Dietary fat and the diabetic state alter insulin binding and the fatty acyl composition of the adipocyte plasma membrane

Catherine J. FIELD,* Edmond A. RYAN,† Alan B. R. THOMSON*† and M. Thomas CLANDININ†‡

*Nutrition and Metabolism Research Group, Faculty of Medicine, 533 Newton Research Building, University of Alberta, Edmonton, Alberta T6G 2C2, and †Muttart Diabetes Research and Training Centre, University of Alberta, Room 8-121B, Clinical Sciences Building, Edmonton, Alberta T6G 2G3, Canada

Control and diabetic rats were fed on semi-purified high-fat diets providing a polyunsaturated/saturated fatty acid ratio (P/S) of 1.0 or 0.25, to examine the effect of diet on the fatty acid composition of major phospholipids of the adipocyte plasma membrane. Feeding the high-P/S diet (P/S = 1.0) compared with the low-P/S diet (P/S = 0.25) increased the content of polyunsaturated fatty acids in membrane phospholipids in both control and diabetic animals. The diabetic state decreased the content of polyunsaturated fatty acids, particularly arachidonic acid, in adipocyte membrane phospholipids. The decrease in arachidonic acid in membrane phospholipids of diabetic animals tended to be normalized to within the control values when high-P/S diets were given. For control animals, altered plasma-membrane composition was associated with change in insulin binding, suggesting that change in plasma-membrane composition may have physiological consequences for insulin-stimulated functions in the adipocyte.

INTRODUCTION

There is convincing evidence in animal models for several tissue types that physiological changes in the type of diet fat consumed changes cell-membrane composition (Clandinin *et al.*, 1985) by mechanisms that can involve altering activity of enzymes of fatty acid desaturation (Garg *et al.*, 1988) and the biosynthesis of membrane phosphatidylcholine *de novo* (Hargreaves & Clandinin, 1987*a,b*). These diet-induced alterations in membrane phospholipid composition influence the function of membrane proteins by changing the lipid environment in which these proteins function (Clandinin *et al.*, 1985). It has also been established in man and animals that normal variation in dietary fatty acid intake can alter the fatty acid composition of adipose-tissue triacylglycerols and phospholipids (Field & Clandinin, 1984). However, little is known about the role of the adipocyte plasma-membrane phospholipid and the influence of physiological shifts in fat intake or disease state on the composition and function of this membrane. A relationship between habitual fatty acid intake and the fatty acid composition of triacylglycerols and phospholipids in human adipose tissue has been established (Field *et al.*, 1985), and suggests that long-term intake of fat may determine the membrane lipid composition in this major organ.

Non-insulin-dependent diabetes is characterized by a cellular resistance to insulin action (Olefsky & Reaven, 1977). Although the mechanisms for insulin resistance are not completely understood, it has been suggested to be the consequence of decreased insulin binding, owing to a decrease in functional insulin receptors (Pedersen, 1984). In the adipocyte, alterations in insulin binding to membrane receptors have also been shown in man (Kolterman *et al.*, 1981; Pedersen, 1984) to be altered in this form of diabetes. However, controversy exists in

the literature as to the existence of this receptor defect in non-insulin-dependent diabetes (Arner *et al.*, 1987; Lonroth *et al.*, 1983).

The insulin receptor is embedded in the lipid bilayer of the plasma membrane (Schlessinger *et al.*, 1978). *In vitro*, the insulin receptor (Ginsberg *et al.*, 1981; Gould *et al.*, 1982) and insulin function (Sandra *et al.*, 1984) appear sensitive to the surrounding lipid environment. The nature of the fat given influenced insulin binding to receptors in erythrocytes (Gould *et al.*, 1982) and Ehrlich ascites cells (Ginsberg *et al.*, 1982), but whole-body physiological tests of this relationship have not been reported, with one exception. When a high polyunsaturated diet was fed to diabetic rats, diet-induced alterations in the form and function of the intestine have been reported to improve the clinical state in this diabetic model (Rajotte *et al.*, 1988).

The diabetic state may also influence the fatty acid composition of cell membranes. Decreased contents of monounsaturated and polyunsaturated fatty acid in liver and adipose tissue have been related to diminished microsomal rates of fatty acid Δ^6 - and Δ^9 -desaturation in experimental models of diabetes mellitus (Faas & Carter, 1980; Worcester *et al.*, 1979). Insulin therapy appears to correct these defects in desaturase activity, and to normalize partially the altered fatty acid composition of microsomal fractions (Faas & Carter, 1980). The physiological importance of these alterations is not clear.

On the basis of the premise that the composition of the adipocyte plasma membrane is an integral determinant of some aspects of insulin-stimulated functions in the adipocyte, the following study was designed to examine the effect of alterations in dietary fatty acid composition on the fatty acid composition of membrane phospholipids and on insulin binding to its receptor in the adipocyte plasma membrane. Dietary transitions representing the

‡ To whom requests for reprints should be sent, at: Department of Foods and Nutrition, Room 318F, Home Economics Building, University of Alberta, Edmonton, Alberta T6G 2M8, Canada.

Table 1. Fatty acid composition of diets

Fatty acid composition of diets is expressed as % (w/w) of total fatty acids and was determined by g.l.c. Abbreviations used: Σ polyunsats, sum of polyunsaturated fatty acids; $\Sigma(\omega-6)$, sum of ($\omega-6$)-unsaturated fatty acids; $\Sigma(\omega-3)$, sum of ($\omega-3$)-unsaturated fatty acids; Σ sats, sum of saturated fatty acids; Σ monounsats, sum of monounsaturated fatty acids; P/S, polyunsaturated/saturated fatty acid ratio.

Fatty acid	Composition (%)	
	P/S = 1.0	P/S = 0.25
C _{14:0}	1.3	2.7
C _{14:1}	0.1	0.1
C _{15:0}	0.2	0.4
C _{16:0}	16.1	22.0
C _{16:1}	0.2	0.3
C _{17:0}	0.9	1.6
C _{18:0}	27.5	47.1
C _{18:1}	8.0	4.2
C _{18:2(9,12)}	43.8	18.2
C _{18:3(6,9,12)}	0.2	0.3
C _{18:3(9,12,15)}	0.9	0.9
C _{20:0}	0.4	0.7
C _{20:1}	0.1	0.1
C _{20:3(5,8,11)}	0.1	0.1
C _{22:0}	0.2	0.2
C _{22:6(4,7,10,13,16,19)}	0.1	0.1
Σ polyunsats	45.0	19.5
$\Sigma(\omega-6)$	44.0	18.5
$\Sigma(\omega-3)$	1.1	1.0
Σ sats	45.7	74.7
Σ monounsats	8.3	4.6
P/S ratio	0.99	0.26

physiological range of dietary fatty acid compositions consumed by humans were used. Control and diabetic rats were compared, to assess whether increasing consumption of polyunsaturated fatty acids would normalize the decreased amounts of desaturase products expected in the diabetic state and would alter insulin binding, an integral membrane function that could regulate insulin-responsiveness of this tissue.

MATERIALS AND METHODS

Animals and diets

Some 40 male Sprague-Dawley rats (4 weeks of age; 140 ± 10 g) were randomly assigned to two groups and fed on a semi-purified high-fat diet containing 27 % (w/w) high-protein casein, 38 % (w/w) carbohydrate as corn starch and 20 % (w/w) fat. The diet was supplemented with the following (per kg): 2.75 g of choline, 6.25 g of inositol, 2.5 g of L-methionine, 10 g of vitamin mix (A.O.A.C.) and 50 g of Bernhart-Tomerelli mineral mix. The compositions of this basal diet, vitamin and mineral mixes have been reported in detail by Hargreaves & Clandinin (1987b). The two diets differed only in the proportion of safflower oil and hydrogenated beef tallow given, to provide a polyunsaturated/saturated fatty acid ratio (P/S) of 0.25 (low P/S) or 1.0 (high P/S), as given in Table 1. Linseed oil was added to both diets to obtain a total $\omega 3$ fatty acid content of 1 % (w/w) of the diet fat.

Both diets were cholesterol-free by analysis. Animals were housed individually in a temperature-controlled room at 21 ± 1 °C and maintained on a 12 h-light/12 h-dark cycle.

After the diet had been given for 14 days, diabetes was induced in half of the animals in each diet group by intravenous injection of streptozotocin (65 mg/kg body wt.; Sigma Chemical Co., St. Louis, MO, U.S.A.) in a citrate buffer, pH 4.5, into a tail vein. Only animals with non-fasting blood glucose greater than 300 mg/dl after 14 days were considered diabetic. Animals were continued on their respective diet treatment for an additional 14–24 days.

Each day, at 09:00 h (2 h after the end of the dark cycle) for a period of 10 days, an animal from each treatment group was killed by decapitation and serum collected for glucose and insulin determinations. Serum glucose and insulin were determined by using a glucose analyser (Glucose Analyzer 2; Beckman Instruments) and by radioimmunoassay (Insulin RIA 100 Kit; Pharmacia, Uppsala, Sweden) against a rat insulin standard. Epididymal fat-pads were collected and adipocytes isolated by incubation with crude collagenase (2.25 mg/g of tissue; Cooper Biomedical, Toronto, Canada) by a modification of the method of Rodbell (1964). The modification included the use of a glucose-free Krebs-Ringer solution buffered with Hepes (Whitesell & Abumrad, 1985) containing 2 % (w/v) Fraction V bovine albumin (Sigma, Canada).

Plasma-membrane isolation

Plasma membranes were prepared from adipocytes by the method of Lewis *et al.* (1979), modified to enable isolation of plasma membranes from small samples of material in 1 ml by using a Beckman TL-100 ultracentrifuge. The modification included loading the second pellet, resuspended in 200 μ l of buffer (0.25 M-sucrose, 1 mM-EDTA, 10 mM-Tris/HCl, pH 7.4) on an 800 μ l linear gradient containing 32–52 % (w/w) sucrose, 1 mM-EDTA and 10 mM-Tris/HCl, pH 7.4, followed by centrifugation at 45000 rev./min (205780 $g_{av.}$) in a Beckman TLS-55 rotor for 30 min at 4 °C. The white band near the top of the gradient containing the plasma-membrane fraction was collected and diluted to a volume of 1 ml in 10 mM-Tris/HCl/1 mM-EDTA buffer (pH 7.4) and centrifuged at 29000 rev./min (30000 $g_{av.}$) for 30 min at 4 °C in a Beckman TL-100 rotor. The membrane pellet was collected, resuspended in 400 μ l of 10 mM-Tris/HCl buffer (pH 7.4) and stored at -70 °C for analysis of lipid composition. Purity of this plasma-membrane fraction was assessed by assaying 5'-nucleotidase (Goldfine *et al.*, 1977) and succinate dehydrogenase (Kun & Abood, 1949) in membrane fractions obtained from control animals fed on high-fat diets. The specific activity of 5'-nucleotidase was highest in the plasma-membrane fraction (6.46 ± 0.04 μ mol of P hydrolysed/h per mg of protein), representing greater than 8-fold enrichment from cell homogenate. Succinate dehydrogenase activity was low in the plasma membrane (1.47 ± 0.12 μ mol of *p*-Iodonitrozoium Violet reduced/h per mg of protein), representing less than 11 % of the activity found in the mitochondrial fraction. The purity of the plasma-membrane fraction was comparable with previous reports (Lewis *et al.*, 1979) and was not affected by diet treatment or the diabetic state (Kahn & Cushman, 1987).

Table 2. Final body weights and serum glucose and insulin contents of animals

Values are group means $\pm$ S.E.M. ($n = 10$). Treatments without a common superscript are significantly different ($P < 0.05$).

Group	Diet	Final body wt. (g)	Serum glucose (mg/100 ml)	Serum insulin (munits/l)
Control	P/S = 1.0	387 $\pm$ 10 ^a	141 $\pm$ 3 ^a	61 $\pm$ 9 ^a
	P/S = 0.25	364 $\pm$ 9 ^a	133 $\pm$ 3 ^a	55 $\pm$ 7 ^a
Diabetic	P/S = 1.0	301 $\pm$ 12 ^b	514 $\pm$ 33 ^b	28 $\pm$ 4 ^b
	P/S = 0.25	288 $\pm$ 6 ^b	501 $\pm$ 36 ^b	23 $\pm$ 3 ^b

Membrane lipid analysis

Lipids were extracted from plasma membranes by a modified Folch procedure (Folch *et al.*, 1957). This extraction procedure was modified as follows: to 400 μ l of 10 mM-Tris/HCl buffer, pH 7.4, containing the membrane pellet, 0.8 ml of methanol, 2.0 ml of chloroform/methanol (1:1, v/v), 2.7 ml of chloroform and 2.5 ml of chloroform/methanol (2:1, v/v) were added in succession. The layers were split by addition of 1.6 ml of 0.1 mM-KCl. The lower phase containing the phospholipids was collected. Individual phospholipids were separated on Whatman HP-K t.l.c. plates (10 cm $\times$ 10 cm) in the solvent system chloroform/methanol/propan-2-ol/0.25% (w/v) KCl/triethylamine (30:9:25:6:18, by vol.) (Touchstone *et al.*, 1980). Separated phospholipids were sprayed with 0.03% (w/v) 2',7'-dichlorofluorescein in 0.01 M-NaOH and detected by comparison under u.v. light with appropriate standards.

Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin fatty acid methyl esters were prepared by using 14% (w/v) BF₃/methanol reagent (Morrison & Smith, 1964) and separated by automated g.l.c. (Vista 6010 G.L.C. and Vista 402 data system; Varian Instruments, Georgetown, Ont., Canada). Chromatography was performed on a fused-silica BP20 capillary column (25 m $\times$ 0.25 mm internal diam.; Varian). Helium was used as the carrier gas at a flow rate of 1.8 ml/min, using a splitless injection mode. The initial oven temperature was 150 °C, increased to 190 °C at 20 °C/min and held for 23 min, then increased to 220 °C at 2 °C/min for a total analysis time of 40 min. The analytical conditions used separate all saturated, mono-, di- and polyunsaturated fatty acid from C₁₄ to C₂₄ in chain length.

Insulin binding

Freshly isolated adipocytes were incubated with ¹²⁵I-labelled pig insulin (New England Nuclear, Du Pont Canada, Quebec, Canada; sp. radioactivity 89.3 μ Ci/ μ g) in buffer described for cell isolation at 24 °C, pH 7.4, as described by Olefsky & Reaven (1975) in a total incubation volume of 600 μ l. Optimal steady-state binding conditions were achieved for both control and diabetic adipocytes at 24 °C after 45 min of incubation and were maintained for at least 1 h further (results not shown). Insulin binding was terminated as described by Gammeltoft & Gliemann (1973) by rapidly centrifuging 250 μ l samples from cell suspensions in polyethylene

microcentrifuge tubes to which 100 μ l of silicone oil had been added in a MSE micro-centrifuge at high speed (570 g for 1 min). The cells were collected, added to 5 ml of Aquasol (New England Nuclear), and radioactivity was measured in a Beckman LS 5801 liquid-scintillation counter at a counting efficiency of 90% as determined by direct counting in a γ -radiation counter. Binding was corrected for non-specific binding by incubating cells with ¹²⁵I-insulin and unlabelled insulin (20 μ g/ml). The non-specific binding was 5–10% of total binding and did not vary with diet treatment or disease state. Cell numbers were determined by 24 h fixation at 36 °C in 2% (w/v) OsO₄ in 0.05 M-collidine (made in 0.15 M-NaCl) as described by Hirsch & Gallian (1968) by using a Coulter ZM counter with 400 μ m aperture (Coulter Electronics, Vancouver, BC, Canada). Binding was calculated on a per-cell basis and expressed as total amount of insulin bound per 2.0×10^5 cells.

Statistical analysis

The effect of diet treatment and disease state on membrane phospholipid fatty acid composition and insulin binding was compared by analysis of variance procedures. A Duncan's multiple-range test was used to discriminate significant differences between individual treatment groups (Steel & Torrie, 1980).

RESULTS

Animals

The diabetic animals weighed less, and had higher serum glucose concentrations and lower serum insulin concentrations as compared with control animals (Table 2). Dietary lipid modification did not significantly alter these three parameters in either control or diabetic animals. Although food consumption was not measured, it has been well documented by others that diabetic animals display hyperphagia (Kobayashi & Olefsky, 1979).

Effect of diet on adipocyte plasma-membrane fatty acyl composition

In the present study, diet significantly ($P < 0.05$) altered the fatty acid composition of the major adipocyte plasma-membrane phospholipids in both control and diabetic animals (Table 3). Feeding a high-polyunsaturated-fat diet (P/S = 1.0) as compared with a low-polyunsaturated-fat diet (P/S = 0.25) increased the total polyunsaturated fatty acid content (Fig. 1a) and P/S ratio (Fig. 1b) in all five membrane phospholipids analysed from control animals. For example, consumption of the high-P/S diet by control animals increased the content of C_{20:4(5,8,11,14)} fatty acid in phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin (Fig. 1c), the content of C_{18:2(9,12)} in phosphatidylcholine and phosphatidylethanolamine, and the total number of double bonds expressed as unsaturation index in fatty acids found in phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine (Table 3).

In diabetic animals the tendency to higher phospholipid content of polyunsaturated fatty acids in animals fed on the high-P/S diet was also evident (Fig. 1). Feeding the high-P/S diet compared with the low-P/S diet to diabetic animals increased the polyunsaturated

Table 3. Effect of dietary fat and the diabetic state on the contents of major fatty acids in adipocyte plasma-membrane phospholipids

Values are means $\pm$ s.e.m. ($n = 10$) for control and diabetic animals given the dietary treatments: P/S, polyunsaturated/saturated fatty acid ratio of 1.0 or 0.25. Only major fatty acids are reported. Abbreviation used: U.I., unsaturation index (total no. of unsaturated bonds). Other abbreviations are defined in the legend to Table 1. Values without a common superscript are significantly different ($P < 0.05$). Values illustrating the effect of treatment on contents of membrane phospholipid, $C_{20:4(6,8,11,14)}$, total polyunsaturated fatty acids and P/S ratio are illustrated in Fig. 1.

Phospholipid group	Diet P/S ratio	Fatty acid (% w/w)								
		C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2(9,12)}	Σsats	Σmonounsats	(ω-6)	(ω-3)	U.I.
Phosphatidylcholine	1.0	16.6 ± 1.6 ^a	35.5 ± 0.9 ^a	6.0 ± 0.5 ^a	25.3 ± 1.7 ^a	53.5 ± 1.3	7.8 ± 0.6 ^a	37.2 ± 1.3 ^a	0.9 ± 0.3	108.6 ± 4 ^a
	0.25	20.0 ± 2.2 ^{ab}	33.2 ± 1.4 ^{ab}	8.1 ± 0.7 ^b	19.1 ± 1.6 ^b	56.3 ± 1.5	10.4 ± 1.0 ^b	30.4 ± 2.2 ^b	1.4 ± 0.4	100.0 ± 6 ^{ab}
	1.0	23.2 ± 2.3 ^b	29.3 ± 1.4 ^c	8.2 ± 1.1 ^b	22.9 ± 2.0 ^{ab}	56.1 ± 1.7	11.5 ± 1.0 ^b	29.8 ± 2.0 ^b	2.1 ± 0.7	91.9 ± 3 ^b
	0.25	23.6 ± 1.3 ^b	29.9 ± 1.0 ^{bc}	9.0 ± 0.8 ^b	20.7 ± 1.4 ^{ab}	56.7 ± 1.3	12.1 ± 1.0 ^b	27.6 ± 1.7 ^b	2.2 ± 0.5	90.8 ± 4 ^b
Phosphatidylethanolamine	1.0	10.5 ± 0.8 ^a	31.5 ± 2.2	10.8 ± 1.2	17.2 ± 1.6 ^a	44.6 ± 1.8 ^a	12.9 ± 1.4	39.7 ± 1.3 ^a	1.6 ± 0.4	145.5 ± 4 ^a
	0.25	19.5 ± 2.4 ^b	31.9 ± 2.7	9.8 ± 0.7	11.9 ± 1.4 ^b	55.4 ± 2.7 ^b	14.8 ± 2.1	25.6 ± 2.1 ^b	2.0 ± 0.2	99.8 ± 6 ^b
	1.0	16.2 ± 2.4 ^b	28.4 ± 1.3	11.1 ± 0.7	16.0 ± 1.8 ^{ab}	48.7 ± 2.0 ^{ab}	14.9 ± 1.2	32.4 ± 3.3 ^c	1.7 ± 0.5	121.6 ± 9 ^c
	0.25	19.0 ± 1.8 ^b	32.2 ± 2.3	11.5 ± 1.2	15.4 ± 1.5 ^{ab}	54.5 ± 0.7 ^b	13.9 ± 1.1	28.1 ± 1.8 ^{bc}	1.8 ± 0.5	104.6 ± 4 ^b
Phosphatidylinositol	1.0	21.4 ± 1.6 ^a	31.0 ± 2.3	10.6 ± 1.2 ^a	7.5 ± 0.8	58.8 ± 1.6 ^a	14.6 ± 1.1 ^a	23.1 ± 1.4 ^a	2.5 ± 0.8	101.7 ± 5 ^a
	0.25	30.5 ± 2.4 ^b	27.8 ± 1.8	15.2 ± 2.0 ^b	6.7 ± 1.1	63.7 ± 2.8 ^{ab}	20.6 ± 1.7 ^b	13.2 ± 1.4 ^b	2.0 ± 0.4	68.6 ± 6 ^{bc}
	1.0	26.8 ± 3.8 ^b	31.1 ± 2.2	10.0 ± 2.1 ^a	7.2 ± 0.9	63.6 ± 3.0 ^{ab}	14.7 ± 2.1 ^a	17.4 ± 2.5 ^b	2.2 ± 0.3	78.0 ± 10 ^b
	0.25	33.0 ± 1.8 ^b	30.0 ± 3.0	10.3 ± 1.1 ^a	8.3 ± 0.8	68.5 ± 1.4 ^b	14.5 ± 1.4 ^a	12.9 ± 1.0 ^b	1.3 ± 0.1	54.6 ± 3 ^c
Phosphatidylserine	1.0	16.2 ± 1.8 ^a	32.6 ± 1.4 ^a	8.1 ± 0.7 ^a	15.2 ± 1.1 ^a	52.3 ± 1.6 ^a	11.2 ± 0.8 ^a	32.7 ± 2.0 ^a	2.3 ± 0.4	121.5 ± 7 ^a
	0.25	23.5 ± 3.2 ^b	32.0 ± 1.0 ^a	9.4 ± 0.9 ^{ab}	11.7 ± 1.3 ^b	59.8 ± 2.4 ^b	12.5 ± 0.7 ^{ab}	22.5 ± 1.9 ^b	2.9 ± 0.8	94.2 ± 8 ^b
	1.0	18.2 ± 2.9 ^{ab}	35.0 ± 0.7 ^b	7.5 ± 0.6 ^a	18.6 ± 0.7 ^a	56.4 ± 2.2 ^{ab}	11.2 ± 0.8 ^a	29.9 ± 2.5 ^a	2.2 ± 0.6	102.0 ± 10 ^{ab}
	0.25	21.9 ± 2.3 ^{ab}	28.2 ± 2.0 ^c	10.4 ± 0.9 ^b	10.9 ± 1.4 ^b	56.3 ± 3.0 ^{ab}	14.5 ± 0.9 ^b	23.0 ± 1.5 ^b	3.5 ± 0.3	96.0 ± 7 ^b
Sphingomyelin	1.0	35.8 ± 1.8	26.0 ± 0.5 ^{ab}	10.2 ± 2.2	7.0 ± 0.8 ^{ab}	67.5 ± 2.0	15.9 ± 2.1	13.1 ± 1.2 ^a	2.9 ± 0.5	64.4 ± 5 ^{ab}
	0.25	38.8 ± 3.0	27.4 ± 1.8 ^{ab}	9.9 ± 1.6	4.6 ± 0.5 ^b	72.7 ± 2.1	15.0 ± 1.9	8.0 ± 0.8 ^b	2.7 ± 0.7	47.6 ± 5 ^b
	1.0	35.5 ± 1.8	29.7 ± 2.6 ^a	6.7 ± 0.6	8.1 ± 1.1 ^a	71.9 ± 2.2	12.8 ± 1.4	13.7 ± 1.1 ^a	2.0 ± 0.3	69.2 ± 9 ^a
	0.25	39.0 ± 3.4	24.4 ± 1.1 ^b	10.6 ± 1.6	6.9 ± 1.1 ^{ab}	70.4 ± 2.1	14.1 ± 1.6	11.1 ± 1.2 ^{ab}	3.4 ± 0.5	60.8 ± 5 ^{ab}

Fig. 1. Effect of dietary fat on the polyunsaturated-fatty-acid composition of adipocyte membrane phospholipids

Values are group means $\pm$ S.E.M. ($n = 10$): $\square$, low-P/S ($P/S = 0.25$) diet; $\blacksquare$, high-P/S ($P/S = 1.0$) diet. For each phospholipid (*) indicates a significant effect of diet treatment ($P < 0.05$), ($\blacksquare$) indicates a significant effect of disease state for animals fed on high-P/S diet ($P < 0.05$), and ($\square$) indicates a significant effect of disease state for animals fed on the low-P/S diet ($P < 0.05$). Abbreviations used; P/S, polyunsaturated/saturated fatty acid ratio; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; C, control; D, diabetic.

fatty acid content in phosphatidylserine and phosphatidylinositol (Fig. 1a), the P/S ratio in phosphatidylethanolamine and phosphatidylinositol (Fig. 1b) and the unsaturation index of phosphatidylinositol and phosphatidylethanolamine (Table 3). A higher content of $C_{18:2(9,12)}$ in phosphatidylserine (Table 3) and $C_{20:4(5,8,11,14)}$ in sphingomyelin (Fig. 1c) was observed in plasma membranes obtained from diabetic animals fed on the high-P/S diet compared with the low-P/S diet.

Effect of the diabetic state on adipocyte plasma-membrane fatty acyl composition

The diabetic state significantly influenced both the fatty acid composition of and the degree of diet-induced alterations occurring in the fatty acyl content of the adipocyte plasma membrane (Table 3). In the present

study, the influence of diet on the polyunsaturated fatty acid content of membrane phospholipids was attenuated by the diabetic state, particularly in phospholipid classes obtained from animals fed on the high-P/S diet. Membranes from diabetic animals fed on the high-P/S diet exhibited significantly lower total polyunsaturated fatty acid content (Fig. 1a), P/S ratio (Fig. 1b) and change in unsaturation index for phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol (Table 3) compared with control animals fed on the same diet. The total polyunsaturated fatty acid content and the P/S ratio in phosphatidylcholine, phosphatidylserine and phosphatidylinositol in diabetic animals fed on the high-P/S diet were decreased to values that were not significantly different from those for control animals fed on the low P/S diet.

With the exception of $C_{20:4(5,8,11,14)}$ acid in phosphatidylcholine (Fig. 1c), the total polyunsaturated, fatty acid content, P/S ratio and $C_{20:4(5,8,11,14)}$ content of membrane phospholipids of diabetic animals fed on the low-P/S diet did not differ significantly from those for diet-matched control animals (Fig. 1). Significantly lower amounts of arachidonic acid were found in phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine obtained from diabetic animals fed on the high-P/S diet than were observed for diet-matched control animals (Fig. 1c). In diabetic animals compared with control animals fed on the low-P/S diet, a significantly lower content of $C_{20:4(5,8,11,14)}$ acid was found in phosphatidylcholine (Fig. 1c).

Effect of diet on insulin binding in normal and diabetic animals

At all insulin concentrations used to test insulin binding, cells from control animals fed on the high-P/S diet bound significantly more insulin than did cells from control animals fed on the low-P/S diet (Fig. 2). From Scatchard analysis (Fig. 2) of binding in control animals, increased insulin binding is associated with an increased number of available insulin-binding sites. An effect of diet on insulin binding was not observed when comparing adipocytes obtained from diabetic animals. At the lower insulin concentrations examined (0.1–10 ng/ml), the adipocytes from diabetic animals bound significantly more insulin than did adipocytes obtained from control animals fed on the low-P/S diet (Fig. 2). However, at higher insulin concentrations (50 or 100 ng/ml) tested, cells from diabetic animals bound significantly less insulin than did control cells from animals fed on the high-P/S diet, but the amount of insulin bound at these concentrations did not differ from that in cells from control animals fed on the low-P/S diet.

DISCUSSION

The present study demonstrated that feeding animals on diets providing a fat content and fatty acid composition analogous to that consumed by segments of the North American population (Field *et al.*, 1985) significantly altered the fatty acyl composition of the major phospholipids of the adipocyte plasma membrane in both control and diabetic animals. Individual phospholipid classes responded to dietary fatty acid manipulation to different degrees (Table 3). Although the molecular control mechanism through which dietary fat composition influences the fatty acyl composition is unknown,

Fig. 2. Relationship between total bound insulin and insulin concentration

Total insulin bound is expressed as the total specifically bound insulin corrected for non-specifically bound insulin, which was 5–10 % of the total. Values at each insulin concentration are group means $\pm$ S.E.M. ($n = 10$). The level of significance (* $P < 0.01$; ** $P < 0.001$; NS, not significant) is indicated for control animals given high-P/S diet ($\bigcirc$ — $\bigcirc$), control animals given low-P/S diet ($\bigcirc$ — $\bigcirc$), diabetic animals given high-P/S diet ($\blacksquare$ — $\blacksquare$) and diabetic animals given low-P/S diet ($\square$ — $\square$). The inset shows Scatchard analysis for group means ($n = 10$).

differences in: rates of synthesis of phospholipids *de novo* (Hargreaves & Clandinin, 1987a), redistribution of fatty acyl chains via phospholipase (Van den Bosch, 1980) or acyltransferases (Hargreaves & Clandinin, 1987b) and direct desaturation of membrane phospholipid-linked fatty acids (Garg *et al.*, 1988) have been demonstrated to be altered by dietary fat composition. The availability of fatty acids at specific sites of synthesis may be influenced by dietary fat, as adipose tissue represents a large pool of available fatty acids, of which the composition to a large extent reflects the dietary fatty acid composition (Field & Clandinin, 1984).

The present study demonstrated that this induction of the diabetic state can rapidly alter the composition of adipocyte membranes in a manner that can to some degree be attenuated by dietary treatment. Streptozotocin-induced diabetes has been shown to decrease the synthesis of polyunsaturated fatty acids, a process which correlated with decreased activity of insulin-dependent microsomal Δ^9 and Δ^6 desaturation in liver (Faas & Carter, 1980; Worcester *et al.*, 1979). Insulin therapy has been shown to increase desaturase activity (Δ^9 and Δ^6) and to correct alterations in the fatty acyl composition, with the notable exception of lower contents of $C_{20:4(5,8,11,14)}$ acid (Faas & Carter, 1980). The present study supports the finding of lower total polyunsaturated fatty acid and $C_{20:4(5,8,11,14)}$ contents in most of the major adipocyte phospholipids from diabetic animals fed on the high-P/S diet compared with their diet-matched controls. In animals given the low-P/S diet, the total polyunsaturated fatty acid and $C_{20:4(5,8,11,14)}$ contents,

with the exception of the lower arachidonic acid content in phosphatidylcholine, were not significantly decreased by the diabetic state. However, a clear effect of diet was observed between the polyunsaturated fatty acid compositions of the diabetic animals fed on high- and low-P/S diets. In the present study, the diabetic state did not produce the dramatic decreases in monounsaturated fatty acids reported by others (Faas & Carter, 1980; Worcester *et al.*, 1979), except for decreased contents of monounsaturated fatty acids in phosphatidylinositol from diabetic animals fed on the low-P/S diet. It is conceivable that feeding high-fat diets in the current experiment, which provided an adequate content of essential fatty acids, may have prevented this diabetes-induced change observed in monounsaturated fatty acids. The greater effect of diabetes on membranes of diabetic animals fed on the high-P/S diet might be partially explained by the observation that high polyunsaturated-fat intakes inhibit Δ^9 and Δ^6 desaturase activities (Garg *et al.*, 1988; Weekes *et al.*, 1986).

The finding that a high-polyunsaturated-fat diet improved insulin binding to control adipocytes supports previous work *in vitro* utilizing cell cultures (Ginsberg *et al.*, 1981) and reconstituted systems (Gould *et al.*, 1982; Pilch *et al.*, 1980), and *in vivo* growing tumour cells in mice fed on different dietary fats (Ginsberg *et al.*, 1982) to alter membrane lipids and insulin binding. Scatchard analysis of binding in control animals, in agreement with the finding by Ginsberg *et al.* (1982), suggesting a more polyunsaturated environment, is associated with an increased number of available binding sites. The mol-

ecular mechanisms by which a more polyunsaturated membrane environment improves insulin binding are not clear, but to date several hypotheses have been proposed. The accessibility of the insulin receptor for its ligand may require conformational changes in the receptor that require a more polyunsaturated environment (Gould *et al.*, 1982), or alterations in the quaternary structure or state of the receptor that are determined by the degree of membrane saturation (Ginsberg *et al.*, 1982). Membrane alterations have also been suggested to alter insulin action after binding by altering the mobility of the receptor within the lipid bilayer (Melchoir & Czech, 1979), changing the activity of the enzymes involved in phosphatidylinositol turnover (Takenawa & Nagai, 1982) and decreasing the generation of a mediator produced by insulin stimulation from the plasma membrane that mimics the effect of the hormone on a variety of intracellular enzymes (Jarett & Kiechle, 1984).

Unlike in control animals, dietary-fat treatment did not alter the amount of insulin bound by cells from diabetic animals. Despite clear resistance to insulin action, compared with control cells, insulin binding to adipocytes has been reported to be significantly increased in streptozotocin-diabetic animals fed on a high-carbohydrate low-fat diet, at both high and low insulin concentrations (Schoenle *et al.*, 1977; Kobayashi & Olefsky, 1979; Sandra & Fyler, 1982). This increase in binding is consistent with the concept that circulating insulin concentrations inversely regulate insulin receptors (Gavin *et al.*, 1974). The results of the present study suggest that feeding a high-fat diet may change the comparison between insulin binding in diabetic animals and control animals. At the lower insulin concentrations, cells from diabetic animals bound more insulin than did those of controls fed on the low-P/S diet. However, at higher insulin concentrations, where preliminary results indicate a clear defect in intracellular insulin action (Field *et al.*, 1987), cells of diabetic animals bound significantly less insulin than did cells from control animals fed on the high-P/S diet.

The present study demonstrates that normal variations in dietary fat intake (1) alter the composition of structural lipids found in the adipose organ, and (2) in the short-term diabetic state alter the membrane response to a high-polyunsaturated-fatty-acid diet. These diet-induced alterations in membrane composition appear to influence insulin binding to adipocyte membranes. It is therefore logical to hypothesize that localized changes in the adipocyte plasma-membrane micro-environment may play some role in modulating insulin action within cells and may be of particular importance in dietary management of diabetes.

This work was supported by grants from the Canadian Diabetes Association and the Natural Sciences and Engineering Research Council of Canada. We are grateful for the technical assistance in fatty acid analysis provided by A. Wierzbicki and by E. Dolinski for serum insulin assays conducted in the Muttart Diabetes Research and Training RIA Laboratory. We also acknowledge the assistance and advice of Dr. Y. K. Goh. C.J.F. was a recipient of a Medical Research Council of Canada Studentship and an Alberta Heritage Foundation for Medical Research Independent Research Allowance. M.T.C. is a Scholar of the Alberta Heritage Foundation for Medical Research.

REFERENCES

- Arner, P., Engfeldt, P., Skarfors, E., Lithell, H. & Bolinder, J. (1987) *Upsala J. Med. Sci.* **92**, 47–58
- Clandinin, M. T., Field, C. J., Hargreaves, K., Morson, L. & Zsigmond, E. (1985) *Can. J. Physiol. Pharmacol.* **63**, 546–556
- Faas, F. H. & Carter, W. J. (1980) *Lipids* **15**, 953–961
- Field, C. J. & Clandinin, M. T. (1984) *Nutr. Res.* **4**, 743–755
- Field, C. J., Angel, A. & Clandinin, M. T. (1985) *Am. J. Clin. Nutr.* **42**, 1206–1220
- Field, C. J., Thomson, A. B. R., Ryan, E. A. & Clandinin, M. T. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 212
- Folch, J., Lees, M. & Sloane Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- Gammeltoft, S. & Gliemann, G. (1973) *Biochim. Biophys. Acta* **690**, 157–164
- Garg, M. L., Sebokova, E., Thomson, A. B. R. & Clandinin, M. T. (1988) *Biochem. J.* **249**, 351–356
- Gavin, J. R., III, Roth, J., Neville, D. M., De Meyts, P. & Buell, D. N. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 84–88
- Ginsberg, B. H., Brown, T. J., Ido, S. & Spector, A. A. (1981) *Diabetes* **30**, 773–780
- Ginsberg, B. H., Jabour, J. & Spector, A. A. (1982) *Biochim. Biophys. Acta* **690**, 157–164
- Goldfine, T. D., Smith, G. J., Wong, K. Y. & Jones, A. L. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1368–1372
- Gould, R. J., Ginsberg, B. H. & Spector, A. A. (1982) *J. Biol. Chem.* **257**, 477–484
- Hargreaves, K. M. & Clandinin, M. T. (1987a) *Biochem. Biophys. Res. Commun.* **145**, 309–315
- Hargreaves, K. M. & Clandinin, M. T. (1987b) *Biochim. Biophys. Acta* **918**, 97–105
- Hirsch, J. & Gallian, E. (1968) *J. Lipid Res.* **9**, 110–119
- Jarett, L. & Kiechle, F. L. (1984) *Vitam. Horm. (NY)* **41**, 51–78
- Kahn, B. B. & Cushman, S. W. (1987) *J. Biol. Chem.* **262**, 5118–5124
- Kobayashi, M. & Olefsky, J. M. (1979) *Diabetes* **28**, 87–95
- Kolterman, O. G., Gray, R. S., Griffin, J., Burstein, P., Insel, J., Scarlett, J. A. & Olefsky, J. M. (1981) *J. Clin. Invest.* **68**, 957–969
- Kun, E. & Abood, L. G. (1949) *Science* **109**, 144–146
- Lewis, D. S., Cellucci, M. D., Masoro, E. J. & Yu, B. P. (1979) *Anal. Biochem.* **96**, 236–245
- Lonroth, P., DiGirolamo, M., Krotkiewski, M. & Smith, U. (1983) *Diabetes* **32**, 748–754
- Melchoir, D. L. & Czech, M. P. (1979) *J. Biol. Chem.* **254**, 8744–8747
- Morrison, W. R. & Smith, L. M. (1964) *J. Lipid Res.* **5**, 600–608
- Olefsky, J. M. & Reaven, G. M. (1975) *Endocrinology (Baltimore)* **96**, 1486–1498
- Olefsky, J. M. & Reaven, G. M. (1977) *Diabetes* **26**, 680–688
- Pedersen, O. (1984) *Dan. Med. Bull.* **31**, 1–32
- Pilch, P. F., Thompson, P. A. & Czech, M. P. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 915–918
- Rajotte, R. V., Erickson, C. L., Clandinin, M. T., Thomson, A. B. R. & Singh, B. (1988) *Diabetes Res.*, in the press
- Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
- Sandra, A. & Fyler, D. J. (1982) *Horm. Metab. Res.* **14**, 638–641
- Sandra, A., Fyler, D. J. & Marshall, S. J. (1984) *Biochim. Biophys. Acta* **778**, 511–515
- Schlessinger, J., Schlechter, Y., Willingham, M. C. & Pastan, I. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 7659–7662
- Schoenle, E., Zapf, J. & Froesch, E. R. (1977) *Diabetologia* **13**, 243–249

- Steel, R. G. D. & Torrie, J. H. (1980) *Principles and Procedures of Statistics*, 2nd edn., McGraw-Hill, New York
- Takenawa, T. & Nagai, Y. (1982) *J. Biochem. (Tokyo)* **91**, 793–799
- Touchstone, J. C., Chen, J. C. & Beaver, K. M. (1980) *Lipids* **15**, 61–62
- Van den Bosch, H. (1980) *Biochim. Biophys. Acta* **604**, 191–246
- Weekes, T. E. C., Wahle, K. W. J. & Lebaijuri, M. B. (1986) *Lipids* **21**, 220–225
- Whitesell, R. R. & Abumrad, N. A. (1985) *J. Biol. Chem.* **260**, 2894–2899
- Worcester, N. A., Bruckdorfer, K. R., Hallinan, T., Wilkins, A. J., Mann, J. A. & Yudkin, J. (1979) *Br. J. Nutr.* **41**, 239–252

Received 27 November 1987/29 February 1988; accepted 24 March 1988