Concentration of 18:1 and 16:1 transunsaturated fatty acids in the adipose body tissue of decedents dying of ischaemic heart disease compared with controls: analysis by gas liquid chromatography

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SUMMARY Proportions of “lower” 16:1 and 18:1 trans acids (T_L) and “higher” C_{20} and C_{22} trans acids (T_H) in samples of depot fat taken at postmortem examination from 136 people who had died of ischaemic heart disease (cases) and from those who had died of unrelated causes (controls) have been determined. Whereas mean percentages 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. We conclude, 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 a lower proportion of ruminant fat than did the controls.

We have previously shown^{1–3} that samples of body fat taken from people who had died of ischaemic heart disease (cases) when compared with controls who had died of other causes had lower mean values of certain odd-number and branched-chain acids (L) characteristic of ruminant-animal fat and higher mean values of total transunsaturated fatty acids (T). There was no difference in the mean values of certain “higher (C_{20} and C_{22} mostly monoenoic) acids” (H). The ratio T/L was significantly higher in the cases than the controls and increased linearly with increases in H in both cases and controls.

We assumed that the similar mean values of H implied that the mean values of “higher trans acids” (T_H) would be similar and that these higher trans acids were not a hazard. Consequently, we were driven to the hypothesis that the observed differences in T and in T/L were due to a difference in “lower trans acids” (T_L)—that is, in 18:1 and 16:1 trans acids—and that any risk attached preferentially to T_L .

We can now report on direct measurements of T_L by gas-liquid chromatography using a more recently developed highly polar liquid phase.

Methods

The methyl esters were those used previously¹ that

were derived from adipose tissue specimens collected over the period 1976–8. The methyl esters (ca 100 mg per sample) were stored in CS_2 solution in sealed containers at $-20^\circ C$ in the presence of tertbutyl hydroquinone antioxidant. Remeasurement of properties (T by infrared spectroscopy, GLC on EGSS-Y polyester) of several 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. Chromatograms were read by laboratory personnel with no knowledge of which samples were cases and which controls.

Fatty-acid compositions were determined with a Perkin-Elmer Model F17 gas chromatograph fitted with dual flame ionisation detectors. The columns were of stainless steel, 40 ft by $\frac{1}{8}$ in diameter, packed with 20% OV-275 (Supelco Inc) coated on 100/120 mesh acid-washed and silanised Chromosorb P.⁴ To eliminate baseline drift all measurements were conducted isothermally using pairs of matched columns. The column temperature was $220^\circ C$; carrier gas, N_2 , flow rate 30 ml/min; optimum ester loading $2 \mu l$ of CS_2 solution (13 mg/ml). Under such conditions, the retention time of 18:1 cis was 60 minutes from the solvent peak.

Slight adjustments in carrier-gas flow were periodically made to keep the retention time of this major peak within narrow limits. Column performance was monitored by periodic analysis of a standard mixture of the methyl esters.

We analysed seven brands of butter and all exhibited five distinct peaks between 16:0 and 18:0 which we identified as follows:

(a) An amount of authentic 17:0 anteisomethyl ester was added to a sample of butter fat esters and the mixture injected on the column; that peak whose area increased relative to others was taken to be 17:0 anteiso. A similar procedure was adopted for other methyl esters.

(b) By determination of equivalent chain lengths (ECL)^{5,6} compared with published values.

We thereby identified the five peaks as:

- Peak 1 17:0 iso, ECL = 16.40
- Peak 2 16:1 trans and 17:0 anteiso of identical ECL = 16.73
- Peak 3 17:0, ECL = 16.99
- Peak 4 16:1 cis, ECL = 17.18
- Peak 5 17:1, ECL = 17.44

This was further confirmed by catalytic hydrogenation,⁷ which resulted in complete disappearance of peaks 4 and 5 and reduction in area of peak 2 by a factor on average ca 2/3.

In those margarines containing hydrogenated marine oil and also in the adipose tissue specimens amounts of 16:1 cis substantially exceeded the amount present in butter fat. In such specimens the 16:1 cis peak broadened and included 17:0 and 17:1 as "shoulders." The ECL values of these components remained, however, such that the *height* of the peak was determined by 16:1 cis only. In specimens with the lowest values of 16:1 trans the baseline separation between peak 2 and the now adjacent large composite 16:1 cis peak was not always achieved. For this reason—and also for strict comparability with the previously derived values of total trans acids¹—percentages 16:1 trans plus 17:0 anteiso were evaluated by measuring appropriate peak heights and retention times.⁸ We labelled the product of peak height and retention time for 16:1 cis as x and that for the 16:1 trans plus 17:0 anteiso peak as y which gives $(\% 16:1 \text{ trans} + \% 17:0 \text{ anteiso}) / (\% 16:1 \text{ cis}) = y/x$.

By rearrangement we get:

$$\begin{aligned} \% 16:1 \text{ trans} &= R(\% 16:1 \text{ cis} + \% 16:1 \text{ trans}) \\ &\quad - (1-R)(\% 17:0 \text{ anteiso}) \\ &= R(\% 16:1 - (1-R)(\% 17:0 \text{ anteiso})) \end{aligned} \quad (1)$$

in which $R = y/(x+y)$. To evaluate the percentage 16:1 trans from this equation, we used the percentage

values of 16:1—combined cis and trans isomers—as previously obtained¹ by use of a EGSS-Y column for 124 specimens (75 cases, 49 controls).

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

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

in which combined percentages of 16:1 and 17:0 br were those previously obtained.¹

Like the other odd-number and branched-chain components of L, the branched-chain C₁₇ acids in adipose samples derive essentially from ruminant-animal fat, and we found accordingly that in the 124 specimens (75 cases, 49 controls) the ratio 17:0 br/L was sensibly constant at 0.22 for both case and control samples. Furthermore, analysis of the seven butters previously mentioned showed that 60% of 17:0 br is 17:0 iso—a proportion that agrees well with that reported by Jensen *et al*⁹ (61%). Equations 1 and 2 therefore finally become

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

$$\begin{aligned} \text{and} \\ \% 16:1 \text{ trans} &= R(\% 16:1 + \% 17:0 \text{ br}) \\ &\quad - (0.09 + 0.13R)L \end{aligned} \quad (4)$$

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 + 17:0 br from EGSS-Y chromatograms as previously reported. 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. Likewise for equation 4.

The 18:1 trans peak showed good but not complete base-line separation from the larger 18:1 cis peak. In view of the well-known difficulty of quantifying by area a smaller peak followed by a larger peak, 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 measured¹ on the EGSS-Y column.

Results

Table 1 records the mean of 18:1 trans, 16:1 trans, T_L, L, and T_L/L for cases and controls in each area of the study. Cases have lower mean values of L in nine of the 10 areas, higher values of T_L in eight areas, and higher values of T_L/L in nine 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—hydrogenated marine oil being the major source of trans acids—16:1 trans and 18:1 trans levels are clearly correlated.

The mean age at death of the cases was 52.7 and of the controls 51.5 years. We calculated the regression coefficients on age for 16:1 trans, 18:1 trans, and T_L/L which were + 0.003, + 0.010, and + 0.004 units a year respectively. We consider that the mean difference in age of 1.2 years would not bias any conclusions we might draw.

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 ($F = 17.7$ $p < 0.001$), 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, we used the method of weighted means of Pearce,¹⁰ and table 2 shows the differences in 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 analysis³ that the differences between cases and controls are significant for T_L but not for T_H .

Discussion

CASE CONTROL LEVELS OF LOWER TRANS ACIDS

The weighted mean value of T_H , as expected, is virtually identical with that of the controls. In contrast, mean percentages of 16:1 trans and 18:1 trans are higher for the case specimens by amounts

Table 1 Area mean values of L, 18:1 trans, 16:1 trans, T_L , and T_L/L , 136 cases (C), 95 controls (K)

Area		No of cases or controls	L	18:1 trans	16:1 trans	T_L	T_L/L
Luton and Dunstable	C	22	2.68	1.92	0.59	2.51	0.99
	K	15	2.70	1.78	0.48	2.26	0.90
West Wales	C	16	2.73	2.11	0.62	2.72	1.12
	K	9	2.83	1.97	0.55	2.52	0.96
South and East England	C	9	2.37	2.35	0.74	3.09	1.41
	K	6	2.43	1.75	0.54	2.28	0.97
Yorkshire and Humberside, hospital 1	C	14	2.33	2.10	0.72	2.82	1.26
	K	7	2.45	1.88	0.61	2.49	1.08
South-east Wales	C	17	2.57	2.38	0.82	3.20	1.29
	K	12	2.82	2.32	0.77	3.09	1.16
Yorkshire and Humberside, hospital 2	C	15	2.21	2.42	0.81	3.23	1.52
	K	16	2.30	2.12	0.62	2.75	1.23
Manchester conurbation, hospital 1	C	15	2.65	2.75	0.76	3.51	1.38
	K	11	3.16	2.99	0.77	3.76	1.27
London (Chatham)	C	12	2.69	3.37	0.88	4.25	1.68
	K	7	3.11	3.08	0.85	3.93	1.28
Manchester conurbation, hospital 2	C	4	2.55	3.10	0.85	3.95	1.55
	K	7	2.78	2.82	0.69	3.51	1.32
Liverpool conurbation	C	12	2.54	2.95	0.82	3.77	1.54
	K	5	2.21	3.33	0.91	4.24	1.88
All centres	C	136					
	K	95					

Table 2 Overall adipose tissue composition: weighted differences cases minus controls

Component trans acids	%	Differences			
		Mean differences	Standard errors	Student's <i>t</i>	<i>p</i>
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
$T_H^\ddagger$	2.222	0.0136	0.1551	0.09	NS
T_L/L	1.259	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 reference 1.

‡ $T_H = T - T_L$.

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 acid. For total lower trans acids T_L , the case mean is higher by an amount 0.21% at significance level $p \sim 0.025$.

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 sources of fat are not identical in structure.

Commercial hydrogenation of vegetable oils¹¹ gives rise not only to trans double bonds dispersed widely along the carbon chain, but also to a variety of positional cis isomers. According to Carpenter *et al*¹² about 30% of the cis 18:1 remaining consists of isomers with their (cis) bonds in a variety of positions other than the w9 position of natural oleic acid. Bearing in mind that 18:1 is the major acid in most margarines and cooking oils/fats, this 30% forms a substantial part of the whole product.

Hydrogenated marine oils also contain 18:1 and 16:1 positional isomers^{13 14}—possibly in amount greater than in hydrogenated vegetable oil.

In ruminant-animal fat the trans double bonds are more centrally placed, and randomisation of the cis bonds is far less pronounced,¹⁵ over 95% of the monoenoic content remaining in “natural” position.

Necessarily, therefore, the 16:1/18:1 trans acid content of hydrogenated fats is positively correlated with amounts of positional cis isomers. In ruminant-animal fats amounts of the latter will be very small in comparison.

Although we have no experimental evidence at present, it also seems likely from consideration of the manufacturing process that amounts of T_L from margarine to margarine will correlate positively with amounts of artificial antioxidants and possibly even with residual Ni catalyst. Addition of such additives to butters sold as such is prohibited. We prefer therefore to use 16:1 trans and 18:1 trans acids as “markers” indicative of certain types of fat without necessarily implicating any particular component acid or additive. In this respect it should be remembered that whereas 16:1 trans is highly characteristic of hydrogenated marine oils,³ only about 40% of 18:1 trans is contributed by hydrogenated fats, the remainder coming from ruminant-animal fat. In consequence amounts of positional isomers per unit amount of 16:1 trans will be considerably greater than amounts per unit amount 18:1 trans. Using lower trans acids as markers, therefore, it is not surprising that whereas

the 16:1 trans content of the case specimens is significantly higher than for controls, mean 18:1 trans levels are more variable and the case minus control difference is not significant.

RATIO OF LOWER TRANS ACIDS TO L AS A MEASURE OF RELATIVITY OF HYDROGENATED FATS TO RUMINANT-ANIMAL FAT

For zero intake of hydrogenated fats, the expected value of T_L/L for otherwise average United Kingdom dietary fat is ca 0.6. (This figure is somewhat lower than the previous figure³ for T_L/L , since we have found that in butter fat about 20% of the total trans content resides within the “higher acid” content.) Inclusion of hydrogenated marine or vegetable oils—of T_L/L values about eight and 60 respectively—will result in sharp increase in this ratio. In agreement with this estimate we have found that of the 231 specimens analysed, only six 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 is 1.175, the difference being significant at $p < 0.005$.

On the reasonable assumption that an amount of butter is replaced by margarine, but consumption of pig/poultry fat remains constant, this difference implies that the cases on average consume 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 hydrogenated marine oil carries a higher risk than hydrogenated vegetable oil containing much 18:1 trans but no 16:1 trans. Whereas this is undoubtedly a possibility, however, precise amounts of positional isomers and additives associated with these two types of hydrogenated fat are at present unknown. Furthermore, since, in fact, 16:1 trans and 18:1 trans are strongly correlated, we believe that it would be premature to apportion risk to the one more than to the other.

For the United Kingdom detailed knowledge relating to relative amounts of the various fatty components making up the total intake of dietary fat is available, and it is possible to calculate the composition of the average fatty diet in England and Wales. From such a calculation it has been shown¹ that the fatty-acid composition of such diet agrees remarkably well (subject only to body desaturation of 16:0 to 16:1 cis and 18:0 to 18:1 cis) with the average composition of 95 control samples of depot fat drawn widely from that population. Agreement extends to such minor compounds as odd-number and branched-chain acids (L) and trans acids. Furthermore, it was shown² that depot fat regional

variations in the ratio trans acids/L correlate satisfactorily with known dietary variations.

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

In addition to the observed within-area case v control differences, differences in area mean values of lower trans acids are of interest (table 1). Although precise comparison is clearly not possible, highest mean values of, for instance, 16:1 trans are found in those areas of highest mortality from ischaemic heart disease—that is, in urban conurbations; lowest mean values are found in South-east England—that is, in a region of lowest mortality from ischaemic heart disease. Intermediate values appertain for Yorkshire and Humberside standard region in agreement with its intermediate SMR.¹⁶ The position of industrialised South-east Wales (compared with the more rural West Wales with its traditional very high butter consumption and quite low SMR) may be of particular interest. Although household consumption of margarine and shortenings in this area is relatively low,¹⁶ it is possible that those hydrogenated fats of high T_L content are for some reason favoured, or that high additional amounts of hydrogenated fat are consumed by purchase and consumption of cakes, pastries, biscuits, etc, outside the private household.

Lastly, we considered the possibility that our case v 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. Medical advice in the light of current opinion, however, would surely have favoured those softer margarines described as “containing only pure vegetable oils.” Such materials would have had a very high content of polyunsaturated acids 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 about 2% only). Our results, however, show that amounts of 16:1 trans are *higher* for the cases, and

that levels of 18:2 are virtually identical for the case and control specimens. Again, the C₁₈-rich hydrogenated vegetable oils are characterised by exceptionally low values of the ratio (16:0 + 16:1)/(18:0 + 18:1) whereas butter fat is relatively rich in C₁₆ acids, but we have found² that the mean value of this ratio is significantly lower ($p < 0.01$) for the *control* group. Indeed, it seems more likely that risk is associated with those hydrogenated materials of relatively low 18:2 content—that is, with hydrogenated marine oils and vegetable oils hardened in greater degree. Both of these types are widely used in the manufacture of cakes, pastries, and biscuits; such items have high fat content and would surely not have been favoured by the dietitian.

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