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Eileen C. Albutt, Graham W. Chance

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### Research Article

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# Plasma and Adipose Tissue Fatty Acids of Diabetic Children on Long-Term Corn Oil Diets

EILEEN C. ALBUTT \* and GRAHAM W. CHANCE

*From the Institute of Child Health and the Children's Hospital, Birmingham, England*

**ABSTRACT** A method is described to determine the fatty acid composition of small samples of subcutaneous adipose tissue, and of fasting plasma free fatty acids (FFA) and triglycerides. These analyses were carried out on samples from five normal children, six diabetic children consuming a standard diabetic diet, 17 diabetic children prescribed a diet rich in corn oil since diagnosis 4-7 years ago, and 2 brothers with familial hypercholesterolemia on a corn oil diet for 3 yr. The results obtained showed that: (1) The composition of adipose tissue triglycerides in the diabetic children on a standard diet was similar to that in the normal children. (2) The 17 diabetic children were consuming different quantities of corn oil. (3) There was a highly significant correlation between the percentage of linoleic acid present in adipose tissue and in the fasting plasma FFA fraction. It is therefore concluded that future assessments of the adherence of these diabetic children to their corn oil diet will be possible by examination of the fasting plasma FFA fraction, obviating the need for repeated adipose tissue biopsies. (4) The sum of the concentration of saturated and monounsaturated fatty acids of the same chain length in adipose tissue was similar to that in the fasting plasma FFA fraction, even though the proportions of individual acids were different in the two fractions.

## INTRODUCTION

There is evidence of an association between high serum lipid levels and coronary artery disease (1, 2). The incidence of vascular degenerative disease is higher in patients with diabetes mellitus than in the general population (3), and hyperlipidemia is a feature of poorly

controlled juvenile diabetes (4). Thus the onset of vascular complications in diabetic children might be delayed by giving optimum insulin dosage to maintain normal serum lipid levels. However, since a diet rich in polyunsaturated fat also lowers serum lipid levels, the use of such a diet could serve as an additional controlling measure (5). For this reason, a diet rich in corn oil and corn oil products (6) has been prescribed to a group of diabetic children at Birmingham Children's Hospital since the time of diagnosis 4-7 yr ago. The children have been examined at regular intervals and their progress compared with that of a group of diabetic children treated similarly, yet consuming a standard diabetic diet (7, 8).

The polyunsaturated fatty acid linoleic acid (18:2)<sup>1</sup> comprises up to 60% of the fatty acids in corn oil. Hirsch, Farquhar, Ahrens, Peterson and Stoffel (9) suggested that since 18:2 is an essential fatty acid, the amount present in adipose tissue reflects the amount consumed over a period of years. Dayton, Hashimoto, and Pearce (10) have shown that after 5 yr on a corn oil diet, adipose tissue 18:2 correlates with adherence to diet.

In order to assess the adherence of our patients to their diet, we have analyzed the fatty acids present in adipose tissue triglyceride and also in fasting plasma free fatty acids (FFA) and triglycerides. The results obtained from these children, from diabetic children on a standard diet, and from normal children are reported in this paper. Data from two boys with hypercholesterolemia who have also been prescribed a corn oil diet is included.

## METHODS

*Children studied.* (A) Five normal children aged 7-12 yr with no family history of diabetes mellitus, coronary artery disease, or obesity. These children were under general anaesthesia for minor surgery at the time of biopsy; fasting blood was obtained before premedication.

\* This work forms part of a B. Sc. thesis (University of Birmingham, 1966).

Address requests for reprints to Miss E. C. Albutt, University of Birmingham, Institute of Child Health, Francis Road, Birmingham 16, England.

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<sup>1</sup> Number of carbon atoms: number of double bonds.

(B) Six insulin-treated diabetic children aged 8-17 yr consuming a standard diabetic diet. Duration of treatment and diet 4-7 yr.

(C) 17 insulin-treated diabetic children aged 7-19 yr consuming a corn oil diet. Duration of treatment and diet 4-7 yr.

(D) Two brothers aged 4 and 6 yr with familial hypercholesterolemia consuming a corn oil diet for 3 yr.

**Lipid extraction: adipose tissue.** A biopsy of buttock subcutaneous adipose tissue weighing 2-4 mg was obtained after an intradermal infiltration of 1% Procaine, by aspirating tissue from an area overlying the right iliac crest with a syringe and Menghini needle 1.6 mm in diameter. The tissue was washed with 0.85% sodium chloride, transferred to a small ground glass tissue grinder,<sup>2</sup> and ground briefly in 2 ml 2:1 v/v chloroform:methanol (11). The extract was transferred to a glass stoppered tube, the grinder washed with 2 ml solvent mixture, and the extracts pooled. After standing at room temperature for 30 min, cell debris was removed by centrifugation.

**Lipid extraction: plasma.** Venous blood, obtained with minimal stasis after an overnight fast, was mixed with solid lithium heparin and placed in an ice-water bath for 10 min before separation and extraction by two methods. (1) For triglyceride analysis, the total lipid was extracted by mixing 1 volume of plasma with 19 volumes of 2:1 chloroform:methanol (11) and standing at room temperature for 30 min. The protein precipitate was removed by filtration. (2) For FFA analysis, plasma was extracted by the method of Dole (12).

Water was also extracted to provide solvent blanks.

**Lipid group separation.** The lipids were isolated by ascending thin-layer chromatography on layers 250  $\mu$  thick prepared using a suspension of Merck silica Gel G in 0.01% rhodamine 6G. The layers were activated by heating at 100°C for 1 hr.

For triglyceride separation, 2 ml of plasma lipid extract (containing the triglyceride extracted from 0.1 ml plasma), 2 ml of blank extract, and 1 ml adipose tissue extract were each evaporated with nitrogen at room temperature. The residues were each dissolved in heptane and loaded on the thin layer under nitrogen. The triglycerides were isolated by ascending chromatography with dichloromethane as solvent.

After separation, the layers were viewed in ultraviolet light (wave length 366  $m\mu$ ) and the spots identified from the positions of known substances run adjacently. The triglyceride spots and appropriate blank areas were removed by aspiration of the silica gel into a microfilter funnel containing a sintered glass disc.<sup>3</sup> The triglycerides were eluted by adding diethyl ether to the funnel and allowing this to drip through into glass stoppered tubes. To minimize oxidation, 0.02  $\mu$ g 2:6 di-tert-butyl-p-cresol was added to each tube to give a concentration of approximately 50 parts antioxidant per million parts lipid (13). Small peaks in fatty acid positions were detected after gas chromatographic analysis of solvent blanks. In order to correct for these impurities, 5  $\mu$ g of heptadecanoic acid (as 0.1 ml of 5 mg/100 ml solution in heptane) was added at this stage to the tubes containing triglycerides and blanks.

For plasma FFA isolation, the 5  $\mu$ g of heptadecanoic acid was added to 2 ml Dole's extracts of plasma (containing the FFA from 0.5 ml plasma) or of water before thin-layer chromatography. The extracts were then concentrated, and loaded on a thin layer and separated using dichloromethane:

diethyl ether:acetic acid (70:30:0.1). In other respects the method of isolation was as described above.

**Methylation.** After solvent evaporation with nitrogen, 2 ml of methanol containing 2% v/v sulphuric acid and 0.5% v/v benzene was added, the tubes tightly stoppered, and heated at 60-65°C for 16 hr (14). 1 ml of water was added and the methyl esters extracted three times with 2 ml heptane. Approximately 150 mg of 4:1 w/w anhydrous sodium sulphate:sodium hydrogen carbonate was then added to dry and neutralize the extracts (15).

**Analysis.** The methyl esters were concentrated into a small volume of heptane and 1  $\mu$ l was analyzed with a Pye Argon Gas Chromatograph.<sup>4</sup> The stationary phase was 10% polyethylene glycol adipate on 100-120 mesh celite at 180°C, detector voltage 1250 volts with attenuation  $\times 3$ , and gas flow 45 ml/min. The peaks were identified by comparing their retention volumes with those of standard mixtures of fatty acids. Peak area was obtained by multiplying peak height by peak width at half the height, and the composition of the mixture calculated.

As a result of these sensitive gas chromatographic conditions, small impurity peaks in fatty acid positions were detected in the solvent blanks. The area of each fatty acid peak in the sample was therefore corrected for the presence of this impurity by subtracting  $(A \times B/C)$  where A = area of the 'fatty acid' peak in the blank, B = area of peak from 5  $\mu$ g 17:0 in sample, C = area of peak from 5  $\mu$ g 17:0 in blank.

Plasma and adipose tissue samples were not analyzed in duplicate. The standard deviations for each fatty acid in other samples that were analyzed in duplicate by this method were less than 0.8% for components present in quantities greater than 17% of total mixture, and less than 0.3% for components comprising less than 17% of total mixture. National Heart Institute fatty acid standards (mixtures B and D) were analyzed at intervals during the study. The results obtained agreed with the stated composition data with a relative error less than 5% for major components ( $> 13\%$  of total mixture) and less than 10% for minor components ( $< 13\%$  of total mixture).

Conditions of elution, methylation, and extraction were established by thin-layer chromatographic examination of products. The effect of the complete analytical procedure on the polyunsaturated fatty acid 18:2 was examined by analyzing solutions containing 5  $\mu$ g 17:0 and either 5 or 10  $\mu$ g 18:2. The area of the 17:0 peak was assumed to be equivalent to a recovery of 100% and, from the area of the 18:2 peak, the recovery of 18:2 was calculated as 100-105%, thus excluding loss of this fatty acid during the procedure. All analyses were completed within 3 days after obtaining the samples. Apparatus was cleaned with lipid solvents, chromic acid and distilled water where possible, and solvents were redistilled and stored over anhydrous sodium sulphate.

## RESULTS

The percentages of the main fatty acids found in each fraction in the four groups of patients are shown in Table I. Trace quantities of other fatty acids were detected, but these are not included. The percentages of each fatty acid in these three fractions in normal children and in the diabetic children on a standard diabetic diet were compared by the *t* test. No significant difference was found in the adipose tissue fatty acids in these two groups, but the mean percentage of 14:0 and 16:1 in the

<sup>3</sup> A. G. Gallenkamp & Co., Ltd. London, England.

<sup>2</sup> Baird and Tatlock Ltd., Chadwell Heath, Essex, England.

<sup>4</sup> Pye Unicam Ltd., Cambridge, England.

TABLE I  
Percentage Fatty Acid Composition\* of Lipid Fractions in Four Groups of Patients

Fraction	Group†	12:0	14:0	16:0	16:1	18:0	18:1§	18:2
Adipose tissue triglyceride	A	0.9 ±0.4	4.4 ±0.6	22.0 ±1.6	8.2 ±1.6	4.8 ±1.2	51.5 ±2.2	8.1 ±0.9
	B	0.5 ±0.3	4.0 ±0.2	22.0 ±1.5	8.9 ±1.7	3.5 ±1.1	53.7 ±2.5	7.5 ±2.0
	C	0.6 ±0.2	2.9 ±0.8	19.6 ±3.4	7.5 ±1.8	3.5 ±1.1	51.5 ±3.3	14.6 ±6.5
	D	0.8, 0.9	1.6, 1.9	14.3, 15.2	4.2, 4.8	2.2, 2.3	41.9, 41.8	35.0, 33.1
Fasting plasma FFA	A	0.6 ±0.2	3.0 ±0.2	26.5 ±0.9	6.4 ±1.3	10.0 ±1.2	44.2 ±1.1	9.4 ±1.0
	B	0.4 ±0.2	2.4 ±0.4	26.5 ±2.1	4.6 ±1.2	12.0 ±1.7	45.6 ±3.6	8.7 ±1.1
	C	0.4 ±0.3	1.9 ±0.6	25.0 ±3.0	3.8 ±1.2	11.4 ±2.8	42.4 ±4.2	15.0 ±6.4
	D	0.9, 0.7	1.2, 1.4	17.0, 17.1	2.5, 2.9	6.1, 6.1	39.1, 36.1	33.2, 35.7
Fasting plasma triglyceride	A	—	2.2 ±0.4	26.1 ±1.6	7.2 ±1.7	2.9 ±0.5	48.8 ±1.3	12.8 ±2.0
	B	—	1.9 ±0.5	29.2 ±1.4	4.6 ±1.1	4.2 ±1.0	48.4 ±3.3	11.8 ±1.9
	C	—	1.7 ±0.8	27.2 ±5.1	4.6 ±1.0	3.9 ±1.0	43.1 ±4.9	19.4 ±8.9
	D	0.2, 0	1.4, 1.6	19.5, 22.0	4.6, 4.6	2.2 3.4	36.4, 31.7	35.7, 36.7

\* Mean ±SD except for Group D.

† Group A, normal children (No. = 5); Group B, Diabetes mellitus, standard diet (No. = 6); Group C, Diabetes mellitus, corn oil diet (No. = 17); Group D, Hypercholesterolemia, corn oil diet (No. = 2).

§ Includes isomers.

|| Significant difference between means for normal children and for diabetic children on a standard diet ( $t = > 2.26$   $P = < 0.05$ ).

fasting plasma FFA and 16:0, 16:1, and 18:0 in the plasma triglycerides were significantly different ( $t = > 2.26$ ,  $P = < 0.05$ ).

Adipose tissue 18:2 in the diabetic children consuming corn oil foods ranged from 7.4 to 31.3%. It was highest in the two boys with familial hypercholesterolemia (33.1 and 35.0%). Duration of corn oil consumption ranged

from 3 yr for the patients with hypercholesterolemia to 4–7 yr for the diabetics. The variations in adipose tissue 18:2 were not related to duration of the corn oil diet (Fig. 1) and indicated varied adherence to this diet. Records of all items of diet consumed by each child at home over a 3–7 day period were subsequently analyzed. The proportion of total calories consumed as corn oil foods by each child over the period ranged from 0 to 40%. The correlation between this value and the concentration of 18:2 in adipose tissue and plasma lipid fractions is shown in Figs. 2 and 3.<sup>5</sup> All correlation coefficients were highly significant.

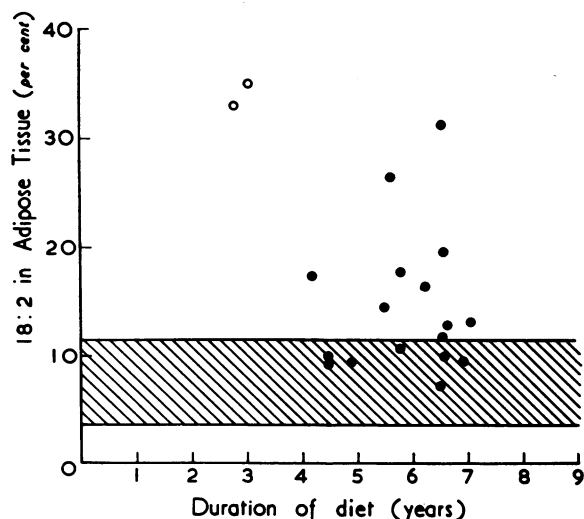


FIGURE 1 Lack of correlation between duration of corn oil diet and 18:2 in adipose tissue confirming varied dietary adherence. The shaded area represents the mean concentration  $\pm 2$  SD of adipose tissue 18:2 in the diabetic children on a standard diet. ● = Diabetes mellitus, corn oil diet; ○ = Hypercholesterolemia, corn oil diet. ( $r = -0.152$ ,  $P = > 0.1$ ).

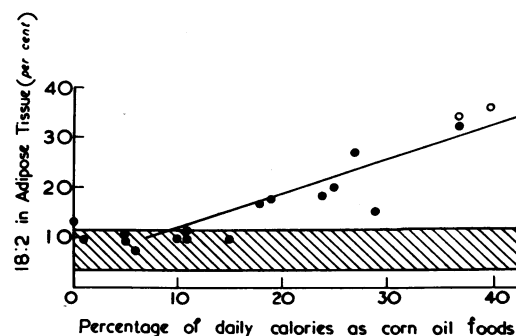


FIGURE 2 Correlation between the percentage of daily calories as corn oil products and 18:2 in adipose tissue. The shaded area represents the mean concentration  $\pm 2$  SD of adipose tissue 18:2 in the diabetic children on a standard diet. ● = Diabetes mellitus, corn oil diet; ○ = Hypercholesterolemia, corn oil diet. ( $r = 0.90$ ,  $P = < 0.001$ ).

<sup>5</sup> All regression lines in the figures have been calculated for  $y$  in terms of  $x$ .

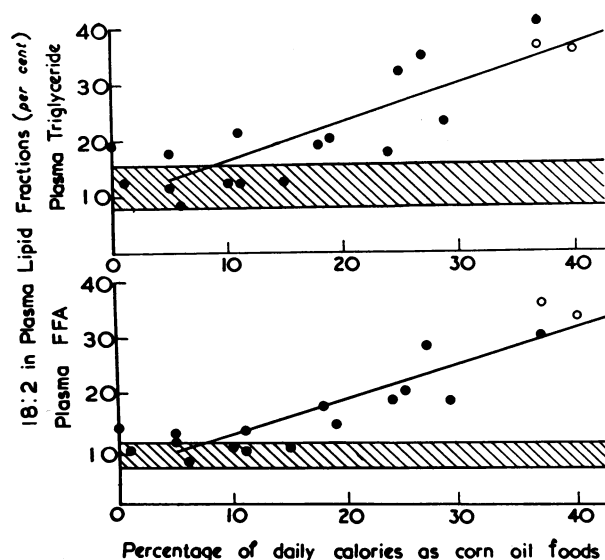


FIGURE 3 Correlation between the percentage of daily calories as corn oil products and 18:2 in fasting plasma FFA and plasma triglyceride. The shaded area represents the mean concentration  $\pm 2$  SD of 18:2 in each fraction in the diabetic children on a standard diet.  $\bullet$  = Diabetes mellitus, corn oil diet;  $\circ$  = Hypercholesterolemia, corn oil diet. (plasma FFA,  $r = 0.90$ ,  $P < 0.001$ ; plasma triglyceride,  $r = 0.85$ ,  $P < 0.001$ ).

Whereas the percentages of 18:2 found in adipose tissue triglyceride and in fasting plasma FFAs were similar in all patients (Fig. 4), the percentage of 18:2 in the plasma triglyceride fraction was, in most children, higher than in adipose tissue (Fig. 5).

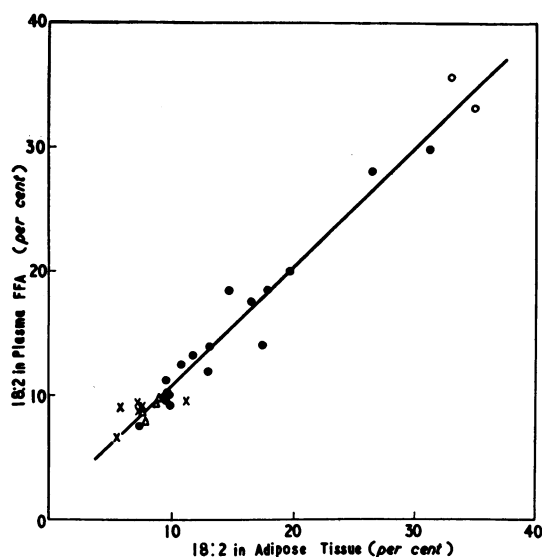


FIGURE 4 Correlation between 18:2 in fasting plasma FFA and adipose tissue.  $\Delta$  = Normal children;  $\times$  = Diabetes mellitus, standard diet;  $\bullet$  = Diabetes mellitus, corn oil diet;  $\circ$  = Hypercholesterolemia, corn oil diet. ( $r = 0.98$ ,  $P < 0.001$ ).

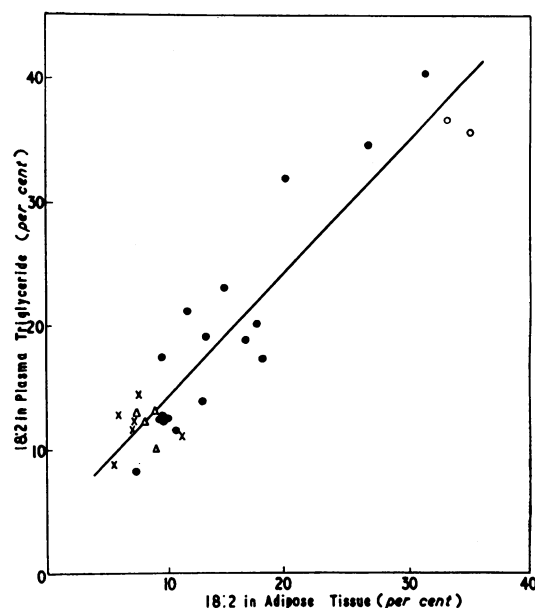


FIGURE 5 Correlation between 18:2 in fasting plasma triglyceride and adipose tissue.  $\Delta$  = Normal children;  $\times$  = Diabetes mellitus, standard diet;  $\bullet$  = Diabetes mellitus, corn oil diet;  $\circ$  = Hypercholesterolemia, corn oil diet. ( $r = 0.94$ ,  $P < 0.001$ ).

The fasting plasma FFA fraction always contained a higher proportion of saturated acids and a lower proportion of monounsaturated acids than did adipose tissue. However, the sum of the percentages of the satu-

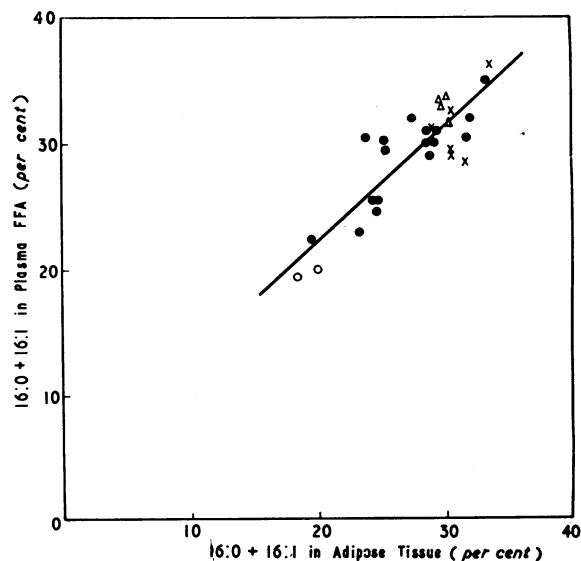


FIGURE 6 Correlation between 16:0 plus 16:1 in fasting plasma FFA and adipose tissue.  $\Delta$  = Normal children;  $\times$  = Diabetes mellitus, standard diet;  $\bullet$  = Diabetes mellitus, corn oil diet;  $\circ$  = Hypercholesterolemia, corn oil diet. ( $r = 0.86$ ,  $P < 0.001$ ).

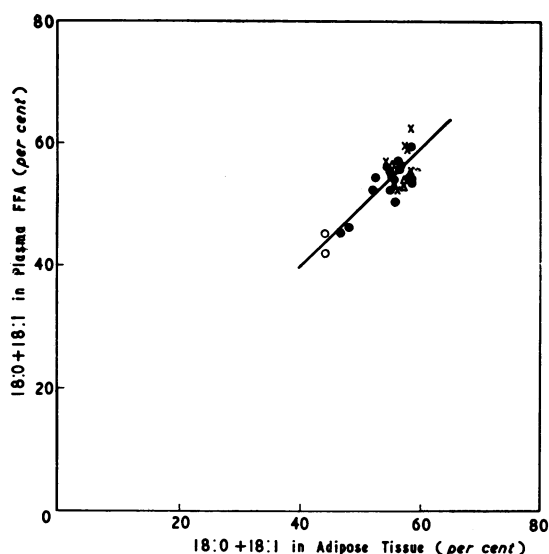


FIGURE 7 Correlation between 18:0 plus 18:1 in fasting plasma FFA and adipose tissue.  $\Delta$  = Normal children;  $\times$  = Diabetes mellitus, standard diet;  $\bullet$  = Diabetes mellitus, corn oil diet;  $\circ$  = Hypercholesterolemia, corn oil diet. ( $r = 0.85$ ,  $P < 0.001$ ).

rated and monounsaturated fatty acids of the same chain length in each of these fractions was similar. The relationship between 16:0 plus 16:1 in the two fractions is shown in Fig. 6 and that between 18:0 plus 18:1 in Fig. 7.

## DISCUSSION

The method described was developed to determine the fatty acid composition of lipids in small samples of adipose tissue and plasma. As a result of the high sensitivity used, impurities from diethyl ether and methylation mixture were detected by gas-liquid chromatography as peaks in fatty acid positions. Other workers have reported the presence of similar contaminants in extracts of methanol-sulphuric acid (16) and filter paper (17). The latter authors suggest that a phthalate ester widely used as a plasticizer might be responsible.

According to Hirsch et al. (9), the seven adipose tissue fatty acids that we have analyzed constitute at least 92% of the total fatty acids in human buttock adipose tissue, and the composition of a needle biopsy of this tissue is representative of adipose tissue throughout the body. Although the numbers of patients in our groups were small, we found no significant difference in the fatty acid composition of adipose tissue in normal children and in insulin-treated diabetic children on a standard diabetic diet. This supports the findings of Hirsch et al. (9) who reported no difference in the composition of adipose tissue in normal and diabetic adults.

It has been reported (18) that the composition of plasma triglycerides is similar in normal young adults (aged 23–38) and juvenile diabetics (aged 20–42, duration of treatment 10–32 years). However, we have found small but significant differences in the fatty acid composition of both plasma triglycerides and FFA between normal and diabetic children. The mean percentage of 16:1 in plasma triglycerides and of 14:0 and 16:1 in plasma FFA were lower in the diabetic children on a standard diet than in normal children, whereas 16:0 and 18:0 in plasma triglycerides were higher in the diabetics. The controlled carbohydrate content of the diabetic child's diet may have influenced the fatty acid composition of these plasma lipid fractions.

The concentrations of adipose tissue 18:2 in the two boys with hypercholesterolemia were similar to levels reported in a group of elderly institutionalized men after 5 yr of good adherence to a corn oil diet (10). Wide variations were found in adipose tissue 18:2 in the diabetic children receiving corn oil. Methods were not available to determine the fatty acid composition of adipose tissue biopsies before dietary treatment commenced some years ago. However, after 4–7 yr on the diet, only 10 of 17 patients had adipose tissue 18:2 concentrations higher than 11.5% (the mean plus 2 sd for the diabetic children on a standard dietary regimen). Estimates of the consumption of corn oil foods by each child confirmed that most of the children were not adhering to the prescribed diet. Some of the older teenage diabetic children were known to object to the added restrictions of the corn oil diet and it had become unpalatable to others.

Kayden, Karmen, and Dumont (19) have shown that plasma triglyceride 18:2 is increased appreciably 8 hr after the ingestion of corn oil. Most of our patients had a higher percentage of 18:2 in plasma triglycerides than in adipose tissue (Fig. 5). Some of the diabetic children were admitted to hospital on the day before the investigations and were known to have consumed the prescribed corn oil diet during this period. Thus the higher percentage of 18:2 in their plasma triglycerides may have resulted from an increased intake of corn oil during the preceding day. Holman, Caster, and Wiese (20) suggested that the 18:2 content of the diet could be calculated from the concentration of plasma triglyceride polyunsaturated fatty acids. Since plasma triglyceride composition so quickly reflects increases in corn oil intake, estimates of long-term corn oil intake by this method could be misleading.

Dayton et al. (10) have shown that adipose tissue 18:2 reflects adherence to corn oil diets after a period of 5 yr. The half-time of 18:2 in adult adipose tissue has been calculated as between 350–750 days (9) and 680 days (21). Hirsch et al. (9) found that the proportion of 18:2 in the fasting plasma FFA fraction of an

adult had risen to 30% after 10 wk on a corn oil diet, but after 20 wk on the diet changes in adipose tissue fatty acids were imperceptible. On the other hand, marked increases have been found in the adipose tissue 18:2 of new-born infants after only 6-8 wk on a diet rich in 18:2 (22, 23). The turnover time for adipose tissue 18:2 is likely to be shorter in infants because of their higher energy expenditure, greater caloric intake in relation to body weight, and rate of deposition of new tissue (22), and in lean adults because of their smaller triglyceride pool (10). In the children reported here, the correlations between corn oil consumption, 18:2 in adipose tissue triglycerides, and 18:2 in fasting plasma FFA suggest that either the children had been maintaining an 18:2 equilibrium by consuming a constant quantity of corn oil for a considerable period and/or that the half-time for the 18:2 equilibrium is much shorter in the growing child than in the adult. In view of the fact that the percentage of 18:2 in adipose tissue and in plasma FFA were almost identical, we suggest that in the future the 18:2 present in the fasting plasma FFA fraction of these diabetic children could be used to assess the degree of their adherence to corn oil diets, thus obviating the need for repeated adipose tissue biopsies.

In the fasting state, plasma FFA are derived mainly from lipolysis of adipose tissue triglyceride (24). We have found a higher proportion of saturated fatty acids and a lower proportion of monounsaturated acids in the fasting plasma FFA fraction than in adipose tissue. Hirsch et al. (9) also reported a lower proportion of 16:1 and 18:1 in plasma FFA than in adipose tissue. The difference between the percentages of saturated and monounsaturated fatty acids in these two fractions may be due to preferential release of saturated fatty acids from adipose tissue, or to preferential extraction of monounsaturated acids from the plasma for oxidation by tissues. The sum of the percentages of 16:0 and 16:1 (and also 18:0 plus 18:1) in adipose tissue was similar to that in the fasting plasma FFA. This suggests that during fasting a fraction of the monounsaturated fatty acid pool in adipose tissue is hydrogenated to saturated acids before release. However, Bloch (25) has stated that hydrogenation of long chain monounsaturated fatty acids is of only minor importance in higher animals. It is possible that the proportions of particular fatty acids released into the plasma from adipose tissue are under the control of adipokinetic hormones, but the mechanisms are yet to be elucidated.

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