0.94		14.6	12.6	6.61
17:0br.	0.00					0.83
17:0	0.10	0.09	0.12			0.63
17:1	0.05	0.04				0.77
18:0	4.29	0.27	36.66	5.1	4.1	6.85
18:1	29.39	2.29		25.2	29.6	41.79
18:2	2.73	1.22		1.3	1.0	7.80
18:3	0.1	0.12		1.8		0.22
20:0	0.2		1.20			0.55
20:1	0.34	0.20				2.64
20:2	0.49	0.22				0.74
20:3	0.21	0.10		3.9		0.20
20:4	0.00			0.3		0.29
22:0	0.82	0.25	1.24			0.21
22:1	0.00					1.00
TOTAL	100.00		100.00	95.4	100.0	100.00
branched-chain	0.0					1.09
odd-numbered straight chain	0.26					2.95
Trans (IR)	0.70					5.83
Trans (GLC)	0.70					

*7 pilot programme specimens (section 4.1)

+Analysis of hydrogenated samples.

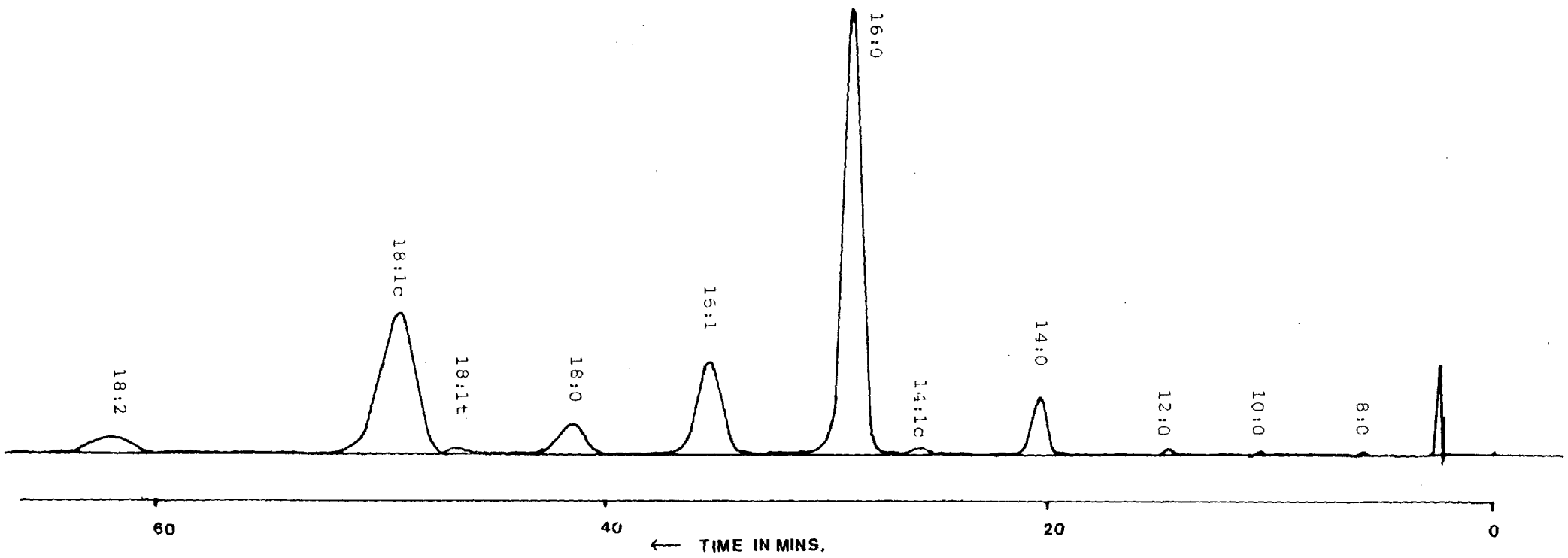


FIGURE 4.1 OV-275 CHROMATOGRAM OF STILLBORN INFANTS' ADIPOSE FATTY ACIDS (AS THE METHYL ESTERS); AT 220°C.

amounts of branched acids have been detected - even upon further GLC analysis with a representative catalytically hydrogenated[48] sample (column 4).

It would seem likely therefore that in human tissue trans acids and L acids are not of endogenous origin and that the (much larger) amounts found in adults are derived essentially from dietary fats.

Levels of C_{16} (16:0 + 16:1) are also much higher - by a factor of 2 times - in stillborn tissue, and this is undoubtedly due to synthesis from carbohydrate[39,62].

It is interesting to confirm earlier findings (see below) that levels of linoleic acid are much lower in the stillborn depot fat. Since it is generally agreed that this acid is entirely of exogenous origin, the lower level must presumably be due to "dilution" of linoleic acid derived from the maternal circulation by de novo synthesis.

Our results closely resemble the literature values[39.63,64] for newborn adipose tissue (for example see Table 4.12 columns 5 and 6) in respect of main components. For more minor components only Hirsch et al[39] to the best of our knowledge has reported 18:3 and C_{20} acids (20:2 and 20:3). The figure for 20:3 quoted by Hirsch et al (on 3 samples) is much larger than ours.

4.5 CASE VERSUS CONTROL STUDY

4.5.1 Compositional analyses and proportions of diagnostic parameters T, L, H (and 18:2)

In addition to the seven original duplicate pairs used to estimate experimental error (see section 4.1), a further 36 specimens were similarly analysed by GLC using both X and Y columns. Of these first 43 specimens, only 25 proved subsequently to have been authentic cases/controls samples. It became apparent from the results of these initial specimens that percentage amounts of 18:3 and 20:4 were sensibly constant at .75% and .25% respectively. Furthermore the values were quite unrelated to values of T, H and 18:2 as is demonstrated in Table 4.13, for example for the first 21 specimens received. In addition, the percentages of main components such as 16:0, 18:0, 18:1 obtained from use of the Y column remained in good agreement with the corresponding levels derived from the X column. At this stage therefore it was decided in the interests of laboratory productivity to conduct further analyses using only the Y column, that column being regarded as the more reliable in the light of (a) its lower polarity, (b) the evidence obtained from analysis of the standard mixtures (section 3.2).

A further 105 specimens (61 cases and 44 controls) were then analysed, H being evaluated by subtraction of the constant (18:3 + 20:4) figure as given above from the total eluted latter than 18:2.

TABLE 4.13

PERCENTAGE VALUES OF 18:3 AND 20:4 FOR FIRST 21 SPECIMENS: LACK OF CORRELATION WITH T, H, AND 18:2, ALL FIGURES TO NEAREST ± 0.05

18:2	RANK ORDER	18:3	20:4	18:3 + 20:4	RANK ORDER	T	H
3.6	1	1.2	.1	1.3	18½	9.45	5.5
4.1	2	.85	.35	1.2	16	3.8	2.7
4.7	3	1.1	.1	1.2	16	3.8	3.6
4.8	4	1.0	.1	1.1	13	3.9	2.9
5.45	5½	.55	.1	.65	3½	5.45	4.1
5.45	5½	.5	.1	.6	2	5.25	5.25
5.50	7	.55	.1	.65	3½	5.45	4.1
5.65	8	.85	.15	1.0	9	3.75	2.75
6.15	9	.7	.1	.8	5	4.6	2.8
6.20	10	1.0	.0	1.0	9	10.25	4.15
6.45	11	.85	.1	.95	7	6.85	3.75
6.55	12	.8	.35	1.15	14	3.95	3.65
6.70	13	.8	.25	1.05	11½	4.8	4.15
6.75	14	.75	.3	1.05	11½	9.1	4.4
6.80	15	.9	.1	1.0	9	5.45	3.3
6.85	16	1.0	.3	1.3	18½	5.65	4.15
6.95	17	1.1	.35	1.45	21	5.1	3.8
7.15	18	1.05	.15	1.2	16	5.65	4.45
7.45	19	.35	.05	.4	1	7.5	5.05
8.50	20	1.05	.25	1.3	20	4.1	3.35
8.70	21	.75	.15	.9	6	5.45	3.3

Correlation of (18:3 + 20:4) with 18:2 R_S = .066 p = .295
Correlation of (18:3 + 20:4) with T R_S = .149 p = .667
Correlation of (18:3 + 20:4) with H R_S = .070 p = .313
} N.S.*

*Not significant

It was later found that as the Y column aged somewhat (but still giving perfectly symmetrical peaks), the composite peaks (18:3 + 20:0) and (22:0 + 20:4) each resolved into their two components, (indentity confirmed by "doping" with authentic fatty acids, as previously described in section 3.2), thus giving percentages, 18:3 and 20:4 directly for the last 101 specimens (58 cases and 43 controls) analysed. The average percentages 20:4 and 18:3 for these last 101 specimens were 0.27 and 0.64 for the 58 case specimens, respectively, and 0.26 and 0.65 for the 43 control specimens (given above). Like the earlier samples, amounts of 18:3 and 20:4 were quite independent of T, H and 18:2 levels.

For reasons of strict comparability and statistical analysis, all 231 values of percentage higher acids were therefore calculated as follows:-

1) For the last 101 specimens, individual 20:4 percentages were subtracted from the total percentages eluted later than 18:2 from the Y column; 2) for the 130 earlier specimens, an amount 0.25 (i.e. the constant figure for percentage 20:4 to the nearest 0.05) was so subtracted.

In other words, the recorded values are of H + 18:3; they differ from true values of H - for cases and controls - by being on average 0.65 higher. It was considered that this inclusion of 18:3 in the parameter would lead to a lower experimental error, especially as for the first 130 specimens analyzed, 18:3 was included in the composite (20:0 + 18:3) peak. Such values of H are listed in Appendix B along with corresponding values of T, L, 18:2 and ages at death; cases

are designated by D and controls by C.

In all a total of 231 specimens were collected from 10 different areas/conurbations. Table 4.14 shows the number of case and control samples in each area. The average ages at death are tabulated accordingly as well as the mean percentage values of the parameters T, H + 18:3, L and 18:2 accompanied by their standard error (S.E.).

For reference purposes full details regarding fatty-acid compositions are recorded in Appendix C, together with the corresponding M.R.C. specimen numbers.

Age at death ranged between 31 to 63 years and the overall mean for the 136 cases was 52.7 years, and for the 95 controls 51.5 years. The percentage values of T found ranged over extreme values 1.85 to 11.6; the ranges in H + 18:3, L and 18:2 were respectively, 2.7 to 8.5, 1.15 to 4.0, and 4.1 to 15.8.

As may have been expected - hydrogenated marine oil being a major source of T in the UK - T is positively correlated with H. This is at once evident (in Table 4.14) by arrangement of the 20 mean values of T in rank order, and comparison with the rank order in mean values of H + 18:3 (Spearman Rank coefficient R_S, 0.748; $p < 0.01$). There is little if any correlation, even within one area, between T and L, or between T and 18:2. This is apparent from the values given in Appendix B which are recorded within each area in rank order of T.

With regard to major components, quite large differences have been found between individual samples, e.g. percentage

TABLE 4.14 MEAN (PERCENT) AND STANDARD ERROR OF T, H + 18:3, L, 18:2 AND AGES AT DEATH, CASES VERSUS CONTROLS, FOR 10 AREAS OR CONURBATIONS

AREA	NO. OF CASES OR CONTROLS	AVERAGE AGE OF DEATH		T		H + 18:3		L		18:2	
		MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
1. WEST WALES	16 cases	54.0	1.3	4.82	0.19	4.07	0.16	2.73	0.19	8.36	0.59
	9 controls	48.3	4.2	4.42	0.46	3.92	0.23	2.83	0.25	8.73	0.46
2. SOUTH AND EAST ENGLAND	9 cases	52.3	3.0	5.82	0.64	5.12	0.42	2.37	0.21	9.83	0.90
	6 controls	58.0	1.9	3.82	0.39	3.93	0.29	2.43	0.16	8.27	1.06
3. LONDON (CHATHAM)	12 cases	53.0	2.4	5.82	0.44	4.30	0.29	2.69	0.16	8.51	0.48
	7 controls	45.9	4.6	5.73	0.55	4.23	0.39	3.11	0.19	8.99	0.95
4. SOUTH EAST WALES	17 cases	52.1	2.2	4.62	0.43	4.35	0.23	2.57	0.16	6.82	0.45
	12 controls	50.8	3.9	4.70	0.28	4.39	0.24	2.82	0.15	7.85	0.62
5. LUTON AND DUNSTABLE	22 cases	56.2	0.9	5.53	0.42	4.44	0.23	2.68	0.19	8.24	0.32
	15 controls	55.7	2.4	5.36	0.36	4.43	0.23	2.70	0.13	8.03	0.56
6. HUDDERSFIELD AND HALIFAX	14 cases	46.4	1.9	5.06	0.32	4.23	0.28	2.33	0.15	8.24	0.54
	7 controls	44.0	4.5	4.99	0.70	4.46	0.44	2.45	0.13	9.34	1.09
7. BRADFORD	15 cases	54.0	1.3	6.08	0.49	4.74	0.34	2.21	0.21	7.56	0.52
	16 controls	50.3	2.1	5.28	0.41	4.66	0.33	2.30	0.13	7.55	0.58
8. MANCHESTER CONURBATION (ASHTON-UNDER-LYNE, ALTRINCHAM	15 cases	51.9	1.9	5.33	0.44	4.38	0.30	2.65	0.13	8.17	0.60
	11 controls	52.9	3.1	5.48	0.35	4.60	0.36	3.16	0.23	8.07	0.49
9. MANCHESTER CONURBATION (ANCOATS)	4 cases	54.5	3.7	5.41	0.36	5.65	0.96	2.54	0.13	8.94	0.84
	7 controls	51.9	2.7	6.28	1.00	6.19	0.52	2.21	0.15	7.09	0.56
10. LIVERPOOL CONURBATION (WALTON)	12 cases	53.6	1.9	6.45	1.76	4.51	0.17	2.55	0.09	7.19	0.51
	5 controls	55.6	3.3	6.82	0.84	5.20	0.40	2.78	0.19	6.35	0.92

16:0 varied between limits 19 to 26; 18:1 between 40 and 54; 16:1 between 3 and 13 and 18:0 between 2 and 8. Despite this however, fatty acid spectra averaged over only quite a small number of samples are remarkably constant as demonstrated in Table 4.15 which shows average (control-specimen) composition for two regions, such differences as exist are due mainly to minor components. This fact has been repeatedly noted by other workers and is undoubtedly due to equalisation effects inherent in variable western style diet and to desaturation of 16:0 and 18:0 to respective monoenoic acids referred to previously (in section 1.5).

Reference to Table 4.14 shows that in respect to H there are no consistant differences between case and control means, which may indicate that at the levels found in the UK at least, higher acids (which it should be remembered are in themselves largely in the trans configuration) carry no hazard. There are also no consistent differences between case and control meansfor 18:2.

With respect to L however, the cases seem to show the lower values - 9 of the 10 area means were lower for the cases than for controls. Overall, the cases appear to have the higher trans acid content.

It is of interest (see Table 4.15) that in Wales (areas 1 + 4) the mean value of L is higher at 2.8% than for Yorkshire and Humberside (areas 6 + 7) at 2.35% by an amount which is significant (Student's "t" = 2.67) at the $p \sim 0.01$ level. The difference clearly reflects the fact that consumption of dairy fat in Wales is known to be well above the average UK

TABLE 4.15

AVERAGE wt % COMPOSITIONS OF CONTROL-SPECIMENS FROM AREA (1 PLUS 4[+]) AND AREA (6 PLUS 7[*]); ALL FIGURES TO NEAREST ± .05

Acids	Wt % Area 1 + 4	Wt % Area 6 + 7	Literature Values	
10:0	.05	.05		
12:0	.55	.55	.5	.7
14:0	3.95	3.55		
14:1+15:0br.	.75	.65	.5	.9
15:0	.55	.4	.5	.6
15:1+16:0br.	.25	.2		
16:0	22.2	21.85		
16:1	7.1	6.7		
17:0br.	.6	.6	1.3	1.0
17:0	.6	.5	.7	.2
17:1	.7	.6		
18:0	5.1	5.4		
18:1	44.95	46.0		
18:2	8.2	8.1		
18:3	.65	.65	.5	.4
20:4	.25	.25	.2	.2
20:0	.55	.5	.2	.6
20:1	1.75	2.05	.5	.6
20:2+20:3	.6	.65	.65	.4
22:0	.1	.1	.05	
22:1	.55	.65	.2	

[+] West Wales plus South East Wales (Wales)

[*] Huddersfield/Halifax plus Bradford (Yorkshire and Humberside)

consumption as previously indicated in section 1.2.

Although there have been a great number of publications dealing with the main components of adipose tissue, analysis of higher acids and minor components are surprisingly few. Although in the earlier stages of development of GLC analysis, the work of Kingsbury et al[66] in 1964 on UK subjects was conducted with great thoroughness and probably remains the definitive work in this area. Their figures for certain odd-numbered and branched-chain acids (column 4 Table 4.15) agree well with our results - as they do for 20:4. There is also satisfactory agreement (column 5) between our results and the classical work of Hirsch et al[39] on Americans.

Our results for higher acids and 18:3 however, are not unexpectedly higher, and agree more with the analysis of Shorland et al[26] on New Zealanders of European origin (18:3, 0.8%; 20:1, 0.95%), and with those of Imaichi et al[59] on Japanese (18:3, 0.8%; 20:1, 2.5%; 20:2 plus 20:3, 0.9% and 22:1, 1.1%).

To the best of our knowledge, the only literature references to amounts of trans components on human tissue appear to be those of Johnston et al[67] who reported the percentage of such acids in adipose tissue on 24 subjects as ranging between 2 and 12%, and those of Kingsbury et al[66] who reported the presence of 1 to 8% trans acids; both figures agree well with our results.

4.5.2 Proportions of lower trans acids T_L

Table 4.16 records the mean values of 18:1 trans, 16:1 trans their sum T_L, T_L/L, with their standard deviations for cases and controls, in each area of the study. For comparison purposes, the previously measured mean values of L are also included (from section 4.5.1 Table 4.14). Individual percentage values of 16:1 trans range from 0.21 to 1.55, 18:1 trans from 0.87 to 4.35 and T_L from 1.21 to 5.58. As expected - HMO being the major source of trans acids - 16:1 trans and 18:1 trans levels are strongly correlated; this can be clearly seen from Table 4.16. It should be noted that whereas controls show the higher value of L in 9 of the 10 areas, they have lower values of T_L in 8 areas and lower values of T_L/L (placed in rank order) in 9 areas.

The mean age at death for the 135 cases was 52.7 years and for the 95 controls 51.5 years.

The regression coefficients on age were calculated for 16:1 trans, 18:1 trans and T_L/L which were + 0.003, + 0.010 and + 0.004 units per year respectively. So it is considered that the mean difference in age of 1.2 years would not influence any conclusions which might be drawn.

Statistical case versus control evaluation for 16:1 trans, 18:1 trans their sum T_L and ratio lower trans acids/L is considered later in section 6 within the wider context of total levels of trans - unsaturated acids.

TABLE 4.16. MEAN AND STANDARD DEVIATION OF 18:1 TRANS, 16:1 TRANS, T_L, T_L/L, 136 CASES (D) VERSUS 95 CONTROLS (C), FOR 10 AREAS OR CONURBATIONS.

Area	No. of cases or con-trols		L	18:1 trans		16:1 trans		*T_L		T_L/L		Rank order in T_L/L for controls	Differences in mean T_L/L cases minus controls
			Mean	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.		
Luton and Dunstable	D	22	2.68	1.92	0.54	0.59	0.23	2.51	0.74	0.99	0.39	1	0.09
	C	15	2.70	1.78	0.37	0.48	0.17	2.26	0.52	0.90	0.38		
West Wales	D	16	2.73	2.11	0.34	0.62	0.20	2.72	0.51	1.12	0.56	2	0.16
	C	9	2.83	1.97	0.51	0.55	0.24	2.52	0.69	0.96	0.45		
South and East England	D	9	2.37	2.35	0.43	0.74	0.15	3.09	0.55	1.41	0.50	3	0.44
	C	6	2.43	1.75	0.38	0.54	0.11	2.28	0.46	0.97	0.28		
Huddersfield and Halifax	D	14	2.33	2.10	0.45	0.72	0.13	2.82	0.51	1.26	0.34	4	0.18
	C	7	2.45	1.88	0.40	0.61	0.20	2.49	0.60	1.08	0.40		
South-East Wales	D	17	2.57	2.38	0.54	0.82	0.21	3.20	0.71	1.29	0.33	5	0.13
	C	12	2.82	2.32	0.64	0.77	0.21	3.09	0.78	1.16	0.50		
Bradford	D	15	2.21	2.42	0.78	0.81	0.31	3.23	0.98	1.52	0.54	6	0.29
	C	16	2.30	2.12	0.51	0.62	0.22	2.75	0.63	1.23	0.30		
Manchester Conurbation(Ashton-under-Lyne and Altrincham)	D	15	2.65	2.75	0.48	0.76	0.16	3.51	0.59	1.38	0.37	7	0.11
	C	11	3.16	2.99	0.40	0.77	0.10	3.76	0.48	1.27	0.44		
London (Chatham)	D	12	2.69	3.37	0.63	0.88	0.26	4.25	0.82	1.68	0.54	8	0.40
	C	7	3.11	3.08	0.47	0.85	0.22	3.93	0.66	1.28	0.25		
Manchester Conurbation(Ancoats)	D	4	2.55	3.10	1.21	0.85	0.33	3.95	1.54	1.55	0.58	9	0.23
	C	7	2.78	2.82	0.54	0.69	0.19	3.51	0.67	1.32	0.45		
Liverpool Conurbation (Walton)	D	12	2.54	2.95	0.57	0.82	0.19	3.77	0.65	1.54	0.44	10	-0.34
	C	5	2.21	3.33	1.04	0.91	0.20	4.24	1.21	1.88	0.31		
All Areas	D	136											
	C	95											

*T_L = 16:1 trans plus 18:1 trans.

5. STATISTICAL EVALUATION OF THE RESULTS, CASE VERSUS CONTROL PERCENTAGES OF COMPONENT FATTY ACIDS

As stated previously a total of 231 specimens were available from 10 areas; 136 were deaths attributed to IHD and 95 were control specimens. The number of cases in each area, and the mean values of T, L and the ratio T/L with their standard errors are shown in Table 5.1. Corresponding mean values of H and 18:2 are as previously recorded (Table 4.14).

The age of death ranged between 31 and 63 years. The overall mean for the 136 cases was 52.7 years and for the 95 controls 51.5 years. Regression coefficients for T, L and H respectively on age were + 0.03,+ 0.02, and + 0.003 units per year so it would appear safe to ignore this slight difference between mean ages.

We were able to obtain the social class status of 115 cases and 76 controls: 2% were in Social Class 1, 12% in class 11, 51% in class 111, 25% in class 1V, and 10% in class V. The difference in status between cases and controls was slight; 12% of the cases and 17% of the controls were in classes 1 and 11, 49% and 54% were in class 111, and 39% and 29% were in classes 1V and V (χ^2 with 2 df = 2.44 $p > 0.2$).

Two way analysis of variance-within cases and controls and between areas - showed highly significant differences between area mean values for L (Fisher's variance ratio F =

TABLE 5.1 MEAN VALUES OF T, L, T/L, 136 CASES (D) VERSUS 95 CONTROLS (C), FOR 10 AREAS/CONURBATIONS

Area	Number of cases/ controls	T Mean	L Mean	T/L Mean	T/L S.E.	Mean Differences cases minus controls L	Mean Differences cases minus controls T/L
1. West Wales	D 16	4.82	2.73	1.96	0.21	-0.10	0.26
	C 9	4.42	2.83	1.70	0.27		
2. South/East England	D 9	5.82	2.37	2.69	0.45	-0.06	1.10
	C 6	3.82	2.43	1.59	0.18		
3. London (Chatham)	D 12	5.82	2.69	2.27	0.22	-0.42	0.43
	C 7	5.73	3.11	1.84	0.12		
4. South East Wales	D 17	4.62	2.57	1.83	0.15	-0.25	0.09
	C 12	4.70	2.82	1.74	0.15		
5. Luton and Dunstable	D 22	5.53	2.68	2.16	0.19	-0.02	0.05
	C 15	5.36	2.70	2.11	0.23		
6. Huddersfield and Halifax	D 14	5.06	2.33	2.25	0.19	-0.12	0.08
	C 7	4.99	2.45	2.17	0.39		
7. Bradford	D 15	6.08	2.21	2.86	0.26	-0.09	0.52
	C 16	5.28	2.30	2.34	0.19		
8. Manchester conurbation(Ashton under-lyne, Altrincham)	D 15	5.33	2.65	2.10	0.21	-0.51	0.21
	C 11	5.48	3.16	1.89	0.25		
9. Manchester conurbation 11 (Ancoats)	D 4	6.45	2.55	2.52	0.66	-0.23	0.02
	C 7	6.82	2.78	2.50	0.32		
10. Liverpool conurbation (Walton)	D 12	5.41	2.54	2.21	0.20	0.33	-0.57
	C 5	6.28	2.21	2.78	0.32		
All Areas	D 136						
	C 95						

3.22: df = 9,211, $p < 0.01$); likewise for T ($F = 2.47$, $p < .025$). There is however no evidence that case/control differences vary between areas ($F = 0.7$ and 1.0 respectively). Mean values of L are higher in the controls than in the cases in 9 out of the 10 areas whereas mean values of T/L are higher for the cases in 9 of the 10 areas.

It has been previously explained (section 1.6) that the value of T/L is determined only by the ratio HF to RAF, the value increasing with the amount of HF relative to RAF. In light of this fact it should be noticed that the general pattern of regional variation in T/L in the controls broadly reflects known dietary patterns. Thus HF consumption is lowest and butter-fat consumption highest in the South, South East and Wales; T/L is lowest in these regions. HF consumption is highest and butter-fat consumption lowest in the North, and North-West; T/L is highest in Yorkshire and Humberside, Manchester and Liverpool.

It would appear therefore that an adequate measure of matching for age, social class and area of residence has been achieved.

In view of the consistent case versus control differences, the results were pooled using the method of weighted means of Pearce[69]. Table 5.2 shows overall adipose tissue composition in respect of the various fatty acids measured together with weighted differences cases minus controls.

The mean % L is higher for the control group, the difference being on the margin of conventional significance

TABLE 5.2.

OVERALL ADIPOSE TISSUE COMPOSITION, WEIGHTED DIFFERENCES CASES MINUS CONTROLS.

Component fatty acids	% to ±.05	Differences (Case - Control)			
		Mean differences	Standard errors	Student's t	p
10:0+12:0	.6	-.037	.043	- .86	NS
14:0	3.85	.075	.108	.70	NS
L	2.6	-.153	.079	- 1.93	~.05
16:0	22.5	.506	.222	2.28	<.025
16:1	7.15	.202	.227	.89	NS
17:0br.	.6	.004	.019	.23	NS
18:0	4.95	-.312	.156	- 2.00	<.05
18:1	44.95	-.266	.318	- .64	NS
18:2	8.05	-.014	.278	- .05	NS
H	3.85	-.007	.144	- .05	NS
18:3	.65	‡			
20:4	.25	‡			
	100.00				
T	5.33	.226	.218	1.04	NS
T/L	2.16	.222	.112	1.98	<.05
R	0.595	.0206	.0073	2.83	<.01
M	55.76	-.135	.427	- .32	NS

‡ Previously shown (4.5.1) to be identical for case and control specimens within the limits of experimental error.

R = (16:0 + 16:1)/(18:0 + 18:1)

M = 16:1 + 18:1 + 0.44 L + 0.65 H.

($t = 1.93$ $p = 0.05$). One explanation is that the controls consumed a higher proportion of RAF in their total fat intake than did the cases. A second possibility is that the controls and cases consumed similar amounts of RAF but that the excess L in the controls was derived from HMO (it should be remembered that vegetable fats have zero L content). But the ratio T/L for HMO is around 15 compared with about unity for RAF. If then the controls had consumed a higher proportion of HMO they would also have had a larger excess of T. In fact however the mean value of T is lower in the controls and their mean value of T/L (2.03) is significantly lower ($t = 1.98$, $p < .05$) than for the cases (2.25) so the excess L could not have derived from HMO.

A third possibility is that the consumption by the two populations of both RAF and HMO is similar but that the excess L in the control population is due to higher consumption of pig and poultry fat. However, since only 0.3% of the 2.8% of L usually derives from pig and poultry fat (as shown in section 1.6), to remain consistent with the above difference in T/L (0.22), the increased consumption would have to be substantial - over 100% higher - which seems very unlikely.

The % 16:0 is significantly higher ($p < .025$) for the cases, whereas % 18:0 is significantly higher for the controls ($p < .05$). This difference in molecular weight is further accentuated if 16:1 and 18:1 are included. Consequently (Table 5.2), the ratio R = (16:0 + 16:1)/(18:0 + 18:1) is higher for the cases at significance level $p < .01$. Although this

difference supports the view that the type of fat consumed is implicated in the incidence of IHD, interpretation in terms of the various types considered in this thesis is difficult. Thus whereas it is true (as shown in section 4.2), that the values of R for RAF (around 0.6) and pig fat (0.5) are substantially lower than the value for HMO (1.0 to 1.4 according to source), the majority of vegetable oils are low in C_{16} and have very low values of R (0.2 to 0.4) whether hydrogenated or not. It is clearly then not possible from this result alone to decide which material is responsible for the observed difference. It would however be difficult to argue in view of these ratios that the observed higher value of R in the cases could be due to a higher consumption of animal fat (ruminant plus pig fat) and lower consumption of HF; if this were so, we would also expect L to be higher for the cases, yet the mean values of L is higher for the control group.

Although percentages of 18:2 in depot fat have been found to vary between 3.7 and 15.8, the weighted mean value for cases is virtually identical for cases and controls. This result lends no support to the currently popular view that higher amounts of polyunsaturated acids (essentially 18:2) which are present in many of the softer margarines have had a beneficial effect. It should be remembered, however, that in the UK until about 1974 consumption of soft margarine "high in polyunsaturated acids" was quite small. In the American diet, according to Call and Sanchez[70] intake of polyunsaturated fatty acids almost doubled during the period 1940

to 1976, and it seems very likely that a further increase has occoured since then.

The weighted mean for the longer - chain acids, H, is virtually identical for the cases and controls and therefore we have no evidence that such "higher acids" at the level consumed in the UK are harmful in themselves. In view of the claims by several authors that the acids erucic (22:1 cis), and probably eicosenoic (20:1 cis), are associated with damage to heart muscle, and that long-chain C_{20} to C_{24} saturated acids are atherogenic, a breakdown of our variable H into its components would seem desirable.

In fact our analyses show that the composition of the higher-acid content of depot fat is remarkably constant from area to area and between cases and controls. Thus for components 20:0, 20:1, 20:2 + 20:3, 22:0 and 22:1, mean values (as fraction, to nearest .01, of total H) are:-

For cases: .15, .50, .19, .02, .14.

For controls: .14, .50, .18, .03, .15.

In respect of the main component (20:1), the 10 case area mean values are 0.47, 0.48, 0.48, 0.48, 0.49, 0.50, 0.50, 0.51, 0.51, 0.53 with a SD of only 0.018; a similar spread appertains for the 10 control means.

Our results therefore show no differences between cases and controls for any particular higher acid, and do not lend any support to the above claims. But it should be added that, with the possible exception of 20:1, the individual components are present in very small amounts: thus 22:1 and 22:0 each

make up on average somewhat less than 1% of the total fatty acid content of adipose tissue. Again, about 90% of higher marine unsaturated acids is in the trans configuration unlike the cis 22:1 and cis 20:1 of rapeseed oil.

Finally, the degree of mono-unsaturation (M) in Table 5.2, is determined principally by the proportion of 16:1 plus 18:1 which together account for 52% of the total spectrum. There are also minor contributions from the mono-enoic content of "higher acids" (65% of H) and L acids (44% of L). The figure for total degree of mono-unsaturation is seen to be virtually identical for case and control specimens.

6. CASE VERSUS CONTROL LEVELS OF TRANS UNSATURATED ACIDS

We have seen that whereas the mean percentage of trans acids (T) is higher for the cases than for the controls, the difference is not statistically significant. This picture of the possible role of trans acids is however too simplistic and two types of trans acids will now be distinguished -

(i) 16:1 trans and 18:1 trans acids which we describe as "lower trans acids" the sum of which we label T_L.

(ii) the trans acids of chain length C_{20} and C_{22} which we describe as "higher trans acids" and label T_H.

These two groups together make up the total trans acids measured by IR spectroscopy, i.e. $T = T_L + T_H$.

The distribution of these types in fats consumed in the UK vary from fat to fat[12,13,14,15,16,23]. These distributions are summarised below:-

1. Ruminant-animal fat (butter-fat, beef, mutton) contain 5-7% trans acids almost entirely as T_L, with 18:1 trans predominating.
2. Pig/poultry fat and other, natural, unhydrogenated fats and oils contain zero trans components.
3. The trans acid content of HVO depends upon the commercial process used. Usually a lower limit of about 12% is set to ensure that the product will keep, (section 1.3), and an upper limit seems to be about 30%. In the UK the average concentration in HVO appears to be about 16% and this consists almost entirely of 18:1 trans acid

(zero 16:1 trans).

4. Hydrogenated marine oils which are much used in Britain[5] have about 2% L acids, and, according to source, 25% to 50% of C_{20} plus C_{22} acids of composition very similar to the H acids of our adipose samples. We have examined 8 leading brands of margarine / cooking fat[21] of high HMO content (ca. 50 to 80%) and it appears that in commercial practice a marine oil of mixed origin and of fairly constant composition is used; its mean H content is 35% and its mean (total) trans content is 50%. The ratio T_H/H in these 8 materials is reasonably constant at 0.9 $\pm$ 0.2 thereby showing that about 90% of the "higher acids" in HMO are themselves in trans configuration - a figure which agrees well with values quoted in the literature in respect of hydrogenated "whale", "seal", and "herring" oils. Of its total trans content (50%) only the smallest part is as T_L, the breakdown being (with standard deviations) -

T_H	=	32 $\pm$ 2%
16:1 trans	=	6 $\pm$ 1.5%
18:1 trans	=	9 $\pm$ 2%
Remainder (mostly 18:2 trans acids)	=	3%
Total trans (T)	=	50%

The T_H content of HMO is therefore relatively constant at 32 $\pm$ 2%. The average T_L content is 15% (compare average 16%, for HVO) but its variability is much greater. The variable content of 18:1 trans is

undoubtedly in part due to the presence of small amounts of HVO also present in these margarines (which are difficult to detect with any degree of confidence). The variable content of 16:1 trans however - which is greater than that of 18:1 trans - is inherent in the hydrogenation process as such.

It is important to realise therefore that in HMO, whereas amounts of total trans and H acids are reasonably constant, amounts of 18:1 trans and (particularly) of 16:1 trans are highly variable. In other words even at similar levels of H and T_H levels of T_L vary greatly from one hydrogenated marine oil to another. Thus for the 8 materials examined, setting H at 35% (the mean value for HMO) the percentage amounts of 16:1 trans acids were found to be:-

3.4, 3.9, 4.5, 5.4, 6.6, 8.9 and 9.5 - a range of almost 3 times from lowest to highest value.

At comparable levels of H and T_H in adipose tissue it is reasonable then to expect levels of 16:1 trans to vary substantially from individual to individual.

It is apparent from the above breakdown that of the total contribution made by hydrogenated fats to the average T content of UK dietary fat (3.3%; see section 1.6) that a substantial part will be in the form of T_H. An estimate shows that % T_H in such diet is about 2.0 so that only about 40% of total trans acids derived from HF is as T_L. Such amount (1.3%) is smaller in fact than that contributed by total RAF (2.1%).

Our method of measurement of trans acids by IR spectroscopy, did not allow us to distinguish directly between T_H and T_L, and initially therefore we approached the problem indirectly[18]. It has been shown (in section 1.6) that at UK average consumption of RAF and pig/poultry fat, the value of T/L will be ca. 0.8 in the event of zero HF intake (5 specimens showed values at about this figure) and the trans acids would then be almost entirely as T_L. The ratio will rise with inclusion of hydrogenated vegetable fat or HMO in the diet and the increase will be accompanied by increasing amounts of T_L. Consumption of HMO will also result in increase in T_H and in H.

It is concluded therefore that since HMO is a major component of British margarine/shortenings compared with HVO (particularly in the period under consideration) the ratio T/L should increase with increase in H.

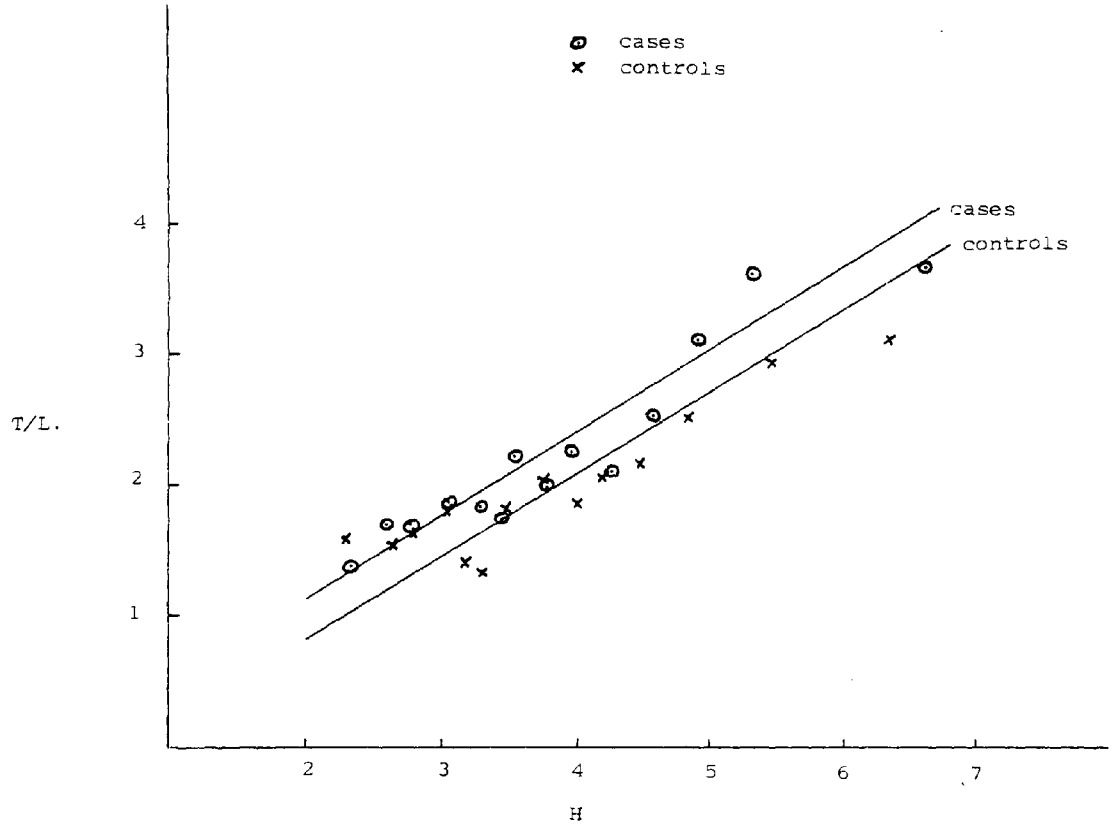
For the cases, T/L ranged from 0.9 to 5.30 and H from 2.15% to 7.45%.

For the controls, T/L ranged from 0.97 to 4.42 and H from 2.05 to 6.95%.

That T/L increases with H for both cases and controls is apparent from the Figure 6.1; the points plotted are mean values of T/L against mean values of H, each mean being that of an approximately constant number of individual values (7 for controls, 10 for cases).

The overall mean percentages of H ($\pm$ standard deviations) were 3.81 ($\pm$ 1.02) in cases and 3.91 ($\pm$ 1.14 for controls; the difference (0.10) was not statistically significant,

Figure 6.1 Association of higher fatty acids (H) with the ratio of transunsaturated acids (T) to L acids in 136 cases and 95 controls.



t = 0.78, p = 0.3. The mean value of T/L was 2.24 ($\pm$ 0.90) in cases and 2.06 ($\pm$ 0.79) in controls, the difference of 0.18 was not significantly different, t = 1.44, p = 0.14. (To avoid confusion, it is added that as shown previously in section 5 - when the decedents were matched for area of residence, the weighted mean value of T/L in cases was significantly higher than in the controls).

Detailed analysis of covariance (of T/L against H, cases versus controls) is given in Table 6.1. There were significant correlations between H and T/L both within the cases (r_1 = 0.705) and within the controls (r_2 = 0.661). The slopes of the two regression lines as shown in Figure 6.1 were b_1 = 0.5841 for cases and b_2= 0.4539 for controls. As the regression lines were not significantly different from one another the two regressions were combined to give a pooled regression b = 0.5266.

When the mean values of T/L are now adjusted for regression on H, the difference between cases and controls becomes highly significant (F = 7.56, p < .01). At comparable levels of H then the cases had a significantly higher value of T/L, and there appears to be no explanation of the result other than:

(a) the cases and controls have similar levels of "higher trans acids" T_H. It would indeed be remarkable in view of the virtual identity in the fatty - acid pattern of these H acids of both case and control specimens (given previously in section 5), if the identity did not extend also to the trans acid content.

(b) the cases have the higher value of T_L relative to L.

TABLE 6.1

ANALYSIS OF COVARIANCE OF REGRESSION OF T/L ON H, CASE AND CONTROLS

	$\sum(H-\bar{H})^2$	$\sum HT/L$	$\sum T/L$	Slope	Correlation	Degrees of freedom	Sum of Squares	Mean Square
Cases	157.66	92.09	108.17	0.5841	0.7052	134	54.39	0.4059
Controls	124.77	56.64	58.78	0.4539	0.6614	93	33.07	0.3566
Within both						227	87.46	0.3853
Between slopes						1	1.18	1.18
Pooled	282.43	148.73	166.95	0.5266	0.6849	228	88.64	0.3888
Between Means						1	2.94	2.94
All specimens	282.95	147.76	168.74	0.5222	0.6722	229	91.57	0.3999

Test	Ratio		F	Degrees of freedom	p	Significance
Between Variances	0.4059/0.3566	=	1.14	93, 134	> .05	NS
Between Slopes	1.18/0.3853	=	3.06	1, 227	> .05	NS
Between Adjusted Means	2.94/0.3888	=	7.56	1, 228	< .01	XX

On the premise that population mean values of trans and L acids in adipose tissue reflects dietary fat (as demonstrated in section 1.5), it is concluded that the cases consumed a higher amount of HF relative to RAF than did the controls. This encouraging conclusion indicated the need to analyse the trans acids in adipose tissue in further detail. At the time of commencement of the project, trans acids were quantifiable only via IR spectroscopy, and this gave only total trans acids irrespective of chain length. More recently it has been possible to measure lower-trans acids (T_L) directly by gas-liquid chromatography employing a high-performance column containing a highly polar liquid phase OV-275[50]. Procedures and experimental results have been given previously (in section 3.3 and 4.5.2 respectively).

Area mean values of 18:1 trans, 16:1 trans, T_L, L and T_L/L for cases and controls for each area of the study have been recorded earlier in Table 4.16. The cases have lower mean values of L in 9 of the 10 areas, higher values of T_L in 8 areas, and higher values of T_L/L in 9 areas. Individual values of 16:1 trans range from 0.21% to 1.55%, 18:1 trans from 0.87% to 4.35% and T_L from 1.21% to 5.58%. As expected - HMO being the major source of trans acids - 16:1 trans and 18:1 trans levels are clearly correlated.

Calculation of the regression coefficients on age of 16:1 trans, 18:1 trans and T_L/L gave + 0.003, + 0.010 and + 0.004 units per year respectively. It is considered that the mean difference in age at death between cases and controls (1.2y) would not bias any conclusions which might be drawn.

Two way analysis of variance within cases and controls and between areas showed highly significant differences between area mean values for 16:1 trans ($F = 6.3$, $p < 0.01$), for 18:1 trans ($F = 17.7$, $p \ll 0.001$) and for T_L/L ($F = 5.5$, $p < 0.01$). There is however no evidence that case/control differences vary between areas ($F = 0.9$, 1.1 and 1.0 respectively). To pool the results therefore the method of weighted means of Pearce[69] was again used, and Table 6.2 shows the difference in (weighted) mean values for cases minus controls for 16:1 trans, 18:1 trans, their sum T_L, for T_H and for various ratios. This reaffirms our previous analysis (that of covariance T/L against H, see above) that the differences between cases and controls are significant for T_L but not for T_H.

TABLE 6.2

OVERALL ADIPOSE TISSUE COMPOSITION: WEIGHTED DIFFERENCES

CASES MINUS CONTROLS

Component trans acids	%	DIFFERENCES			p
		Mean Differences	Standard errors	Student's "t"	
16:1 trans	0.708	0.0820	0.0279	2.94	<0.005
18:1 trans	2.397	0.1308	0.0746	1.75	NS
*T_L	3.105	0.2128	0.0952	2.23	~0.025
+Total trans acids (T)	5.327	0.2264	0.2185	1.04	NS
‡T_H	2.222	0.0136	0.1551	0.09	NS
T_L/L	1.159	0.1670	0.0578	2.89	<0.005
16:1 trans/L	0.290	0.0532	0.0159	3.35	<0.001

*T_L = 16:1 trans plus 18:1 trans

+Taken from Table 5.1.

‡T_H = T - T_L

7. CONCLUSIONS AND DISCUSSION

7.1 Case versus control differences

The weighted mean values of T_H (2.22%) is, as expected, virtually identical for cases and controls. In contrast, mean percentages of 16:1 trans and 18:1 trans acids are higher for the case specimens by amounts 0.08% and 0.13% respectively, the difference being significant at $p < 0.005$ for the former, but only at the 8% level for the latter acids. For total lower - trans acids, T_L, the mean percentage for cases (3.21) is higher than that for the controls (3.00) by an amount significant at $p \sim 0.025$.

An obvious interpretation of the results is that whereas there is no evidence that "higher-trans" acids are harmful, risk attaches to "lower-trans" acids (particularly to 16:1 trans) purely in virtue of these having a trans configuration irrespective of other structural considerations. Whereas this is undoubtedly a possibility, it ignores the fact that trans acids from differing fat - sources are not identical in structure. Thus according to Dutton[71] and Carpenter et al[72] who have examined a number of commercial hydrogenated vegetable margarines and shortenings, the 18:1 trans bonds of HF are widely dispersed along the carbon chain between positions w4 and w12 (numbered from the methyl end). A similar picture emerges for the 18:1 and also 16:1 trans acid content of hydrogenated marine oils. In RAF on the other hand, the 18:1 trans bonds are mostly in the w7

and w9 central positions[73]. For this and other reasons which will be apparent, it was therefore preferred to use lower trans (and L) acids as "markers" indicative of the relativity of HF to RAF, without necessarily implicating any particular constituent(s).

Probably of greater significance than the above structural difference in the trans acids of HF and RAF is the fact that commercial hydrogenation of vegetable oils gives rise to a variety of cis isomers. Thus according to Dutton[71] and Carpenter et al[72], about 25% of the cis 18:1 remaining in HVO consists of isomers with their (cis) double bonds in a variety of positions other than the central cis w9, position of natural oleic acid. Bearing in mind that 18:1 is the major acid of margarine and shortenings, this 25% forms a substantial part of the whole product.

Hydrogenated marine oils also contain 18:1 and 16:1 positional cis isomers[13,16] - possibly in amount greater than in HVO.

In RAF on the other hand, over 95% of the mono-enoic content remains in the "natural" position[23,73].

Necessarily therefore, the 16:1 trans and 18:1 trans content of HF is positively correlated with amounts of positional cis isomers. To date we have not separately quantified amounts of cis isomers, but they appear to be intuitively more likely to carry risk than trans isomers - if for no other reason that whereas trans acids closely resemble saturated acids in shape, introduction of cis bonds greatly changes molecular geometry.

In addition to the presence of chemically - altered mono-enoic fatty acids there are other possible hazards associated with HF which are thought worthy in our opinion of thorough investigation.

These are:

1. Except for babies and infants, a variety of artificial anti-oxidants are added to margarine and branded cooking fats and oils, and to manufactured foods containing such oils and fats[74]. Their addition to butter intended for retail sale as such is however prohibited. Due to the necessity of protecting highly unsaturated materials from autoxidation (see section 2.2.2), it seems probable that the softer margarines of higher 18:2 content are likely to contain such additives in higher amounts.
2. Hydrogenated materials are known to contain residual amounts of nickel catalyst over which there is apparently no statutory control. It is generally assumed that nickel is relatively non - toxic[75]. On the other hand, Koch et al[76] reported relatively high levels in cardiac muscle, and D'Alonzo and Peel[77] reported the intriguing fact that 19 of 20 MI patients newly admitted to hospital exhibited "abnormally high levels of serum nickel", whereas "only 4 of the 20 controls exhibited high levels".
3. There have been many protestations to the effect that if PUFA consumption is to be increased, amounts of natural anti-oxidant, Vitamin E, should be increased

in due proportion. Conditions normally associated with refinement and processing of vegetable oils would appear to lead to destruction of vitamin E in at least some degree.

4. The special status of linoleic acid (18:2cc) as an essential fatty acid (EFA) and precursor of arachidonic acid and prostaglandins, raises the interesting possibility that isomeric 18:2 acids may interfere with important enzymic pathways. In view of the necessity of reducing the high levels of 18:3 (mentioned previously in section 1.3), it appears that such isomers are present in hydrogenated soya in fair amounts. The exact amount and its precise nature is difficult to establish with a degree of confidence. The well - known lipoxidase determination of EFA - as employed recently by Nazir et al[24] leads to a figure ca. 7 - 10% for lipoxidase inactive 18:2 in American margarine, but it seems likely that this figure could well be an over - estimate. In 8 leading brands of margarine of high HMO content previously analysed (section 4.2) amounts of (non-conjugated) 18:2 trans acids have been found[53] at about the 2% levels. However, in hydrogenated vegetable oil based materials amounts of trans acids appear to be higher[21], with one material (of linoleic acid content 27%) having a figure of 9% (i.e. a quarter of the total 18:2 content).

These analyses serve to demonstrate that increasing dietary levels of linoleic acid achieved through

consumption of softer, HVO - based margarines will therefore be accompanied by increase in isomeric 18:2. It is known too[78] that all - cis linolenic acids (18:3) is converted by the conditions normally used for de-odourization of soya oil to trans forms in substantial measure (25%), and certainly the presence of such isomers has been detected[21] in UK margarine. Returning now to use of T_L and L as "markers", in the event of zero intake of HF, the expected value of T_L/L for otherwise average UK fatty - diet is ca. 0.6 (This figure is somewhat lower than the previous figure of 0.8 for T/L given in section 1.6, since it has been found[21] that in butter fat about 20% of the total trans content resides within the "higher acid" content). Inclusion of HMO or HVO - of T_L/L values about 8 and 60 respectively - will result in sharp increase in this ratio. In agreement with this estimate it has been found that of the 231 specimens analysed, only 6 have values of T_L/L between 0.5 and 0.6, the rest falling between 0.7 and the highest value 2.9. The weighted mean value of T_L/L for the cases is 1.342 and that for the controls 1.175, the difference (0.167) being significant at $p < 0.005$.

On the resonable assumption that an amount of butter is replaced by margarine in the diet, but consumption of pig/poultry fat remains constant, this difference implies that the cases consumed about 30% more (average - type) margarine and 10% less butter fat than the control group.

In respect of ratio 16:1 trans/L, the difference between

case and control specimens is significant at $p < 0.001$, and it might be inferred that HMO carries a higher risk than HVO containing much 18:1 trans but no 16:1 trans. Whereas this is undoubtedly a possibility we believe it would be premature to apportion risk to the one than to the other for the following reasons. (a) precise amounts of positional isomers and additives associated with these two types of HF are at present unknown, (b) whereas 16:1 trans derives almost entirely from HMO, the 18:1 trans derives not only from HF but also, in large measure, from RAF. In other words, for strict comparability we require to know only that portion of 18:1 trans which derives from HF. Unfortunately at present there appears to be no way in which this can be unambiguously achieved.

On the premise therefore that population mean levels of T_L and L acids in adipose tissue reflect dietary intakes it is concluded that the cases consumed a higher amount of hydrogenated fat relative to RAF than did the controls. Put in another way - since consumption of HF is in general known to be negatively correlated with consumption of RAF[3] - the cases consumed on average the higher amount of HF and the lower amount of RAF. Further it follows that on a weight to weight basis, those hydrogenated fats having higher content of "lower trans" acids will present the greater risk. In this last respect it is possible that some hydrogenated vegetable oils may be more harmful than hydrogenated marine oils.

7.2 Area comparisons

In addition to the determined within area case versus control differences, it should also be observed (Table 4.16 of section 4.5.2) that, although admittedly based on small numbers of samples, the rank order in area mean values of T_L/L reflect relative mortalities from IHD in England and Wales rather well. Thus the ratio is lowest for Luton and Dunstable, South and East England, and "rural" West Wales; mortality is lowest for such areas (see SMR ages 25-64, 1968-71 of Table 1.2, in section 1.2) with Luton and Dunstable having one of the lowest standardised mortality ratios amongst 115 country and London boroughs of England and Wales[79]. (The rank position of Chatham as a South East region hospital however seems anomalous). Values of T_L/L and of SMR are intermediate for the Yorkshire and Humberside region (i.e. Huddersfield/Halifax and Bradford). Higher values of T_L/L are shown by the Manchester and Liverpool hospitals in keeping with the very high mortality experience of the provincial conurbations.

The position of industrialised South East Wales compared with the more rural Welsh area (with its traditional very high butter consumption) is of particular interest. Previous work referred to earlier of Thomas (in section 1.5), based on National Food Survey Committee data for household consumption of margarine (available unfortunately only for the whole Principality) showed South East Wales to be highly anomalous. It now appears possible that although its all-brand consumption of margarine is relatively low, those

margarines having higher content of "lower trans"acids relative to "higher trans" acids are for some reason favoured. Another possibility (although we have no sound evidence for this) is that extra T_L is consumed outside the private household through purchase of cakes, biscuits etc.. These possibilities are supported by the fact - taking HMO as the main source of "industrial" trans acids in the UK - that the mean value for the ratios 16:1 trans/T and 16:1 trans/T_H for both cases and controls is higher for South East Wales than for any other area examined. (The area having the lowest values of these ratios is Luton/Dunstable). The reason(s) for these peculiar preferences however remains obscure at present.

In further conclusion it is felt that the observed differences in T_L and in L between cases and controls, co with the fact that area mortality experience satisfactorily correlates with area T_L/L levels, suggests that consumption of certain margarines/shortenings in particular could well be a major causative factor of IHD. The many other "risk factors" which have at various times been suggested would reasonably have been expected to outweigh this dietary factor in at least some of the widely - differing areas examined were they of greater import.

7.3 A possible artefact

Lastly, we consider the possibility that our case versus control differences are an artefact of case - selection in the sense that a fraction of the cases may well have suffered previous incidents of heart disease and, acting on medical advice, changed diet in favour of margarine. There are however in our opinion good reasons for supposing that the observed differences cannot be explained on such a basis:

Thus -

1. Medical advice in the light of current opinion might reasonably be supposed to have favoured those softer margarines described as "containing only pure vegetable oils". Such materials would have had a very high content of PUFA and no 16:1 trans. (Analysis of 10 such margarines purchased over the period 1976-81 in our laboratory gave a mean percentage 18:2 of 31%; the 18:2 content of butter fat is only ca. 2%). The results however show that amounts of 16:1 trans are <u>higher</u> for the <u>cases</u> and that levels of 18:2 are virtually identical for the case and control specimens. Again the C_{18} - rich vegetable oils are characterised by exceptionally low values of the acid ratio R i.e. (16:0 + 16:1)/ (18:0 + 18:1), whereas butter fat is relatively rich in C_{16} acids, but we have found that the mean value of the ratio is significantly <u>lower</u> for the <u>control</u> group.
2. Hydrogenated marine oils and vegetable oils hardened in greater degree are widely used in the manufacture of

cakes, pastries and biscuits. Such items have a high fat content and would surely not have been favoured by the dietician - in which case a decrease in 16:1 trans in particular may have been expected.

3. It should be remembered that until quite recently (see section 1.5), the higher - priced vegetable - oil based margarines were not widely available in the UK, in which case medical advice would have resulted in increasing consumption of the harder margarines based largely on HMO. If HMO carries no hazard, levels of T_L T_H and H would have increased in relative amounts reflecting average HMO composition. The fact however, is that whereas there is an excess in the case specimens of 16:1 trans in particular no case/control difference is observed in T_H or in H. We can see no explanation of this peculiarity other than that only the lower trans acids carry the hazard. Neither the Doctor nor the consumer would be aware of the exact composition of the brand or brands chosen; indeed it was shown in section 6, that the ratio 16:1 trans/T_H is highly variable and it seems very likely that a given brand may vary from batch to batch according to the exact conditions appertaining in the hydrogenation process. Per unit amount of H and T_H therefore, amounts of T_L consumed will be open to chance-with cases having the higher intake.

APPENDIX A

EQUIVALENT CHAIN LENGTH (ECL) VALUES OF OLEFINIC, BRANCHED CHAIN AND OTHER ISOMERIC ESTERS

FATTY ACID*	STATIONARY PHASE		
	8% EGSS-X	15% EGSS-Y	20% OV275
i. 14:0	13.48	13.60	13.79
ai. 14:0	13.71	13.74	13.60
14:1t	14.78	14.60	-
14:1c	14.78	14.60	15.55
i. 15:0	14.50	14.50	14.55
ai. 15:0	14.72	14.75	14.89
15:1t	15.70	15.55	-
15:1c	15.70	15.55	16.30
i. 16:0	15.46	15.48	15.48
ai. 16:0	15.68	15.70	15.79
16:1t w7	16.64	16.53	16.70
16:1c w7	16.64	16.53	17.23
i. 17:0	16.51	16.47	16.44
ai. 17:0	16.72	16.69	16.83
17:1t	17.65	17.45	17.66
17:1c	17.65	17.45	18.15
i. 18:0	17.47	17.48	17.40
ai. 18:0	17.72	17.74	-
18:1t w9	18.60	18.47	18.64
18:1c w9	18.60	18.47	19.12
18:2tt w9	19.40	19.16	19.50
18:2ct w9	19.40	19.16	20.17
18:2cc w9**	19.40	19.16	20.45
conj. 18:2ct w9**	20.48	20.24	22.20
conj. 18:2ct w8	20.93	20.57	22.57
18:3ttt w9	20.48	19.90	20.99
18:3ctt w9	20.48	19.90	21.36
18:3cct w9	20.48	19.90	21.57
18:3ccc w9	20.48	19.90	21.95
19:1t w8	19.64	19.47	19.70
19:1c w8	19.64	19.47	20.21
20:1t w9	20.56	20.48	20.75
20:1c w9	20.56	20.48	21.27+
20:2tt w9	21.14	20.87	21.25+
20:2ct w9	21.14	20.87	21.80+
20:2cc w9	21.14	20.87	22.20+
20:2tt w6	21.40	21.18	21.55
20:2ct w6	21.40	21.18	22.00
20:2cc w6	21.40	21.18	22.40
20.3ccc w9	21.69	21.20	23.55
20:3ccc w6	22.06	21.50	23.91
20:3ccc w3	22.45	21.97	24.43
20:4cccc w6	22.45	22.05	24.03
22:1t w9	22.54	22.54	23.02
22:1c w9	22.54	22.54	23.53

*Fatty acids are designated by number of carbon atoms : number of double bonds. The letters C (cis) and t (trans) specify the configuration of the double bond(s) present. The digit after w states the number of carbon atoms from the methyl end of the acyl chain to the nearest double bond. The latter designation is omitted when the position of the double bond is unknown.

ai. = anteiso

i. = iso

conj. = conjugated; prepared according to AOCS Standard Method Cd 7-59.

**mixed geometric (cis/trans) isomer positions not determined.

+ correlated using semilog plots[43].

APPENDIX B

VALUES OF PARAMETERS T*, H + 18:3, L AND 18:2 FOR 136 CASE-SPECIMENS (D) AND 95 CONTROL-SPECIMENS (C) COLLECTED FROM 10 DIFFERENT REGIONS/CONURBATIONS; ALL FIGURES TO NEAREST ± 0.05

Age at death in Y	Case or Control	T	H + 18:3	L	18:2
	1. WEST WALES				
34	C	2.6	4.8	2.1	7.95
46	C	3.1	2.7	3.15	10.85
48	D	3.35	4.05	2.95	9.85
45	C	3.6	4.1	3.1	7.35
40	C	3.6	3.4	3.2	7.65
60	D	3.8	4.2	3.15	5.55
53	D	4.3	3.45	3.8	6.0
63	C	4.4	3.6	3.9	7.05
50	D	4.4	2.9	3.5	6.8
60	D	4.5	4.05	2.55	15.25
61	D	4.5	5.0	2.65	9.1
51	D	4.55	4.55	1.75	6.8
69	C	4.6	4.1	2.8	9.7
55	D	4.75	3.9	3.15	9.35
57	D	4.75	4.6	1.4	8.75
52	D	4.8	4.4	2.8	5.95
60	C	4.95	4.35	1.6	9.0
43	D	4.95	3.4	3.35	7.45
51	D	5.0	4.7	2.05	9.6
54	D	5.05	3.25	3.05	6.7
63	D	5.9	4.3	1.55	9.8
51	D	6.15	3.35	3.75	7.5
44	C	6.2	3.4	2.05	8.55
55	D	6.3	5.0	2.2	9.35
34	C	6.75	4.8	3.55	10.45
	2. SOUTH AND EAST ENGLAND				
60	C	2.45	5.15	2.35	5.65
60	C	3.25	3.45	2.35	12.8
49	C	3.3	3.15	2.25	9.75
37	D	3.85	3.0	1.95	9.8
38	D	4.2	4.4	2.55	6.85
62	D	4.35	5.15	2.3	7.95
57	C	4.5	4.3	1.95	7.2
61	C	4.55	3.85	3.15	7.7
54	D	4.75	4.15	3.2	7.9
61	C	4.85	3.7	2.55	6.55
52	D	5.3	5.4	2.35	9.15
61	D	5.75	4.65	3.3	8.55
57	D	7.15	5.9	1.35	15.8
57	D	7.4	6.0	1.85	11.1
53	D	9.65	7.4	2.45	11.4
	3. LONDON (CHATHAM)				
54	D	4.15	3.95	2.8	10.75
58	D	4.15	4.55	2.3	7.15
41	D	4.5	3.45	3.45	7.35
57	C	4.65	3.4	2.4	14.5
53	C	4.7	2.95	2.85	9.05
33	C	4.8	4.2	3.55	7.5
59	D	4.8	3.3	2.1	7.85
37	D	4.85	3.95	2.4	8.15
44	C	5.15	3.35	3.15	8.85
53	D	5.45	3.15	4.0	7.9
62	D	5.7	4.45	2.35	7.7
35	C	5.9	5.1	3.1	7.75
54	D	6.0	4.2	2.35	12.2
35	C	6.2	5.65	3.05	7.15
60	D	6.2	5.3	1.7	9.45
42	D	7.3	3.35	3.55	9.4
57	D	7.95	5.8	2.45	6.45
64	C	8.7	4.95	3.65	8.15
59	D	8.75	6.2	2.85	7.75
	4. SOUTH EAST WALES				
46	D	1.85	3.0	2.05	6.3
34	C	2.85	3.8	2.5	6.25
45	D	3.15	4.5	1.85	7.3
45	D	3.3	3.95	1.55	7.6
36	D	3.35	5.1	2.0	7.2
40	C	3.45	3.65	2.75	6.3
57	D	3.75	3.5	2.5	5.65
58	D	3.8	3.55	2.5	4.1
44	C	3.85	5.1	2.15	8.6
62	D	3.9	3.9	3.85	4.8
56	C	4.1	4.25	3.65	6.15
47	D	4.1	3.85	2.85	6.35
47	D	4.15	4.0	2.25	8.05
38	D	4.3	4.35	2.3	6.6
63	C	4.45	3.3	3.6	7.15
32	C	4.55	3.1	1.65	13.05
46	D	4.55	4.35	2.85	10.3
63	D	4.6	3.4	3.0	6.15
63	D	5.1	4.9	2.85	6.95
33	C	5.2	4.2	2.8	9.25
70	C	5.25	4.65	2.5	5.8
61	C	5.35	6.05	2.55	5.9
53	D	5.6	5.35	3.65	10.2
63	C	5.65	5.1	2.55	9.75
52	C	5.7	4.75	3.35	8.85
62	C	5.95	4.7	3.75	7.1
58	D	6.2	3.9	2.0	8.7
58	D	7.3	5.7	2.65	6.1
63	D	9.45	6.7	3.0	3.6

* T arranged in rank order within each area.

Age at death in Y	Case or control	T	H + 18:3	L	18:2	Age at death in Y	Case or control	T	H + 18:3	L	18:2
	5. LUTON AND DUNSTABLE						6. HUDDERSFIELD AND HALIFAX				
56	D	2.5	4.15	1.15	9.95	36	D	3.2	3.3	2.0	8.05
56	D	3.15	3.25	1.85	8.0	51	D	3.35	3.1	1.8	8.15
51	C	3.45	2.95	2.5	8.0	36	C	3.45	3.75	2.35	8.7
60	C	4.0	3.65	2.8	4.95	51	C	3.5	3.85	2.2	15.75
45	C	4.05	4.55	2.9	8.6	31	C	3.65	3.25	2.75	8.3
54	D	4.05	3.5	3.4	6.85	49	C	3.85	4.1	3.5	8.65
57	D	4.15	3.8	2.15	9.15	47	D	4.1	2.8	2.95	8.9
56	D	4.2	4.05	3.0	6.6	58	D	4.2	3.85	2.45	7.2
63	D	4.35	4.0	2.9	8.95	43	D	4.25	4.2	1.7	4.55
51	D	4.4	3.0	3.95	6.3	49	D	4.45	4.1	2.3	9.25
38	C	4.5	3.85	3.55	7.45	33	D	4.75	4.0	2.85	11.7
57	D	4.5	3.7	2.9	7.7	52	D	5.35	3.55	2.85	7.9
62	C	4.55	4.55	3.0	8.35	40	D	5.35	5.4	1.6	11.25
60	D	4.65	4.05	2.7	5.75	48	D	5.4	3.4	2.8	7.6
65	C	4.75	4.6	2.55	5.2	45	C	5.95	4.2	2.0	7.35
59	D	4.75	3.85	2.65	8.55	44	D	6.35	5.25	2.25	7.2
63	C	4.9	3.65	2.2	7.7	32	C	6.45	5.3	1.8	7.6
62	C	4.95	3.85	2.65	7.1	44	D	6.45	5.6	1.7	4.9
57	D	5.0	5.0	2.95	8.4	57	D	6.7	6.45	2.4	9.55
64	C	5.05	4.65	2.9	6.2	48	D	6.9	4.25	2.95	9.1
46	D	5.1	3.85	2.8	8.3	64	C	8.1	6.75	2.55	9.05
50	D	5.1	4.35	2.55	8.55						
60	C	5.25	3.55	3.7	7.55						
59	D	5.25	4.0	3.9	6.95						
55	D	5.3	3.8	3.05	7.3						
54	C	5.6	4.4	1.9	6.3						
60	D	5.95	4.5	2.4	11.8						
41	C	6.05	4.8	1.85	13.05						
51	D	6.85	5.2	2.25	9.95						
61	D	7.2	4.8	2.55	8.75						
63	D	7.3	5.3	2.15	7.85						
64	C	7.65	5.55	3.2	10.3						
63	C	7.7	5.45	3.0	8.65						
44	C	7.95	6.45	1.8	11.1						
52	D	8.2	5.85	3.05	6.7						
56	D	9.8	5.5	2.1	7.95						
56	D	10.0	8.1	2.65	11.05						

Age at death in Y	Case or control	T	H + 18:3	L	18:2
		7. BRADFORD			
48	C	2.8	2.85	2.0	7.45
64	C	3.1	3.35	1.6	4.0
49	D	3.2	3.2	2.9	4.4
49	C	3.45	3.25	1.95	4.65
60	D	3.8	3.3	1.95	6.25
52	C	4.1	3.7	1.6	10.9
35	C	4.35	4.05	2.9	8.5
44	C	4.4	3.3	2.5	8.45
48	D	4.5	5.6	2.0	6.3
51	C	4.55	4.1	2.0	5.35
54	D	4.55	3.35	1.65	6.8
57	D	4.7	4.2	1.65	4.2
47	C	4.95	4.4	2.4	8.1
58	D	5.1	3.25	2.45	8.95
57	C	5.15	4.9	2.6	11.5
41	C	5.6	4.1	2.3	11.7
60	D	5.6	4.55	2.45	6.4
35	C	5.7	6.3	2.65	8.55
57	D	5.7	3.6	1.5	6.75
54	D	6.0	4.05	2.75	8.05
56	C	6.35	5.85	1.6	6.3
48	D	6.4	4.6	2.65	8.4
54	C	6.55	5.1	3.35	5.65
42	D	6.95	6.95	1.65	7.45
52	C	7.7	6.8	2.75	6.7
58	C	7.8	7.2	2.05	6.25
54	D	7.9	6.75	2.1	9.05
62	C	7.95	5.25	2.5	6.7
55	D	8.1	5.7	2.55	8.5
55	D	9.1	6.35	1.8	10.55
59	D	9.55	5.6	3.15	11.3

Age at death in Y	Case or control	T	H + 18:3	L	18:2
		8. MANCHESTER CONURBATION 1			
49	D	2.95	3.0	2.85	7.1
59	D	3.6	3.95	3.2	7.25
63	C	3.65	4.0	3.35	9.6
44	D	3.8	4.5	2.2	5.8
54	D	4.2	3.15	3.4	5.1
33	C	4.3	3.65	2.15	6.75
37	D	4.55	3.1	3.05	8.25
66	C	4.85	3.8	3.1	7.8
46	C	4.85	3.9	4.15	6.5
53	C	4.85	3.95	3.55	6.45
55	D	4.85	4.25	3.25	7.8
52	C	4.9	3.3	3.95	8.1
58	D	4.9	4.6	3.1	7.1
44	D	4.9	3.5	2.1	10.8
46	D	5.3	4.2	1.85	7.5
57	D	5.75	4.75	2.05	9.55
63	D	5.75	5.15	2.75	6.6
54	D	5.9	5.15	2.0	7.7
51	D	6.05	3.3	2.75	14.0
43	C	6.2	3.9	3.95	7.1
57	C	6.3	6.05	2.95	7.1
45	C	6.4	5.5	3.35	11.15
66	C	6.55	6.9	1.7	7.75
60	D	7.35	6.0	2.5	6.65
58	C	7.4	5.65	2.55	10.45
48	D	10.05	7.15	2.75	11.3
		9. MANCHESTER CONURBATION 11			
47	C	3.1	4.1	1.65	3.7
50	D	3.4	4.1	1.95	3.85
58	D	3.8	4.5	3.25	8.45
61	D	4.0	3.55	2.6	7.8
40	D	4.05	4.8	2.5	6.15
62	C	5.3	4.6	2.2	5.75
55	D	5.5	4.6	2.6	4.6
54	D	5.7	4.6	2.9	8.9
58	D	6.1	4.95	2.7	7.1
58	D	6.1	4.15	2.9	7.55
52	D	6.15	3.9	1.7	10.15
43	D	6.45	4.2	2.3	6.1
55	C	6.5	5.7	2.5	5.6
60	D	6.75	5.75	2.15	8.15
54	D	6.95	5.0	2.9	7.45
50	C	7.5	5.2	2.25	9.0
64	C	9.0	6.4	2.45	7.7
		10. LIVERPOOL CONURBATION			
58	C	3.2	3.8	2.85	5.75
58	D	4.2	4.25	2.7	6.7
55	D	4.2	4.85	2.3	8.7
49	C	4.5	4.8	2.5	9.55
44	D	5.8	5.0	2.6	10.6
64	C	6.9	7.15	1.85	6.8
46	C	7.3	6.5	3.0	5.6
54	C	7.8	6.35	2.7	6.65
45	C	8.85	7.15	3.2	7.1
47	C	9.2	7.6	3.35	8.15
61	D	11.6	8.5	2.6	9.75

APPENDIX C

Fatty acid composition of the 231 case/control adipose specimens, with corresponding values of age at death, T, H + 18:3, L and trans unsaturated acids 16:1 and 18:1; collected from 10 different regions/conurbations.

MRC no.	AREA†	TYPE‡	AGE	T	L	H + 18:3	14:0	18:2	10:0 + 12:0	16:0	16:1	17:0 br.	18:0	18:1t	16:1t	18:1
3003	9	1	57	3.75	2.50	3.50	4.45	5.65	0.60	24.80	7.40	0.30	4.90	2.29	0.75	54.35
3008	9	1	58	6.20	2.00	3.90	2.90	8.70	0.35	21.05	7.45	0.40	3.30	2.72	0.98	50.30
461	13	1	53	5.45	4.00	3.15	4.70	7.90	0.40	23.45	7.65	1.10	4.05	3.17	0.93	56.65
474	13	1	54	6.00	2.35	4.20	3.75	12.20	0.85	22.45	5.10	0.60	4.75	4.18	0.72	56.50
1132	13	1	42	7.50	3.55	3.35	4.10	9.40	0.65	22.05	8.30	0.65	4.60	3.49	1.30	56.90
2014	9	2	70	5.25	2.50	4.65	4.45	5.80	0.95	20.85	5.50	0.35	7.10	2.30	0.72	52.40
192	10	1	46	1.85	2.05	3.00	2.20	6.30	0.15	22.75	10.85	0.75	3.55	1.47	0.60	51.85
702	1	2	46	3.10	3.15	2.70	3.05	10.85	0.20	21.15	7.90	0.75	3.20	1.28	0.26	53.20
2013	9	1	58	3.80	2.50	3.55	4.25	4.10	0.55	22.30	8.55	0.35	6.20	2.33	0.70	52.60
721	1	2	45	3.60	3.10	4.10	4.75	7.35	0.85	22.55	6.70	0.45	6.40	1.94	0.64	56.50
723	1	2	69	4.60	2.80	4.10	3.15	9.70	0.65	20.50	8.00	0.40	3.75	1.62	0.46	53.30
724	1	2	63	4.40	3.90	3.60	5.20	7.05	0.10	25.65	6.60	0.85	5.95	2.13	0.89	59.15
1278	1	2	34	6.75	3.55	4.80	4.35	10.45	0.70	19.30	8.15	0.70	4.50	2.62	0.40	56.75
435	18	2	49	4.50	2.50	4.80	2.55	9.55	0.35	20.90	5.90	0.60	4.45	2.51	0.63	51.85
1290	1	2	44	6.20	2.05	3.40	4.70	8.55	0.75	23.05	7.55	0.55	3.85	2.42	0.86	54.70
1291	1	2	40	3.60	3.20	3.40	3.95	7.65	0.50	24.00	5.20	0.75	7.00	2.22	0.65	55.90
106	2	2	34	2.60	2.10	4.80	2.80	7.95	0.30	22.25	7.05	0.60	3.75	1.16	0.21	51.85
147	3	2	60	4.95	1.60	4.35	3.20	9.00	0.35	23.05	6.40	0.60	4.35	2.30	0.62	53.15
703	1	1	61	4.50	2.65	5.00	3.80	9.10	0.40	21.50	6.40	0.60	4.55	1.99	0.49	54.25
707	1	1	55	6.30	2.20	5.00	3.75	9.35	0.55	24.60	4.50	0.60	6.05	2.27	0.87	56.85
710	1	1	55	4.75	3.15	3.70	4.15	9.35	0.40	21.45	8.50	0.55	4.50	2.08	0.69	56.20
432	18	2	47	9.20	3.35	7.60	3.80	8.15	0.65	21.15	6.85	0.60	5.25	3.13	0.66	57.65
715	1	1	60	4.50	2.55	4.05	4.05	15.25	0.60	22.55	4.35	0.60	5.35	1.81	0.27	59.60
718	1	1	50	4.40	3.50	2.90	4.50	6.80	0.55	24.15	6.45	1.00	5.70	2.11	0.65	55.80
720	1	1	51	6.15	3.75	3.35	5.65	7.50	1.00	22.75	7.00	0.70	5.75	2.49	0.69	57.70
722	1	1	63	5.90	1.55	4.30	4.15	9.80	0.90	21.80	6.25	0.60	4.40	2.90	0.91	54.60
1282	1	1	57	4.75	1.40	4.60	4.25	8.75	0.55	22.35	5.45	0.60	6.15	2.53	0.70	54.35
1283	1	1	54	5.05	3.05	3.25	4.40	6.70	0.35	21.95	8.80	1.05	4.25	2.16	0.72	54.05
1294	1	1	43	4.95	3.35	3.40	3.85	7.45	0.50	20.35	8.90	0.70	3.75	1.86	0.75	52.50
103	2	1	53	4.30	3.80	3.45	5.75	6.00	0.60	25.60	6.40	0.95	5.80	2.33	0.65	58.60
430	18	2	46	7.30	3.00	6.50	3.90	5.60	0.35	23.20	5.35	0.60	5.70	3.01	0.72	54.45
105	2	1	51	5.00	2.05	4.70	4.25	9.60	0.75	22.80	6.30	0.60	4.95	1.97	0.51	56.25
107	2	1	52	4.80	2.80	4.40	3.85	5.95	0.65	20.35	8.05	0.60	4.40	2.03	0.47	51.30
108	2	1	48	3.35	2.95	4.05	3.95	9.85	0.55	25.50	7.60	0.80	3.85	1.49	0.24	59.15
427	18	2	58	3.20	2.85	3.80	3.40	5.75	0.45	21.10	9.95	0.60	3.70	1.73	0.42	51.85
142	3	1	60	3.80	3.15	4.20	4.15	5.55	0.40	22.55	6.85	0.60	5.55	1.75	0.46	53.25
441	18	2	54	7.80	2.70	6.35	4.60	6.65	1.05	19.85	7.75	0.60	5.30	3.30	0.61	55.10
150	3	1	51	4.55	1.75	4.55	3.45	6.80	0.35	24.10	8.05	0.60	5.95	1.97	0.76	53.85
388	4	1	61	5.75	3.30	4.65	4.30	8.55	0.95	21.65	6.25	0.70	5.70	2.46	0.77	56.30
355	16	1	55	4.85	3.25	4.25	2.85	7.80	0.45	22.95	7.40	0.85	4.85	2.80	0.61	54.90
394	4	1	54	4.75	3.20	4.15	3.95	7.90	0.60	20.50	7.75	0.25	4.75	1.91	0.60	53.30
736	5	1	62	4.35	2.30	5.15	4.25	7.95	0.55	23.55	5.50	0.60	5.40	1.85	0.53	55.50
978	6	1	57	7.15	1.35	5.90	2.30	15.80	0.25	21.65	7.15	0.60	3.25	2.44	0.72	58.50
979	6	1	52	5.30	2.35	5.40	4.30	9.15	1.25	21.00	5.65	0.60	6.35	2.79	0.59	56.30
984	6	1	38	4.20	2.55	4.40	4.65	6.85	0.65	24.35	5.20	0.60	5.85	1.91	0.65	55.35
411	7	1	53	9.65	2.45	7.40	4.10	11.40	0.80	21.40	5.90	0.60	5.20	3.17	1.04	59.50
423	7	1	37	3.85	1.95	3.00	4.10	9.80	0.50	23.65	7.15	0.60	2.90	2.29	0.83	53.90
25	8	1	63	7.30	2.15	5.30	4.45	7.85	0.60	22.35	6.30	0.30	5.20	2.19	0.95	54.75
26	8	1	60	5.10	2.55	4.35	3.65	8.55	0.35	23.45	7.95	0.25	3.55	1.76	0.55	54.90
29	8	1	54	4.05	3.40	3.50	3.85	6.85	0.70	23.30	6.10	0.85	5.60	1.54	0.43	54.40
30	8	1	62	8.20	3.05	5.85	5.30	6.70	0.95	20.90	6.90	0.70	6.05	2.24	0.60	56.65
34	8	1	60	5.95	2.40	4.50	3.95	11.80	1.10	21.45	6.20	0.85	3.05	2.45	0.37	56.15
35	8	1	57	4.15	2.15	3.80	3.90	9.15	0.60	23.05	5.50	0.65	4.95	1.95	0.71	54.00
36	8	1	56	10.00	2.65	8.10	4.60	11.05	1.60	19.05	7.20	0.40	4.05	2.50	0.87	58.95
38	8	1	51	6.85	2.25	5.20	2.60	9.95	0.40	23.40	4.90	0.70	5.85	2.42	0.70	55.50
39	8	1	57	4.50	2.90	3.70	5.10	7.70	0.70	23.05	4.25	1.00	4.75	1.63	0.44	57.40
40	8	1	59	4.75	2.65	3.85	3.40	8.55	0.45	22.45	7.25	0.60	3.75	1.90	0.71	53.20
42	8	1	57	5.00	2.95	5.00	4.20	8.40	0.90	22.95	6.05	0.60	7.35	2.08	0.62	58.65
353	16	1	44	3.80	2.20	4.50	3.20	5.80	0.25	24.35	9.20	0.60	3.05	2.21	0.61	53.40
2	8	2	60	4.00	2.80	3.65	4.10	4.95	0.65	24.95	10.00	0.30	4.45	1.18	0.16	56.10
4	8	2	64	7.65	3.20	5.55	3.80	10.30	0.70	21.15	5.75	0.50	5.35	2.22	0.50	56.55
13	8	2	62	4.55	3.00	4.55	3.00	8.35	0.15	19.05	8.40	0.40	5.40	1.72	0.48	51.05
22	8	2	62	4.95	2.65	3.85	4.35	7.10	0.40	23.90	8.85	0.25	4.05	1.61	0.59	55.85
27	8	2	45	4.05	2.90	4.55	3.25	8.60	0.45	22.95	4.35	0.40	7.90	1.71	0.50	55.60
28	8	2	60	5.25	3.70	3.55	2.85	7.55	0.45	22.25	8.10	0.60	3.65	1.56	0.44	52.45

†Area/Hospital code number - see p. 158

‡Controls = 1, Cases = 2.

MRC no.	AREA	TYPE	AGE	T	L	H + 18:3	14:0	18:2	10:0 + 12:0	16:0	16:1	17:0 br.	18:0	18:1t	16:1t	18:1
85	12	1	52	6.15	1.70	3.90	2.85	10.15	0.60	21.90	5.60	0.75	4.25	3.46	0.71	51.95
86	12	1	58	6.10	2.70	4.95	4.65	7.10	0.70	22.75	5.10	0.75	6.90	3.64	0.75	55.85
2008	9	1	63	5.10	2.85	4.90	3.65	6.95	0.45	20.95	8.90	0.50	3.75	2.18	0.76	53.15
94	12	1	54	6.95	2.90	5.00	3.95	7.45	0.55	22.00	7.35	0.55	5.25	3.28	0.84	55.25
95	12	1	55	5.50	2.60	4.60	3.25	4.60	0.35	23.95	9.00	0.35	3.85	2.32	0.79	52.80
100	12	1	58	3.80	3.25	4.50	3.35	8.45	0.40	22.85	7.65	0.70	4.95	2.30	0.44	56.35
452	13	1	37	4.85	2.40	3.95	3.90	8.15	0.35	24.75	8.45	0.55	3.45	2.96	0.80	56.20
178	10	2	52	5.70	3.35	4.75	3.35	8.85	0.50	22.70	6.40	0.60	4.05	2.87	0.77	54.80
456	13	1	62	5.70	2.35	4.45	5.10	7.70	1.25	21.40	6.05	0.40	7.30	3.54	0.74	56.25
460	13	1	54	4.15	2.80	3.95	3.70	10.75	0.60	22.55	6.30	0.70	4.80	3.05	0.83	56.40
1021	19	1	55	8.10	2.55	5.70	3.65	8.50	0.45	23.35	6.15	0.60	5.00	2.88	0.85	56.20
1023	19	1	59	9.55	3.15	5.60	4.00	11.30	0.65	19.70	6.50	0.35	5.05	4.35	0.89	56.55
1024	19	1	58	5.10	2.45	3.25	2.85	8.95	0.35	22.85	6.80	0.60	3.75	2.65	0.58	52.10
1025	19	1	54	6.00	2.75	4.05	3.70	8.05	0.55	22.20	6.60	0.65	4.70	2.34	0.54	53.50
1353	19	1	57	4.70	1.65	4.20	2.75	4.20	0.30	23.20	12.80	0.60	2.40	1.69	0.63	52.35
1357	19	1	57	5.70	1.50	3.60	3.65	6.75	0.60	23.95	7.10	0.60	5.50	2.43	1.07	53.50
1358	19	1	42	6.95	1.65	6.95	3.85	7.45	0.95	21.05	6.10	0.60	6.45	2.88	0.87	55.30
1364	19	1	54	4.55	1.65	3.35	3.40	6.80	0.35	24.35	9.65	0.60	3.90	1.22	0.56	54.30
1365	19	1	48	4.50	2.00	5.60	3.70	6.30	0.55	25.00	4.75	0.60	5.55	2.55	1.15	54.30
1002	19	2	62	7.95	2.50	5.25	4.80	6.70	1.00	23.75	6.50	0.60	5.05	2.83	1.04	56.40
1006	19	2	57	5.15	2.60	4.90	4.30	11.50	0.10	20.10	5.85	0.60	6.30	2.68	0.33	56.50
1009	19	2	44	4.40	2.50	3.30	3.20	8.45	0.60	20.80	7.05	0.80	5.10	1.62	0.31	52.05
1010	19	2	49	3.45	1.95	3.25	3.50	4.65	0.65	24.30	9.40	0.60	3.75	1.51	0.53	52.30
1013	19	2	35	4.35	2.90	4.05	4.55	8.50	0.90	21.80	5.60	0.60	5.95	2.20	0.50	55.10
1014	19	2	52	4.10	1.60	3.70	2.10	10.90	0.25	23.45	4.45	0.50	7.80	1.99	0.78	55.00
1016	19	2	47	4.95	2.40	4.40	4.00	8.10	0.95	22.30	6.25	0.60	6.25	2.39	0.79	55.50
1018	19	2	48	2.80	2.00	2.85	2.45	7.45	0.25	22.30	10.85	0.60	2.60	1.53	0.61	51.60
1352	19	2	64	3.10	1.60	3.35	2.20	4.00	0.20	19.25	12.45	0.60	2.30	1.39	0.47	46.20
1355	19	2	58	7.80	2.05	7.20	5.80	6.25	2.25	23.75	6.75	0.60	4.75	2.79	0.81	59.65
1356	19	2	52	7.70	2.75	6.80	3.65	6.70	0.40	23.85	4.10	0.60	6.05	2.40	0.86	55.15
1078	15	1	36	3.20	2.00	3.30	3.10	8.05	0.25	22.50	8.85	0.85	3.60	1.49	0.60	52.75
1081	15	1	57	6.70	2.40	6.45	3.70	9.55	0.80	19.95	5.85	0.60	6.45	2.27	0.62	56.00
1087	15	1	58	4.20	2.45	3.85	3.95	7.20	0.40	24.10	7.35	0.60	3.50	1.62	0.82	53.65
1089	15	1	48	5.40	2.80	3.40	6.10	7.60	1.30	25.05	4.90	0.65	6.35	2.85	0.83	58.40
1092	15	1	33	4.75	2.85	4.00	2.60	11.70	0.10	22.75	6.90	0.85	3.95	1.83	0.80	55.95
1093	15	1	44	6.35	2.25	5.25	4.05	7.20	0.95	21.30	6.80	0.60	6.00	2.35	0.62	54.65
1094	15	1	47	4.10	2.95	2.80	3.90	8.90	0.80	20.35	6.45	0.60	6.20	1.90	0.48	53.20
1099	15	1	52	5.35	2.85	3.55	3.50	7.90	0.50	26.20	5.20	0.60	7.10	1.94	0.73	57.65
352	16	2	66	6.55	1.70	6.90	4.05	7.75	0.55	23.20	5.10	0.60	7.75	3.25	0.77	57.85
358	16	2	45	6.40	3.35	5.50	4.05	11.15	0.85	19.05	7.10	0.25	5.55	2.78	0.59	57.10
366	16	2	66	4.85	3.10	3.80	3.75	7.80	0.45	19.25	6.35	0.70	4.30	3.16	0.79	49.75
369	16	2	46	4.85	4.15	3.90	4.45	6.50	0.60	21.85	6.45	0.90	4.70	2.89	0.60	53.75
370	16	2	57	6.30	2.95	6.05	4.80	7.10	0.95	22.80	8.50	0.60	4.40	2.69	0.75	58.40
372	16	2	43	6.20	3.95	3.90	5.05	7.10	0.70	24.25	6.85	0.60	4.50	3.12	0.92	57.15
1207	16	2	63	3.65	3.35	4.00	3.90	9.60	0.85	21.70	6.75	0.60	4.40	2.45	0.71	55.40
1211	16	2	33	4.30	2.15	3.65	4.35	6.75	0.60	22.60	6.90	0.60	6.15	2.45	0.66	54.00
1215	16	2	53	4.85	3.55	3.95	4.55	6.45	0.60	21.50	7.90	1.15	5.05	2.94	0.67	54.95
1220	16	2	58	7.40	2.55	5.65	3.65	10.45	0.45	26.35	5.00	0.55	4.25	3.52	0.89	59.15
331	16	2	52	4.90	3.95	3.30	4.20	8.10	0.85	21.55	8.80	0.95	5.50	3.66	0.90	57.45
167	10	1	47	4.15	2.25	4.00	4.45	8.05	0.50	23.90	8.00	0.60	5.35	2.60	1.01	57.35
1150	13	2	33	4.80	3.55	4.20	4.50	7.50	1.05	21.85	8.40	0.35	4.55	3.14	0.92	56.20
195	10	1	38	4.30	2.30	4.35	5.15	6.60	1.45	23.50	7.55	0.25	5.25	2.72	0.96	56.65
1064	15	2	49	3.85	3.50	4.10	4.40	8.65	0.85	21.60	5.60	0.75	5.60	1.48	0.37	55.50
166	10	1	47	4.10	2.85	3.85	4.65	6.35	0.85	22.20	8.80	0.60	5.35	2.27	0.69	55.75
189	10	1	46	4.55	2.85	4.35	3.70	10.30	0.55	22.70	5.75	0.60	4.90	2.39	0.74	55.95
1077	14	2	51	3.50	2.20	3.85	2.75	15.75	0.40	21.10	3.65	0.60	6.30	1.75	0.59	56.85
3001	9	1	63	4.60	3.00	3.40	3.75	6.15	0.25	23.15	6.95	0.60	4.55	2.22	0.68	52.05
1084	14	2	45	5.95	2.00	4.20	3.35	7.35	0.35	22.10	6.80	0.60	5.15	2.32	0.85	52.15
322	11	2	61	5.35	2.55	6.05	3.90	5.90	0.50	20.75	5.90	0.60	6.70	2.39	0.70	53.10
1086	14	2	31	3.65	2.75	3.25	3.15	8.30	0.55	23.45	6.00	0.75	6.00	1.47	0.31	54.45
23	8	1	59	5.25	3.90	4.00	4.50	6.95	0.70	23.20	9.35	0.75	4.10	1.64	0.71	57.70
1096	14	2	64	8.10	2.55	6.75	3.70	9.05	0.60	19.15	6.30	0.60	6.40	2.49	0.84	55.35
1097	14	2	32	6.45	1.80	5.30	2.80	7.60	0.45	22.00	8.05	0.60	6.85	1.99	0.66	53.70
1098	14	2	36	3.45	2.35	3.75	2.85	8.70	0.60	21.35	6.95	0.55	4.90	1.65	0.63	52.25
1063	15	1	51	3.35	1.80	3.10	3.10	8.15	0.50	22.10	4.55	0.70	7.45	1.96	0.58	51.70
1066	15	1	48	6.90	2.95	4.25	4.80	9.10	0.55	25.35	5.45	0.60	6.40	2.92	0.92	59.70
1070	15	1	44	6.45	1.70	5.60	3.20	4.90	0.65	23.75	7.75	0.60	4.80	2.31	0.91	53.20
1072	15	1	43	4.25	1.70	4.20	3.30	4.55	0.45	23.00	8.45	0.60	4.10	1.68	0.68	50.60
1075	15	1	40	5.35	1.60	5.40	3.75	11.25	0.70	22.80	4.90	0.60	5.90	2.51	0.67	57.15
364	16	1	59	3.60	3.20	3.95	3.75	7.25	0.45	23.30	8.70	0.60	3.65	2.46	0.56	55.10
367	16	1	60	7.35	2.50	6.00	3.85	6.65	0.55	21.95	7.75	0.60	4.80	3.46	0.93	54.90
374	16	1	57	5.75	2.05	4.75	4.15	9.55	0.65	23.35	5.05	0.60	5.55	3.35	0.70	55.95
1209	16	1	44	4.90	2.10	3.50	3.25	10.80	0.55	22.60	7.50	0.50	3.70	2.70	0.70	54.75
1210	16	1	51	6.05	2.75	3.30	4.55	14.00	0.65	20.75	5.50	0.75	5.35	3.25	0.60	57.85
1223	16	1	49	2.95	2.85	3.00	2.75	7.10	0.25	22.30	9.70	0.60	3.90	2.72	0.79	52.90
446	18	2	45	8.35	3.20	7.15	5.15	7.10	1.25	21.75	6.30	0.60	5.75	2.96	0.74	58.50
1119	18	2	64	6.90	1.85	7.15	4.05	6.80	0.60	21.50	7.50	0.60	4.30	3.08	1.05	54.60
439	18	1	61	11.60	2.60	8.50	3.90	9.75	0.60	19.75	6.00	0.60	5.15	4.34	1.24	57.10
1103	18	1	58	4.20	2.70	4.25	2.40	6.70	0.20	21.25	8.40	0.60	3.35	1.95	0.51	50.10
1124	18	1	55	4.20	2.30	4.85	3.05	8.70	0.40	22.75	6.30	0.60	4.35	2.18	0.66	53.55
1125	18	1	44	5.80	2.60	5.00	3.60	10.60	0.55	19.60	7.50	0.60	4.65	3.93	1.00	54.95
4000	13	2	64	8.70	3.65	4.95	4.10	8.15	0.70	20.55	8.40	0.80	4.60	3.77	1.16	56.15
1003	19	1	55	9.10	1.80	6.35	3.45	10.55	0.50	22.05	5.20	0.60	5.80	3.01	1.55	56.55
1005	19	1	54	7.90	2.10	6.75	3.55	9.05	0.80	20.85	7.05	0.60	5.45	2.44	1.14	56.45
1011	19	1	60	3.80	1.95	3.30	4.15	6.25	0.70	23.65	9.40	0.50	3.85	1.40	0.46	54.00
1012	19	1	49	3.20	2.90	3.20	5.00	4.40	0.55	25.50	8.35	0.60	5.10	1.46	0.57	55.85
1015	19	1	60	5.60	2.45	4.55	7.15	6.40	0.95	23.75	5.50	0.60	5.65	2.35	0.79	57.65
1126	13	2	53	4.70	2.65	2.95	3.85	9.05	0.65	22.75	8.05	0.85	4.10	2.60	0.61	55.35

MRC no.	AREA	TYPE	AGE	T	L	H + 18:3	14:0	18:2	10:0 + 12:0	16:0	16:1	17:0 br.	18:0	18:1t	16:1 t	18:1
1020	19	1	48	6.40	2.65	4.60	4.55	8.40	0.55	24.90	5.00	0.60	6.25	2.72	0.68	57.75
937	12	2	64	9.00	2.45	6.40	3.25	7.70	0.50	20.85	7.10	0.60	4.35	3.86	1.16	53.45
370	4	2	60	2.45	2.35	5.15	3.05	5.65	0.35	22.20	10.05	0.60	4.05	1.08	0.36	53.70
400	4	2	61	4.55	3.15	3.85	3.60	7.70	0.35	21.05	8.55	0.65	4.45	1.61	0.51	53.60
731	5	2	61	4.85	2.55	3.70	4.20	6.55	0.45	25.60	5.20	0.60	5.65	2.11	0.55	54.75
734	5	1	57	7.40	1.85	6.00	2.55	11.10	0.50	20.80	6.80	0.60	3.60	2.33	0.87	54.05
735	5	2	60	3.25	2.35	3.45	3.35	12.80	0.25	23.00	4.60	0.60	4.50	1.69	0.62	55.15
976	6	2	49	3.30	2.25	3.15	3.70	9.75	0.50	24.50	6.40	0.60	4.40	1.99	0.53	55.50
981	6	2	57	4.50	1.95	4.30	2.85	7.20	0.30	23.90	8.40	0.60	4.05	2.00	0.66	53.80
302	11	1	45	3.30	1.55	3.95	3.15	7.60	0.65	25.20	5.45	0.60	5.70	1.63	0.65	54.10
1	8	1	46	5.10	2.80	3.85	3.30	8.30	0.40	24.30	6.45	0.40	4.80	1.88	0.44	54.85
3	8	1	55	5.30	3.05	3.80	4.90	7.30	0.65	25.95	5.15	0.55	6.30	1.93	0.47	57.90
7	8	1	63	4.35	2.90	4.00	3.05	8.95	0.55	21.70	7.15	0.45	4.45	1.65	0.36	53.45
305	11	1	56	3.35	2.00	5.10	3.40	7.20	0.30	24.65	8.30	0.60	3.25	2.63	0.99	55.05
9	8	1	61	7.20	2.55	4.80	4.35	8.75	0.60	22.95	6.90	0.25	4.60	1.99	0.76	56.00
10	8	1	51	4.40	3.95	3.00	4.90	6.30	0.65	24.20	8.30	0.80	4.45	1.63	0.44	56.80
11	8	1	60	4.65	2.70	4.05	4.80	5.75	1.00	23.55	7.90	0.40	6.10	1.56	0.41	56.50
14	8	1	56	4.20	3.00	4.05	2.80	6.60	0.20	20.20	7.75	0.75	5.15	1.66	0.50	50.75
16	8	1	56	2.50	1.15	4.15	1.70	9.95	0.20	19.85	6.35	0.15	4.90	0.97	0.24	48.65
312	11	2	32	4.55	1.65	3.10	3.30	13.05	0.80	22.10	5.40	0.60	6.90	3.30	0.94	57.15
19	8	1	56	9.80	2.10	5.50	3.10	7.95	0.35	18.80	7.45	0.50	4.25	3.62	1.14	50.25
21	8	1	56	3.15	1.85	3.25	3.40	8.00	0.15	21.35	9.65	0.85	4.40	1.13	0.29	53.15
1360	19	2	56	6.35	1.60	5.85	3.35	6.30	0.45	19.35	7.40	0.60	5.65	1.91	0.78	50.80
1363	19	2	41	5.60	2.30	4.10	3.60	11.70	0.65	23.10	4.05	0.60	6.85	1.84	0.65	57.20
1367	19	2	35	5.70	2.65	6.30	3.15	8.55	0.50	20.25	5.25	0.60	5.60	2.60	0.52	53.10
319	11	2	40	3.45	2.75	3.65	4.40	6.30	0.50	24.90	5.70	0.60	6.30	2.30	0.58	55.35
1372	19	2	54	6.55	3.35	5.10	5.10	5.65	0.80	21.75	5.80	0.60	6.60	2.69	0.58	55.00
1373	19	2	51	4.55	2.00	4.10	3.45	5.35	0.45	20.25	10.50	0.60	2.20	1.62	0.72	49.15
1138	13	1	41	4.50	3.45	3.45	3.50	7.35	0.90	23.55	7.85	0.90	4.05	2.21	0.34	55.25
1140	13	1	57	7.95	2.45	5.80	5.05	6.45	0.85	22.70	6.50	0.60	6.75	4.35	1.19	57.40
1152	13	1	60	6.20	1.70	5.30	3.50	9.45	0.65	25.85	3.85	0.60	7.10	3.70	1.03	58.25
1155	13	1	58	4.15	2.30	4.55	3.30	7.15	0.35	24.10	6.95	0.60	4.90	3.03	0.80	54.45
1158	13	1	59	8.75	2.85	6.20	4.30	7.75	0.70	20.45	7.60	0.60	4.65	4.00	1.15	55.35
1163	13	1	59	4.80	2.10	3.30	3.15	7.85	0.30	22.05	9.50	0.65	4.00	2.74	0.77	53.15
310	11	1	58	7.30	2.65	5.70	6.05	6.10	1.95	21.20	6.90	0.60	6.70	2.06	0.61	58.10
315	11	1	45	3.15	1.85	4.50	3.85	7.30	0.35	22.60	7.90	0.60	4.35	1.80	0.59	53.55
324	11	1	53	5.60	3.65	5.35	4.55	10.20	0.75	20.20	6.00	0.40	5.50	2.75	0.67	56.85
53	12	2	55	6.50	2.50	5.70	4.85	5.60	0.70	21.10	6.20	0.40	6.15	4.31	1.04	53.45
68	12	2	62	5.30	2.20	4.60	5.25	5.75	1.05	22.65	8.85	0.35	6.65	3.35	0.84	57.60
1091	14	1	49	4.45	2.30	4.10	3.70	9.25	0.30	22.25	8.30	0.60	3.20	1.77	0.82	54.25
459	13	2	35	6.20	3.05	5.65	3.75	7.15	0.55	23.15	6.00	0.60	6.50	3.43	1.08	56.65
467	13	2	35	5.90	3.10	5.10	4.50	7.75	1.00	22.95	8.50	0.65	5.05	2.66	0.62	58.85
207	9	2	33	5.20	2.80	4.20	5.15	9.25	1.15	23.65	5.90	0.60	6.05	2.36	0.76	59.00
468	13	2	57	4.65	2.40	3.40	3.85	14.50	0.65	22.35	5.00	0.65	5.55	3.39	0.74	58.60
471	13	2	44	5.15	3.15	3.35	4.70	8.85	0.65	24.55	7.30	0.75	3.95	2.59	0.82	57.50
210	9	2	63	5.65	2.55	5.10	3.90	9.75	0.75	24.30	7.10	0.45	4.80	2.76	0.96	58.95
223	9	2	34	2.85	2.50	3.80	2.55	6.25	0.30	19.55	11.90	0.45	2.55	0.87	0.54	50.10
180	10	2	56	4.10	3.65	4.25	4.55	6.15	0.90	21.55	9.45	0.60	4.05	2.63	0.79	55.40
182	10	2	53	4.45	3.60	3.30	4.30	7.15	1.05	21.05	8.40	0.70	5.90	2.41	1.22	55.70
31	8	2	53	4.90	2.20	3.65	3.25	7.70	0.30	25.05	6.00	0.45	5.30	1.91	0.57	54.15
33	8	2	63	7.70	3.00	5.45	3.75	8.65	0.70	19.10	9.15	0.60	3.05	2.21	0.60	53.70
45	8	2	64	5.05	2.90	4.65	3.50	6.20	0.55	21.00	7.15	0.60	5.00	1.70	0.41	51.60
46	8	2	54	5.60	1.90	4.40	4.05	6.30	0.45	25.05	4.90	0.60	4.80	1.87	0.58	52.70
1324	8	2	65	4.75	2.55	4.60	3.20	5.20	0.40	20.20	10.55	0.60	4.40	1.41	0.41	51.95
1501	8	2	38	4.50	3.55	3.85	3.95	7.45	0.70	22.60	8.30	0.60	6.10	1.61	0.37	57.35
351	16	1	54	5.90	2.00	5.15	3.50	7.70	0.55	23.60	8.05	0.60	3.95	2.51	0.61	55.35
1503	8	2	51	3.45	2.50	2.95	4.60	8.00	2.50	20.30	5.10	0.60	7.35	1.32	0.21	54.15
354	16	1	46	5.30	1.85	4.20	2.80	7.50	0.20	24.25	8.95	0.60	3.70	2.50	0.86	54.30
1523	8	2	41	6.05	1.85	4.80	2.85	13.05	0.65	24.90	3.35	0.60	6.15	2.12	0.47	58.45
359	16	1	48	10.05	2.75	7.15	3.50	11.30	0.40	20.55	7.30	0.55	3.85	3.65	1.18	57.60
360	16	1	54	4.20	3.40	3.15	4.50	5.10	0.70	23.70	10.15	0.80	4.05	2.11	0.72	55.90
361	16	1	58	4.90	3.10	4.60	3.15	7.10	0.50	22.05	9.10	0.55	3.80	2.30	0.59	54.20
362	16	1	37	4.55	3.05	3.10	3.65	8.25	0.60	23.70	8.95	0.70	3.10	2.37	0.71	55.35
1529	8	2	44	7.95	1.80	6.45	3.55	11.10	0.80	20.40	5.90	0.60	4.95	2.56	0.80	55.80
2006	9	1	62	3.90	3.85	3.90	5.00	4.80	0.55	25.80	9.40	0.60	4.70	2.51	0.92	58.85
203	9	2	44	3.85	2.15	5.10	2.95	8.60	0.25	22.55	6.75	0.50	4.45	1.44	0.41	53.55
205	9	2	62	5.95	3.75	4.70	4.95	7.10	0.85	22.00	7.25	0.60	5.25	2.24	0.84	56.70
363	16	1	63	5.75	2.75	5.15	4.35	6.60	0.65	24.20	6.10	0.60	5.55	2.88	0.79	56.20
93	12	2	47	3.10	1.65	4.10	2.70	3.70	0.25	22.50	11.80	0.45	2.75	1.59	0.64	50.15
98	12	2	50	7.50	2.25	5.20	2.95	9.00	0.30	23.95	5.45	0.80	5.90	3.52	0.85	56.05
51	12	1	50	3.40	1.95	4.10	3.15	3.85	0.20	21.65	10.80	0.55	4.10	2.26	0.79	50.80
2010	9	1	63	9.45	3.00	6.70	5.65	3.60	0.90	24.00	9.50	0.25	6.10	3.87	1.39	59.95
54	12	1	60	6.75	2.15	5.75	3.40	8.15	0.35	21.70	7.05	0.45	4.55	3.73	1.05	53.80
56	12	1	40	4.05	2.50	4.80	3.55	6.15	0.20	22.60	11.05	0.60	3.45	2.44	1.13	55.15
61	12	1	43	6.45	2.50	4.20	3.10	6.10	0.45	21.85	9.80	0.65	3.35	2.72	0.90	52.05
69	12	1	54	5.70	2.90	4.60	4.15	8.90	0.45	23.10	6.55	0.25	4.90	3.35	0.73	56.05
76	12	1	58	6.10	2.90	4.15	4.35	7.55	0.45	26.10	5.50	0.65	5.90	3.31	1.02	57.90
81	12	1	61	4.00	2.60	3.55	3.20	7.80	0.30	23.80	9.20	0.60	2.70	2.51	0.72	54.00

Code Number	AREA/HOSPITAL	STUDY AREA/CONURBATION
1	Carmarthen	West Wales
2	Llanelli	
3	Aberystwyth	
4	Winchester	South/East England
5	Folkstone	
6	Orpington	
7	Poole	
8	Luton and Dunstable	Luton and Dunstable
9	Cardiff	South East Wales
10	Bridgend	
11	Church Village	
12	Walton	Liverpool
13	Chatham	London
14	Huddersfield	Huddersfield and Halifax
15	Halifax	
16	Ashton-under-Lyne	Manchester 1
17	Altrincham	
18	Ancoats	Manchester 11
19	Bradford	Bradford

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