Skeletal Muscle Membrane Lipid Composition Is Related to Adiposity and Insulin Action

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Abstract

The cellular basis of insulin resistance is still unknown, however, relationships have been demonstrated between insulin action in muscle and the fatty acid profile of the major membrane structural lipid (phospholipid). The present study aimed to further investigate the hypothesis that insulin action and adiposity are associated with changes in the structural lipid composition of the cell. In 52 adult male Pima Indians, insulin action (euglycemic clamp), percentage body fat (pFAT; underwater weighing), and muscle phospholipid fatty acid composition (percutaneous biopsy of vastus lateralis) were determined. Insulin action (high-dose clamp; MZ) correlated with composite measures of membrane unsaturation (% C20–22 polyunsaturated fatty acids [$r = 0.463, P < 0.001$], unsaturation index [$r = -0.369$, $P < 0.01$]), a number of individual fatty acids and with $\Delta5$ desaturase activity ($r = 0.451, P < 0.001$). pFAT (range 14–53%) correlated with a number of individual fatty acids and $\Delta5$ desaturase activity ($r = -0.610, P < 0.0001$). Indices of elongase activity ($r = -0.467, P < 0.001$), and $\Delta9$ desaturase activity ($r = 0.332, P < 0.05$) were also related to pFAT but not insulin action. The results demonstrate that $\Delta5$ desaturase activity is independently related to both insulin resistance and obesity. While determining the mechanisms underlying this relationship is important for future investigations, strategies aimed at restoring "normal" enzyme activities, and membrane unsaturation, may have therapeutic importance in the "syndromes of insulin resistance." (*J. Clin. Invest.* 1995. 96:2802–2808.) Key words: dietary fats • euglycemic clamp • body composition • elongase activity • desaturase activity

Introduction

Impaired insulin action (insulin resistance) is central to a cluster of prevalent diseases including non-insulin-dependent diabetes mellitus (NIDDM),[1] obesity, hypertension, dyslipidemias and cardiovascular disease (1–3). However, the basic mechanisms underlying insulin resistance are not known.

Skeletal muscle is the primary site of insulin stimulated glucose disposal at euglycemia (4, 5). Recently, a relationship between the fatty acid composition of skeletal muscle membrane structural lipid (phospholipid) and measures of insulin resistance have been demonstrated in both experimental animals and humans (6–8). This has demonstrated that the greater the percentage of polyunsaturated fatty acids (PUFA) in muscle membranes, the better the insulin action. With the exception of some marine oils, dietary fats must be desaturated and elongated to become the long-chain PUFA of muscle membranes. The enzymes involved in these transformations, that we have focussed on in this paper, include: $\Delta9$ desaturase, which inserts a double bond at the ninth carbon from the carboxyl terminal; $\Delta5$ desaturase, which inserts a double bond at the fifth carbon from the carboxyl terminal; and the ubiquitous elongase enzyme, which inserts two carbon units at the carboxy terminal of the fatty acid (9). How these factors relate to insulin action or to body composition, which itself is related to insulin resistance, has not been fully explored.

There is an emerging body of evidence to suggest that dietary fat profile is a determining factor in weight gain and adiposity (10–13). Dietary fat content has been shown to act at the molecular level, having a direct effect on gene expression including both hepatic lipogenesis (14) and desaturase activity (15).

The present study was aimed at investigating the relationship between insulin action and adiposity and the structural lipid composition of the cell membrane. The results demonstrate that both impaired insulin action and obesity are independently associated with reduced $\Delta5$ desaturase activity. In contrast, increased adiposity was additionally found to be associated with reduced elongase activity and higher $\Delta9$ desaturase activity.

Methods

Subjects. Individuals in this study were 52 male volunteer Pima Indians of the Gila River Indian Community who were participating in a longitudinal study of the development of NIDDM (16). The metabolic studies were performed in the clinical research unit of the National Institutes of Health (NIH) in Phoenix, Arizona. Subjects were 44 years of age

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1. *Abbreviations used in this paper:* C20–22 PUFA, the total percentage of long chain PUFA with ≥ 20 carbon units; UI, the unsaturation index; LFPIns, log 10 fasting plasma insulin; M, low-dose in vivo insulin mediated glucose disposal rate; MZ, high-dose in vivo insulin mediated glucose disposal rate; NIDDM, non-insulin-dependent diabetes mellitus; PUFA, polyunsaturated fatty acids.

or younger and in good health as assessed by medical history and physical examination. The current analysis was limited to individuals with a mean fasting plasma glucose concentration of less than 7.8 mM. Subject characteristics are listed in Table I. All subjects gave informed consent, and the studies were approved by the ethics committees of the NIH, the Indian Health Service and the Gila River Indian Community.

Upon admission to the clinical research unit, all subjects received a weight maintenance diet consisting of 50% carbohydrate, 30% fat, and 20% protein. A 75 gram oral glucose-tolerance test was performed after ≥2 d on the diet and diabetes mellitus was diagnosed according to World Health Organization criteria (17). At this base-line test, glucose tolerance was normal in all but one subject who was marginally glucose intolerant. Body composition (pFAT) was estimated by hydrodensitometry with simultaneous determination of lung residual volume (18, 19).

Euglycemic clamp. In vivo insulin-mediated glucose disposal rate was measured by a two-step euglycemic-hyperinsulinemic clamp according to a modification of the method of DeFronzo et al. (20) which has been described previously (21). The clamp was performed by a primed continuous low- and high-dose insulin infusion (290 and 2900 $pmol/min.m^2$ respectively) each of which were continued for 100 min while the plasma glucose was maintained at ~ 5.5 mmol/liter. It is unlikely that a true "steady state" is obtained during this length of insulin infusion, although the glucose uptake approaches "steady state" after 60 min. Nevertheless, the data does allow for relative comparisons between individuals to be made. The physiology for its own sake is not addressed and a pure "steady state" is not required for this analysis. The in vivo insulin action was determined during the period from 60 to 100 min. Both the low-dose or physiological insulin stimulation level (M) and high-dose or maximal insulin stimulation level (MZ) use the units of mg/min.kg fat-free mass +17.7 (22). Plasma glucose was measured by the glucose oxidase method using a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA) and insulin concentrations by radioimmunoassay using a radioassay analyzer (Concept 4; ICN, Horsham, PA).

Indirect calorimetry. 40 min before the initial insulin infusion and for the last 40 min of each insulin infusion, oxygen consumption and carbon dioxide production were determined by open circuit indirect calorimetry (23).

Muscle biopsy. 2 d after the euglycemic hyperinsulinemic clamp, a percutaneous biopsy of the vastus lateralis muscle was obtained using a Bergström needle (Depuy, Phoenix, AZ). The specimen was immediately frozen and stored in liquid nitrogen for later analysis.

Phospholipid fatty acid analysis. Extraction and derivatization of the fatty acid components of muscle phospholipids has been described elsewhere (12). In brief, muscle tissue was homogenized in 2:1(vol/vol) chloroform:methanol and total lipid extracts prepared according to Folch et al. (24). Phospholipids were isolated from less polar lipids by solid-phase extraction on Sep-Pak silica cartridges (Waters, Milford, MA). The phospholipids were transmethylated and the methyl fatty acids separated, identified and quantitated by gas chromatography.

Fatty acid data analysis. The content of individual fatty acids in the skeletal muscle phospholipids was expressed as a percentage of the total fatty acids identified. (For a review on the nomenclature and function of the principal PUFA and their metabolic interconversions see references 25 and 26). Two fatty acid indices were derived from the primary data: the average degree of fatty acid unsaturation (the unsaturation index; UI), which was calculated as the average number of double bonds per fatty acid residue multiplied by 100; and the total percentage of long chain PUFA with ≥ 20 carbon units (C20–22 PUFA). The activity of a number of the enzymes of fatty acid biosynthesis was estimated according to the product precursor ratios of the percentage of individual fatty acids. The estimated enzyme activities include: the ubiquitous elongase, calculated from the ratio of the percentage of 18:0 (stearic acid) to 16:0 (palmitic acid); and the Δ5 desaturase, calculated from the ratio of 20:4n-6 (arachidonic acid) to 20:3n-6; and Δ9 desaturase, calculated from the ratio of 18:1n-9 (oleic acid) to 18:0.

Statistics. All data are expressed as the mean±SEM. All statistical analyses were performed using Statview 512+ statistical package (Aba-

Table I. Subject Characteristics

Variable	Mean*	Minimum	Maximum
n	52		
Age (years)	27.5±0.7	18.1	43.8
Height (cm)	171.4±0.8	150.5	182.5
Weight (kg)	96.8±4.0	56.1	257.3
Body Mass Index (wt/ht²)	32.9±1.3	19.0	81.2
Percent body fat	28±1	14	53
Waist/thigh (cm)	1.7±0.1	1.3	2.3
Fasting plasma glucose (mM)	5.0±0.1	4.1	6.6
Fasting plasma insulin (pM)	215±15	83	677
Total M (mg/min.kgFFM + 17.7)	3.3±0.2	1.6	11.4
Total MZ (mg/min.kgFFM + 17.7)	9.0±0.3	3.9	16.3

cus Concepts Inc., Berkeley, CA). The relations between variables were analyzed by both simple and multiple regression with significance determined by F value.

Results

Mean values and ranges obtained for body size and insulin action are shown in Table I. The group is an overweight population as indicated by the mean BMI and pFAT of 32.9±1.3 and 28±1% respectively. The fatty acid profile of muscle phospholipids and correlations with fasting plasma insulin, clamp-derived M and MZ values, pFAT BMI and waist thigh ratio are listed in Table II. The relationships between the metabolic determinants and derived fatty acid indices are presented in Table III and the salient points are highlighted below.

Insulin action was significantly related to membrane phospholipid fatty acid proportions. This was true of measures of insulin action reflected by fasting insulin (LFPIns), glucose uptake at physiological insulin (M) or glucose uptake at supra physiological (MZ; maximally stimulating) insulin concentrations (Table III). Measures of insulin action were well correlated with each other and with measures of obesity (Table III). Similarly, measures of obesity were significantly correlated with each other and to membrane phospholipid fatty acid proportions. In contrast to these relationships, no significant relationships were observed between values for either basal lipid (0.58 ±0.03 mg/min.kg FFM; range 0.14–0.97) or carbohydrate (1.65±0.07mg/min.kg FFM; range 0.70–3.10) oxidation and any measure of insulin action or obesity.

Insulin action and fatty acid variables. As shown in Table III, significant correlations were found between MZ and: (*a*) the percentage of C20–22 PUFA; (*b*) Δ5 desaturase activity; and (*c*) the composite measure of unsaturation, UI. Similarly, insulin action at the low dose insulin infusion (M) was also positively correlated with the percentage of C20–22 PUFA and Δ5 desaturase activity (Fig. 1 *a*) but not UI.

Obesity and fatty acid variables. A number of individual fatty acids and derived indices were significantly correlated with measures of adiposity (Tables II and III). The Δ5 desaturase activity, related strongly to measures of adiposity (Fig. 1 *b*) as did the ratio 18:0/16:0, an index of elongase activity (Fig. 2). Furthermore, a significant ($P < 0.05$) relationship was observed

Table II. The Profile of Fatty Acids in the Phospholipid Fraction of Skeletal Muscle and Simple Correlations with Fasting Plasma Insulin, Insulin Action Indices, Percentage Body Fat, Body Mass Index and Waist to Thigh Ratio

Variable	Mean*	LFPIns[‡]	Log M[§]	Log MZ[‖]	pFAT[¶]	BMI**	Waist/thigh
16:0	15.3±0.2	0.156	−0.009	−0.094	0.334[a]	0.320[a]	0.153
18:0	13.1±0.2	−0.092	0.273	0.117	−0.286[a]	−0.288[a]	−0.168
16:1	1.2±0.1	0.304[a]	−0.263	−0.207	0.145	0.201	0.115
18:1n9	7.9±0.2	0.062	−0.147	0.065	0.251	0.189	0.156
18:2n-6	36.2±0.3	0.199	−0.201	−0.297[a]	0.069	0.121	0.149
20:3n-6	2.0±0.0	0.444[c]	−0.479[c]	−0.266	0.587[d]	0.528[d]	0.400[b]
20:4n-6	16.8±0.2	−0.416[b]	0.292[a]	0.391[b]	−0.322[a]	−0.348[b]	−0.132
22:5n-6	1.0±0.1	−0.299[a]	0.254	0.314[a]	−0.024	−0.081	−0.401[b]
22:6:n-3	1.3±0.1	−0.101	0.112	0.119	−0.254	−0.306[a]	−0.298[a]

* Values are means±SEM expressed as a percentage of total fatty acids. [‡] Log10 of the fasting plasma insulin (pM). [§] Log10 of the low-dose insulin-(290 pmol/min.m^2) mediated glucose disposal rate (mg/min/kgFFM + 17.7). [‖] Log10 of the high-dose insulin-(2900 pmol/min.m^2) mediated glucose disposal rate (mg/min/kgFFM + 17.7). [¶] Percentage body fat determined by densitometry. ** Body mass index (wt/ht^2). [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; [d] $P < 0.0001$.

between percentage body fat and Δ9 desaturase activity (18:1n-9/18:0).

Insulin action and obesity. Measures of insulin action were related to each other as well as to measures of obesity (Table III). Δ5 desaturase activity was the only lipid variable that correlated with all the measures of insulin action and all the obesity measures (Table III).

Table IV shows possible permutations of the independent relationships between Δ5, insulin action and adiposity. Δ5 related to LFPIns, M and MZ independent of pFAT, BMI and waist/thigh. In turn, Δ5 related to pFAT and BMI but not waist thigh ratio, independently of all three measures of insulin action.

The C20–22 PUFA and UI were related to insulin action but not to any direct measure of adiposity or central adiposity (Table III). On the other hand elongase and Δ9 were significantly related to BMI and pFAT but not at all to insulin action (Table III).

Discussion

The present study has demonstrated significant relationships between skeletal muscle membrane phospholipid fatty acid composition and both insulin action and adiposity in a population with the highest reported incidence of NIDDM in the world. Diabetes mellitus in this discrete population has been extensively characterized and roles for obesity, insulin receptor and post receptor function, insulin resistance, family history and genetic make-up in NIDDM have been determined (16, 27–33). The initial lesion that leads to diabetes in this population appears to be insulin resistance in skeletal muscle and possibly liver. The current studies relating obesity, insulin action and membrane phospholipid were performed in a search for possible underlying mechanisms for these previous clinical observations. These studies extend earlier findings in Caucasians showing relationships between insulin action and skeletal muscle membrane lipid composition (7, 8). The present data are unable to define the cause for this insulin resistance or the effect of obesity on insulin resistance. However, since membrane function is central to most physiological processes, the current findings have far reaching implications for possible causes of diabetes and obesity.

Cell membranes are dynamic lipid bilayers which separate the cell from the extracellular milieu and surround intracellular organelles. The major component is phospholipid which consti-

Table III. Correlation Table of Metabolic Determinants and Derived Fatty Acid Indices

Variable	Mean*	LFPIns[‡]	Log M[§]	Log MZ[‖]	pFAT[¶]	BMI**	Waist/thigh	UI	C20-22	Elongase	Δ9	Δ5
LFPIns	2.3±1.1	1										
Log M	0.45±0.02	−0.788[d]	1									
Log MZ	0.92±0.01	−0.854[d]	0.789[d]	1								
pFAT	28±1	0.649[d]	−0.558[d]	−0.458[c]	1							
BMI	32.9±1.3	0.557[d]	−0.447[b]	−0.408[b]	0.879[d]	1						
Waist/thigh	1.7±0.2	0.555[d]	−0.471[c]	−0.490[c]	0.572[d]	0.579[d]	1					
Unsaturation index	176.6±0.9	−0.371[b]	0.214	0.369[b]	−0.187	−0.306[a]	−0.193	1				
C20-22 polyunsaturated	22.7±0.3	−0.396[b]	0.296[a]	0.463[b]	−0.169	−0.284[a]	−0.245	0.839[d]	1			
Elongase activity (18:0/16:0)	0.85±0.0	−0.170	0.190	0.137	−0.467[c]	−0.422[b]	−0.240	0.129	0.069	1		
Δ9 activity (18:1n-9/18:0)	0.63±0.0	−0.108	−0.219	−0.008	0.332[a]	0.275[a]	0.223	−0.032	−0.079	−0.592[d]	1	
Δ5 activity (20:4n-6/20:3n-6)	8.02±0.2	−0.589[d]	0.563[d]	0.451[c]	−0.610[d]	−0.576[d]	−0.348[b]	0.421[b]	0.351[b]	0.365[b]	−0.341[a]	1

Mean values are means±SEM. Other values as described in Table II [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; [d] $P < 0.0001$.
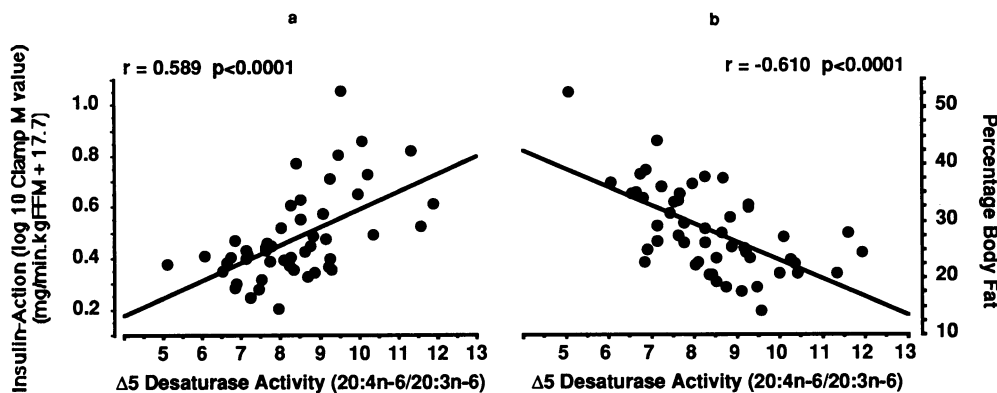
Figure 1. Insulin-action (M; a) and percentage body fat (b) in relation to Δ5 desaturase activity. The index of insulin-action at physiological insulin level (M; 290 pmol/min.m²) was derived from the hyperinsulinemic eu-glycemic clamp technique and is expressed in terms of the number of milligrams of glucose infused per minute per kg of fat free mass + 17.7. Percentage body fat was determined by hydrodensitometry. Δ5 desaturase activity was esti-mated according to the fatty acid product precursor ratio 20:4n-6/20:3 n-6.

tutes ~ 60% of the plasma membrane and > 90% of some organelle membranes such as mitochondria (34). Membranes permit the maintenance of ionic gradients, potential differences and modulate the passage of hormones, substrates, nutrients and intracellular signals. As such, the fatty acid composition of membrane phospholipids themselves are instrumental determi-nants of cellular metabolism. The current studies used total muscle membranes. Isolation of subcellular membrane fraction would doubtless provide even greater insight into the role of membranes in insulin action and obesity.

NIDDM is a genetic disease that becomes manifest under particular environmental conditions (27–30, 35, 36). The envi-ronmental component of NIDDM is demonstrated by the secular trends in diabetes prevalence this century, particularly in devel-oping societies (37, 38). Obesity has long been associated with the development of NIDDM and along with diet composition, may constitute one of the major "environmental" determinants of the development of the disease. The incidence of NIDDM in the Pima Indians is proportional to the degree of obesity as well as the family history of the disease (27) consistent with environmental and genetic causes. Obesity also correlates with the degree of insulin resistance and it seems possible that the impact of obesity on NIDDM may be mediated via insulin resistance (22, 33). Membrane lipid composition has the poten-tial to be influenced by both genetic and lifestyle factors and therefore may be the focal point at which these factors act in concert to determine insulin action. This could also be true for

example if the activities of the enzymes controlling the forma-tion of long-chain PUFA or their insertion into the structural lipids of cell membranes, were under strong genetic influence. In addition, dietary lipid profile (12, 39–41) and alcohol (42) are environmental factors capable of modifying membrane lipid composition.

With the exception of certain marine oils, the major dietary fatty acids must all be substantially elongated and desaturated to be transformed into the long chain PUFA that we have shown to be associated with leanness and insulin sensitivity. In this study, enzyme activities were determined indirectly by product precursor ratios. A strong relationship was observed between reduced Δ5 desaturase activity (20:4n-6/20:3n-6) and both re-duced insulin action and increased adiposity. Both insulin action and obesity are independently related to Δ5 and both obesity and Δ5 independently correlate with M values. This means that the strong relationship of the Δ5 to insulin resistance is not merely an epiphenomenon of the effects of obesity and therefore deserves consideration as part of the genetic predisposition to insulin resistance (28). On the other hand, the C20–22 PUFA and UI independently correlate with measures of insulin action but not obesity. This could be due to effects of either genotype or diet directly on insulin action.

The elongase activity (18:0/16:0), which inserts two carbon units onto the fatty acid backbone, showed a relationship to obesity but not to insulin action. The elongase enzyme is thought to be ubiquitous throughout all fatty acid biosynthetic pathways and it must work in concert with the desaturase en-zymes as requisite steps between the major dietary n-6 and n-3 fatty acids (linoleic and α-linolenic) and the formation of the important long chain PUFA.

Δ9 desaturase, which inserts a double bond at carbon nine of the fatty acid chain, was also associated with obesity but not insulin action. This is a novel finding in humans but entirely consistent with results in animal models of obesity (13). Though the etiological significance of increased Δ9 desaturase activity in obesity is unclear it may relate to an attempt to compensate for major decreases in other desaturase enzymes in order to maintain cell membrane fluidity within some regulated range. Certainly there is clear evidence for major (and unchrac-teristic) increases in the n-9 class of fatty acids during essential (n-6 and/or n-3) fatty acid deficiencies (43, 44). Studies aimed at measuring enzyme activity before and after intervention (re-sulting in both weight loss or weight gain) would be instructive
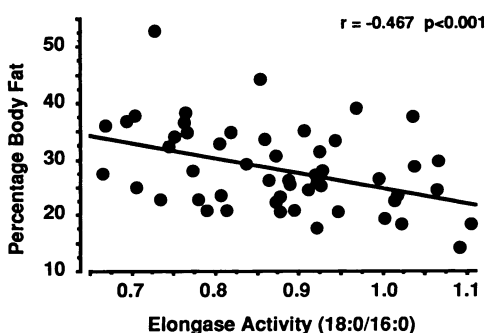

Figure 2. Percentage body fat in relation to elongase activity. Elongase activity was estimated from the product precursor ratio of the percent-ages of individual fatty acids 18:0/16:0.

Table IV. Multiple Regression Results

Independent variables	Δ5 = y t	Δ5 = y P	Independent variables	Δ5 = y t	Δ5 = y P	Independent variables	Δ5 = y t	Δ5 = y P	Independent variables	LFPIns = y t	LFPIns = y P	Independent variables	Log M = y t	Log M = y P	Independent variables	Log MZ = y t	Log MZ = y P
pFAT	2.623	P < 0.0116	BMI	2.659	P < 0.0106	W/T	0.204	P < 0.8394	pFAT	3.586	P < 0.0008	pFAT	2.382	P < 0.0213	pFAT	1.833	P < 0.0731
LFPIns	2.517	P < 0.0152	LFPIns	3.139	P < 0.0029	LFPIns	4.215	P < 0.0001	Δ5	2.517	P < 0.0152	Δ5	2.461	P < 0.0176	Δ5	1.718	P < 0.0923
pFAT	3.280	P < 0.0002	BMI	3.350	P < 0.0016	W/T	0.784	P < 0.4372	BMI	2.467	P < 0.0172	BMI	1.270	P < 0.2103	BMI	1.423	P < 0.1643
Log M	2.461	P < 0.0176	Log M	3.148	P < 0.0029	Log M	3.774	P < 0.0004	Δ5	3.139	P < 0.0029	Δ5	3.148	P < 0.0029	Δ5	2.067	P < 0.0442
pFAT	4.054	P < 0.0002	BMI	3.767	P < 0.0005	W/T	1.139	P < 0.2607	W/T	3.671	P < 0.0006	W/T	2.608	P < 0.0122	W/T	2.971	P < 0.0047
Log MZ	1.718	P < 0.0923	Log MZ	2.067	P < 0.0442	Log MZ	2.500	P < 0.0160	Δ5	4.215	P < 0.0001	Δ5	3.774	P < 0.0004	Δ5	2.500	P < 0.0160

These are the multiple regressions for an equation $y = x_1 + x_2$. Included are the t and P values which are the significance of the co-efficients for the x_1 and x_2 variables (i.e., their independent effects as predictors).

in elucidating whether altered $\Delta9$ or desaturase activities are the likely cause or effect in adiposity.

The results indicate that some aspects of fatty acid metabolism are of considerable importance in the well recognized obesity-insulin action relationship while others are not. This is consistent with the observations that insulin action has determinants that are independent of obesity (28) and that obesity bears a complex relationship with insulin resistance (45).

Given the relationships between adiposity and phospholipid biosynthetic enzyme activities what are the possible mechanisms which might subtend such relationships? Increases in ion "leakage" and Na^+/K^+ ATPase activity, (i.e., increased whole-body metabolic rate) have been directly associated with increased PUFA content in comparative studies between endotherms and ectotherms (46, 47). In animals and humans, increasing the dietary polyunsaturated to saturated ratio resulted in increased membrane unsaturation (12, 41) and increased basal metabolic rate (10, 26). The effect of leaking membranes may not be limited to energy expenditure. Leaking membranes are the basis of spontaneous membrane depolarization which may affect neuronal firing rates and in turn muscle gene expression affecting muscle fibre type (48, 49). Altered ion flux may also influence central nervous system activity and hence the regulation of numerous processes including appetite.

The overall control of the partitioning of dietary fats between storage and oxidation is not clear. However, work in rodents has shown very clear differences between the oxidation rates of different fatty acids (50). Longer-chain PUFA are oxidized at much faster rates than are saturated fats. Alteration in enzyme activities, as are here associated with obesity, would favor an increased proportion of the less readily oxidized fatty acids, and thus may cause, or at least exacerbate, the tendency towards decreased fat oxidation in the obese. This study found no relationship between lipid oxidation rate and phospholipid fatty acid composition. This may reflect the fact that most of the lipid for oxidation is coming from storage triglyceride and the relationship of its fatty acid profile to that of muscle membrane phospholipid is unknown.

Docosahexaenoic acid (22:6n-3) is the most highly unsaturated and longest chain of any fatty acid found in skeletal muscle. It comprises the bulk proportion of total n-3 fatty acids. Unless provided in the diet (primarily as marine oils), its precursors must be substantially elongated and desaturated to form this fatty acid. One striking difference between the muscle phospholipid fatty acid composition in the Pima Indians and an Australian, largely Caucasian, population (7) is the low percentage of long chain n-3 PUFA (22:6n-3: $1.2\pm0.1\%$ versus $2.5\pm0.7\%$ in the Australian population). This finding may reflect a very low dietary intake of n-3 in the Pima Indians. However, among the Australian population even individuals with little or no discernible n-3 intake (unpublished observations) had muscle membrane n-3 levels much higher than the Pima study group. The low levels of n-3 in the Pimas may then reflect a genetic reluctance to incorporate this important class of fatty acids into membranes, thus predisposing this population to the "syndromes of insulin resistance." Further to this point, an intestinal fatty acid binding protein loci on chromosome 4q has been significantly linked with insulin resistance in Pima Indians (32). It is tempting to speculate that the "thrifty gene(s)" (51, 52) may therefore include, those which control the binding and uptake of certain fatty acids, the incorporation of specific PUFA into membrane lipids, and/or regulation of

the production and activity of the enzymes that elongate and desaturate fatty acids.

This study is unable to determine which are the cause and effects in any of the relationships described. The possibility that $\Delta 5$ desaturase activity could be part of the genetic regulation of insulin resistance needs to be explored. Whether any effect of diet on insulin resistance could be mediated through this enzyme or the fatty acid levels it regulates deserves study. Finally, it is possible that obesity directly affects elongase and $\Delta 9$ desaturase activity, but it should also be considered that such alterations of activity might lead to obesity.

Acknowledgments

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