Serum Phospholipid Fatty Acid Composition and Insulin Action in Type 2 Diabetic Patients

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Relationships have been demonstrated between insulin sensitivity and the fatty acid (FA) composition of serum and tissue lipids in adult humans. The present study aimed to investigate the above relationships in different groups of type 2 diabetic patients (DM2). The FA composition of serum phospholipids (S-PL) measured by gas liquid chromatography and insulin action during a 2-step hyperinsulinemic isoglycemic clamp (1 and 10 mU/kg · min) were determined in 21 newly diagnosed DM2 subjects (DMN), in groups of long-term DM2 patients treated with hypoglycemic agents (DMH; n = 21) or diet alone (DMD; n = 11), and in 24 healthy subjects (HS). In diabetics, the metabolic clearance rates of glucose at both insulin levels (MCR_{glu}submax and MCR_{glu}max) were significantly reduced compared with HS (MCR_{glu}submax DMN, 5.35 ± 2.7 mL · kg^{-1} · min^{-1}, DMH, 5.38 ± 2.17 mL · kg^{-1} · min^{-1}; DMD, 5.48 ± 2.35 mL · kg^{-1} · min^{-1} *v* HS, 10.9 ± 3.3 mL · kg^{-1} · min^{-1}; *P* < .01; MCR_{glu}max DMN, 13.3 ± 3.3 mL · kg^{-1} · min^{-1}; DMH, 12.5 ± 3.0 mL · kg^{-1} · min^{-1}; DMD, 13.3 ± 3.0 mL · kg^{-1} · min^{-1} *v* HS, 17.4 ± 3.8 mL · kg^{-1} · min^{-1}; *P* < .05). Increased contents of highly unsaturated n-6 family FA (*P* < .01), arachidonic acid in particular (DMN, 10.98% ± 1.79%; DMD, 10.78% ± 1.64%; DMH, 10.97% ± 1.7% *v* HS, 8.51% ± 1.53%; *P* < .001), were found in all groups of diabetics compared with HS, while lower levels of linoleic acid were seen in DMN (*P* < .001) and DMH (*P* < .05). The contents of saturated FA and monounsaturated FA were comparable in HS, DMN, and DMD. While in HS there were significant negative correlations between MCR_{glu} and the contents of saturated FA and a positive association between insulin action and proportions of linoleic and arachidonic acids, no significant relationships were found in diabetic subjects. Different groups of DM2 patients show an altered FA pattern of S-PL, which is not related to insulin action. The above data support the hypothesis that changes in FA composition may play a role in modulating insulin action in peripheral tissues, but cannot explain the insulin resistance (IR) in DM2 patients.

INSULIN RESISTANCE (IR) represents a major abnormality underlying cardiovascular disease. It is strongly linked to the development of a cluster of prevalent diseases including dyslipidemia, hypertension, central obesity, type 2 diabetes mellitus (DM2), endothelial dysfunction, and hemostatic disturbances, all of which are associated with an increased risk of atherosclerosis.[1-3] However, the basic mechanisms of IR are not known.

Dysregulation of fatty acid (FA) metabolism may be close to the center of the pathophysiology of IR, at least as it relates to the risk for cardiovascular disease.[2] Substantial evidence has now accumulated for a major role of fat subtypes in insulin action and atherogenesis. Studies, both in experimental animals and adult humans, show that IR resistance may be influenced by the amount and quality of dietary fats.[4-7] Our group first pointed to the significant relationships between the FA composition of serum phospholipids (S-PL) and insulin action, as measured in vivo using the hyperinsulinemic euglycemic clamp in healthy humans.[8] Similar associations between insulin action and the FA pattern of serum cholesterol esters have been reported by other investigators.[9,10] Skeletal muscle is the main site of insulin-stimulated glucose uptake,[11] and studies in adult humans have shown relationships between skeletal muscle membrane phospholipids and insulin action.[9,12,13] In essence, a higher proportion of saturated FA is associated with IR, whereas a higher proportion of long-chain polyunsaturated FA is associated with insulin sensitivity. Significant relationships have been demonstrated not only in healthy adults but, also, in insulin-resistant subjects, such as patients with ischemic heart disease[12] and in Pima Indians.[13]

IR is considered to be a basic abnormality underlying DM2. It plays a critical role in the pathogenesis of the disease in terms of glucose intolerance, but IR also seems to be important as a factor associated with an increased risk of cardiovascular morbidity and mortality among diabetic patients.[14,15] Human studies have shown that the FA composition of serum lipids of DM2 patients differs from that of subjects with normal glucose tolerance. When newly diagnosed DM2 patients were investigated and compared with healthy controls, they were found to have considerably higher proportions of saturated FA[16] and lower proportions of linoleic acid[16,17] in serum cholesterol esters and phospholipids. However, whether or not the FA pattern of lipids is directly related to insulin sensitivity in DM2 patients is yet to be examined.

The aims of the present study were (1) to analyze the FA composition of S-PL in healthy subjects and in groups of DM2 patients with varying duration of the disease, degree of glucose intolerance, and blood glucose control, as well as the type of treatment and (2) to investigate the relationships between FA composition and insulin action, as assessed by a 2-step hyperinsulinemic isoglycemic clamp technique, to test the hypothesis that altered FA pattern is responsible for insulin resistance in DM2 subjects. Because IR is influenced by body weight, only nonobese subjects were included, and the study groups were comparable in terms of body mass index (BMI).

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SUBJECTS AND METHODS

Subjects

The study group consisted of 53 men with DM2 as defined by the criteria of the American Diabetes Association.[18] Twenty-one of them were diabetics whose condition was diagnosed within 1 year since screening, who were less than 45 years of age, treated with a diet, with a BMI less than 30 kg/m^2 (DMN). The remaining patients were older individuals with a comparable BMI, with disease duration of more than 8 years, and treated with a diet (DMD; n = 11) or glibenclamide (10 mg/day) (DMH; n = 21). None had a history or clinical evidence of a significant cardiovascular disease, diabetic nephropathy, or proliferative retinopathy, as demonstrated by routine fundoscopic examination. Fifteen patients reported labile hypertension in their history. The group of healthy subjects (HS) comprised 24 weight- and sex-matched volunteers. They were not taking any drugs, and none had a family history of diabetes. Normal glucose tolerance was confirmed by the oral glucose tolerance test (OGTT). All subjects were euthyroid, and none had a concomitant disease. Exclusion criteria included respiratory, renal, hepatic, gastrointestinal or other diseases, alcoholism, and drug abuse. The clinical and metabolic characteristics of the study groups are summarized in Table 1, which shows that the groups had a comparable dietary intake, based on records of all meals and drinks taken by the subjects for 3 consecutive days before examination.[19] Informed consent was obtained from all the individuals after the purpose, nature, and potential risks of the study had been explained to them.

Procedures

The subjects were examined on an outpatient basis, after overnight fasting with only tap water allowed ad libitum. They were instructed to adhere to their ordinary lifestyle and avoid changes in food intake, alcohol consumption, and exercise.

The 2-step hyperinsulinemic isoglycemic clamp study, taking 5 hours to complete, was conducted as previously described.[20] Briefly, a Teflon cannula (Venflon; Viggo, Helsingborg, Sweden) was inserted into an antecubital vein for the infusion of all test substances. A second cannula was inserted retrogradely into a wrist vein for blood sampling, and the hand was placed in a heated (65°C) box to achieve venous blood arterialization. A stepwise primed-continuous insulin infusion (1 and 10 mU/kg · min of Actrapid HM; NovoNordisk, Copenhagen, Denmark) was administered to acutely raise and maintain the plasma concentrations of insulin. Plasma glucose concentrations during the clamp were maintained at the fasting levels by continuous infusion of 15% glucose. Blood samples for plasma immunoreactive insulin (IRI) determination were taken before (0 minute) and at 150, 175, 180, 270, 285, and 300 minutes of the clamp study.

The OGTT was performed by giving the subjects 100g of glucose dissolved in 400 mL of water. Blood for measuring plasma glucose, IRI, and C-peptide concentrations was taken from antecubital veins before and at 30, 60, and 120 minutes after glucose load.

Analytical Methods

Plasma glucose concentrations were measured on a Beckman analyzer (Beckman Instruments, Fullerton, CA) using glucose oxidase method. IRI was determined by radioimmunoassay using an IMMUNOTECH Insulin IRMA kit (IMMUNOTECH as, Prague, Czech Republic). C-peptide was analyzed by radioimmunoassay using an IMMUNOTECH C-Peptide IRMA kit (IMMUNOTECH as). Glycosylated hemoglobin was measured by a Bio-Rad Haemoglobin A_{1c} Column Test (Bio-Rad Laboratories GmbH, Munich, Germany).

Serum lipids were extracted according to Folch et al.[21] Lipid classes were separated by thin layer chromatography using hexane-diethyl ether-acetic acid (80:20:3, vol/vol) as the solvent system. FA in S-PL were converted to methyl esters using a modification of the method of Stoffel at al.[22] FA of S-PL scraped off the plate were transmethylated with HCl-methanol in the presence of silica gel in sealed ampoules. FA methyl esters were eluted with hexane. Gas chromatography of FA methyl esters was performed on a GS 5890A (Hewlett Packard, Avondale, PA) instrument equipped with a flame-ionization detector. A carbowax fused silica capillary column (25 m × 0.25 mm internal diameter) was used. The column temperature was 150° to 225°C (2°C/min), and hydrogen was used as the carrier gas. Individual peaks of FA methyl esters were identified by comparing the retention times with those of authentic standards (Sigma, Prague, Czech Republic).

All FA expressed as mol% were divided into the following groups: saturated FA (SFA: 14:0, 16:0, 17:0, 18:0, 22:0, 24:0); monounsaturated FA (MUFA: 14:1, 16:1, 18:1, 24:1); polyunsaturated FA of the n-6 family (PUFA n-6: 18:2, 18:3, 20:3, 20:4, 22:4); highly unsaturated

Table 1. Characteristics of Study Groups

	Healthy Subjects (n = 24)	DMN (n = 21)	DMD (n = 11)	DMH (n = 21)
Age (yr)	39.8 ± 3.1	41 ± 2.6	46.1 ± 3.6*	51.8 ± 6.1*
BMI (kg/m^2)	25.3 ± 2.2	26.2 ± 3.2	26.0 ± 2.1	26.7 ± 2.2
Fasting plasma glucose (mmol/L)	4.8 ± 0.35†	8.0 ± 1.69	8.29 ± 2.2	8.2 ± 2.5
Plasma glucose 120 (mmol/L)	4.4 ± 1.04†	16.2 ± 4.0	10.3 ± 4.33‖	15.5 ± 3.86§
AUC_{IRI}	4,975 ± 1,961	4,915 ± 2,804	5,428 ± 1,934	4,591 ± 1,681
$AUC_{C\text{-}peptide}$	169 ± 78	158 ± 82	205 ± 78	154 ± 72
HbA_{1c}(%)	5.4 ± 1.1†	9.4 ± 2.9	6.94 ± 1.6‖	8.7 ± 1.2§
S-cholesterol (mmol/L)	5.1 ± 1.2‡	5.9 ± 1.1	6.17 ± 1.17	6.4 ± 1.2
S-triglycerides (mmol/L)	0.98 ± 0.23†	2.09 ± 1.58	2.18 ± 1.32	1.83 ± 1.02
Diabetes duration (yr)		0.4 ± 0.4	10.5 ± 1.3	9.8 ± 1.4
Dietary energy intake (MJ/d)	10.2 ± 2.9	9.6 ± 2.5	9.3 ± 2.8	9.1 ± 2.6
Carbohydrates (%)	43.9 ± 3.2	43.6 ± 5.1	40.1 ± 7.1	40.6 ± 6.2
Fats (%)	41.1 ± 5.8	38.3 ± 5.5	41.3 ± 6.8	39.9 ± 5.1
Proteins (%)	15.0 ± 2.2	19.9 ± 4.6	18.6 ± 2.0	19.4 ± 1.9

Abbreviations: DMN, newly detected type 2 diabetics; DMD, type 2 diabetics treated with diet only; DMH, type 2 diabetics treated with glibenclamide; plasma glucose 120′, plasma glucose at 120 minutes of OGTT; AUC_{IRI}, area under the insulin curve during OGTT; $AUC_{C\text{-}peptide}$, area under the C-peptide curve during OGTT.

Statistical significance: *$P < .01$ *v* healthy subjects; †$P < .001$ *v* DMN, DMH, and DMD; ‡$P < .01$ *v* DMN, DMH, and DMD; §$P < .01$ *v* DMN; ‖$P < .001$ *v* DMD.

daughter FA of the n-6 family (DPUFA n-6; PUFA n-6 minus 18:2 n-6); and polyunsaturated FA of the n-3 family (PUFA n-3; 8:3, 20:5, 22:5, 22:6). The product/precursor ratios were used to calculate the activities of enzymes involved in FA metabolism: elognase (18:0/16:0), Δ6 desaturase (18:3 n-6/18:2 n-6), Δ5 desaturase (20:4 n-6/20:3 n-6), and Δ9 desaturase (16:1 n-7/16:0).

Data Analysis

The isoglycemic rather than the euglycemic insulin clamp protocols were used to assess insulin sensitivity to study the diabetic patient in their native (hyperglycemic) conditions and to avoid the confounding effect of insulin preinfusion (to achieve euglycemia). Insulin action was estimated as the metabolic clearance rate of glucose calculated at minutes 140 to 180 of the clamp at the insulin infusion rate of 1 mU/kg · min (MCR_{glu}submax), and from minutes 240 to 300 at the insulin infusion rate of 10 mU/kg · min (MCR_{glu}max) as the rates of glucose infusion, after correction for changes in glucose pool size and urinary glucose loss, divided by ambient glucose concentration.[20] The metabolic clearance rates of glucose in diabetic patients are independent of glycemia over a wide range.[23] Hepatic glucose production was not measured in this study. MCR values may have been slightly underestimated in DM2 patients, as we were unable to exclude impaired suppression of hepatic glucose production by insulin in this group. Sensitivity index (SI) was calculated in the 140- to 180-minute time period as another measure of insulin action. SI = MCR_{glu}submax (mL/kg · min)/IRI (μU/mL, mean concentration in the 140- to 180-minute time period) × 100. The areas under the curve for IRI (AUC_{IRI}) and C-peptide ($AUC_{C\text{-}peptide}$) during the OGTT were calculated by the trapezoidal method.

Statistical analysis. The data were analyzed by Kruskal-Wallis 1-way analysis of variance (ANOVA). Spearman rank correlation was used for testing the relationships between variables. The courses of IRI and C-peptide during the OGTT were evaluated using ANOVA with repeated measures and grouping factor. All data are expressed as means ± SD.

RESULTS

The FA composition of S-PL is shown in Table 2. There is a significant decrease in the proportion of linoleic acid (18:2 n-6) in DMN ($P < .001$) DMD ($P < .05$) compared with healthy subjects. A tendency to decrease is seen also in DMH, but it is not statistically significant. On the other hand, the contents of n-6 DPUFA, in particular the proportion of arachidonic acid (20:4 n-6), are higher in all groups of diabetics compared with HS ($P < .001$), a finding consistent with increased desaturation activity. The total contents of n-6 PUFA remain unaltered in DMN and DMD. In DMH, the proportion of n-6 PUFA is higher compared with that of healthy subjects ($P < .01$) and DMD ($P < .05$), who are of the same age and with the same duration of diabetes. The groups are comparable in proportions of n-3 PUFA. The contents of saturated FA are similar in DMN, DMD, and in HS, while in DMH the proportions of saturated FA are lower compared with those of controls ($P < .001$) and DMD ($P < .001$). The proportions of monounsaturated FA are unchanged in DMD and DMH, whereas they are higher in DMN than in HS ($P < .01$) and DMH ($P < .05$). Activities of the enzymes involved in FA metabolism are unchanged in diabetics.

Long-term blood glucose control (HbA_{1c}) is worse in DMN and DMH compared with DMD. The concentrations of IRI and C-peptide during the OGTT are shown in Fig 1. Although the AUC_{IRI} and $AUC_{C\text{-}peptide}$ are comparable in all groups (Table 1), the courses of both IRI and C-peptide during the OGTT are different in diabetics and HS (IRI, $P < .001$; C-peptide, $P < .001$). DMN, DMD, and DMH have fasting hyperinsulinemia and altered dynamics of insulin secretion after glucose load.

Mean IRI concentrations during the 2-step isoglycemic hyperinsulinemic clamps were held constant at similar plateaus in HS, DMN, and DMD (140 to 180 minutes: 80 ± 19 *v* 73 ± 24 *v* 71 ± 17 μU/mL; 260 to 300 minutes: 1,510 ± 431 *v* 1,483 ± 451 *v* 1,612 ± 277 μU/mL). In DMH, they were significantly higher during both periods compared with the other groups (140 to 180 minutes: 115 ± 41 μU/mL, $P < .01$; 260 to 300 minutes: 2,046 ± 693 μU/mL, $P < .05$).

MCR_{glu}submax, MCR_{glu}max (Fig 2), and HSI ($P < .01$) (HS: 13.54 ± 3.2 *v* DMN: 8.1 ± 4.7; DMD: 6.12 ± 4.9; DMH: 7.7 ± 4.3$mL^2 \cdot kg^{-1} \cdot min^{-1} \cdot \mu U^{-1}$) were significantly lower in all groups of diabetics compared with HS. Despite the clinical differences between the groups of diabetics, the degrees of insulin resistance were comparable in DMN, DMD, and DMH.

In the group of HS, significant positive correlations were found between the contents of n-6 PUFA and insulin action: linoleic acid (18:2 n-6) and MCR_{glu}submax ($r = +.59$; $P < .05$); linoleic acid and MCR_{glu}max ($r = +.66$; $P < .01$); arachidonic acid (20:4 n-6) and MCR_{glu}submax ($r = +.63$; $P < .05$). Conversely, the content of saturated FA negatively correlates with MCR_{glu}submax ($r = -.52$; $P < .05$) and MCR_{glu}max ($r = -.76$; $P < .01$). Figure 3 shows the relationships between insulin action and the ratio of 18:2 n-6 to saturated FA. No significant correlations were found between the FA composition of S-PL and estimates of insulin sensitivity in diabetics.

DISCUSSION

The results of this study demonstrate an altered FA composition of S-PL in groups of DM2 patients with different mean ages, duration of disease, degrees of glucose intolerance, blood glucose control, and type of treatment. The shifts in the proportions of n-6 family FA were a general finding in diabetic subjects, while no differences were seen in the contents of saturated FA, monounsaturated FA, and n-3 family FA. A lower content of linoleic acid and, by contrast, higher proportions of their daughter FA (DPUFA n-6) were found in diabetics compared with HS. The diabetics treated with glibenclamide (DMH), the patients with worse blood glucose control, and the most severe glucose intolerance showed similar trends in FA changes, but the decrease in the content of linoleic acid was only marginal compared with DMN and DMD; and the proportions of saturated FA were even lower, and the contents of monounsaturated FA higher compared with those in HS. Our results indicate that shifts in the FA composition of lipids can be seen in different groups of type 2 diabetics, and that they lasted during the course of the disease.

Low concentrations of linoleic acid and high proportions of DPUFA n-6 were also found in cholesterol esters and membrane phospholipids of erythrocytes in newly detected DM2 patients.[16,17] A similar FA pattern was seen in patients with long-term diabetes in erythrocyte phosphatidylcholine[24] and platelet phospholipids, where the high content of arachidonic

Table 2. FA Composition of S-PL

	Healthy Subjects (n = 24)	DMN (n = 21)	DMD (n = 11)	DMH (n = 21)
FA (%)				
14:0	0.57 ± 0.28	0.54 ± 0.30	0.46 ± 0.14	0.57 ± 0.52
16:0	28.29 ± 2.44	28.29 ± 1.61*†	30.31 ± 3.52	26.04 ± 1.94‡§
17:0	0.46 ± 0.11	0.51 ± 0.18	0.40 ± 0.17	0.45 ± 0.17
18:0	13.37 ± 1.25	12.23 ± 1.72‖†	13.61 ± 1.11	12.32 ± 0.96†¶
24:0	1.43 ± 0.49	1.17 ± 0.73‡‡	1.37 ± 0.40	1.57 ± 0.38
Saturated FA	45.35 ± 3.67	43.56 ± 2.07*¶	46.39 ± 3.80	41.11 ± 2.30**§
16:1	1.33 ± 0.24	1.64 ± 0.35†	1.20 ± 0.73	1.48 ± 0.41
18:1	11.78 ± 1.56	12.35 ± 1.80	11.71 ± 1.93	12.09 ± 1.19
24:1	2.80 ± 0.77	3.77 ± 0.89**‡‡¶	2.69 ± 0.98	3.14 ± 0.62
Monounsaturated FA	15.92 ± 2.08	17.77 ± 2.25‡†	15.61 ± 2.34	16.71 ± 1.33
18:2 n-6	18.52 ± 2.49	15.49 ± 1.92**††	16.47 ± 2.67‖	17.92 ± 3.60
18:3 n-6	0.69 ± 0.99	0.34 ± 0.26	0.22 ± 0.22	0.34 ± 0.29
20:3 n-6	2.02 ± 0.38	2.99 ± 1.09**	2.36 ± 1.03	2.86 ± 0.72**
20:3 n-6	2.02 ± 0.38	2.99 ± 1.09**	2.36 ± 1.03	2.86 ± 0.72**
20:4 n-6	8.51 ± 1.53	10.98 ± 1.79**	10.78 ± 1.64**	10.97 ± 1.70**
20:5 n-6	0.91 ± 0.69	1.11 ± 0.56¶	0.24 ± 0.54	1.17 ± 0.90¶
n-6 PUFA	30.04 ± 3.65	30.92 ± 3.62	30.07 ± 4.47	33.28 ± 4.00‡†
n-6 DPUFA	11.52 ± 1.32	15.43 ± 1.56**	13.6 ± 1.8‡	15.36 ± 1.87**
18:3 n-3	0.29 ± 0.14	0.20 ± 0.19	0.24 ± 0.19	0.17 ± 0.22‖
20:5 n-3	0.82 ± 0.41	1.04 ± 0.54	1.17 ± 0.78	1.36 ± 1.13‖
20:5 n-3	0.82 ± 0.41	1.04 ± 0.54	1.17 ± 0.78	1.36 ± 1.13‖
22:5 n-3	1.56 ± 1.20	1.12 ± 1.29	1.34 ± 1.54	0.79 ± 0.20‡
22:6 n-3	2.11 ± 0.49	3.04 ± 0.87**†	2.32 ± 0.83	3.17 ± 1.04**†
22:6 n-3	2.11 ± 0.49	3.04 ± 0.87**†	2.32 ± 0.83	3.17 ± 1.04**†
n-3 PUFA	4.67 ± 1.39	5.40 ± 1.75	5.07 ± 1.07	5.49 ± 2.07
Elongase	0.47 ± 0.04	0.43 ± 0.72	0.46 ± 0.04	0.45 ± 0.06
Δ6 desaturase	0.039 ± 0.061	0.024 ± 0.019	0.015 ± 0.015	0.011 ± 0.012
Δ5 desaturase	4.34 ± 1.09	4.30 ± 2.26	3.84 ± 0.907	4.88 ± 2.33
Δ9 desaturase	0.47 ± 0.01	0.057 ± 0.111	0.054 ± 0.017	0.41 ± 0.025

Abbreviations: DMN, newly detected type 2 diabetics; DMD, type 2 diabetics treated with diet only; DMH, type 2 diabetics treated with glibenclamide.

*$P < .001$ *v* DMH.
†$P < .05$ *v* DMD.
‡$P < .01$ *v* healthy.
§$P < .001$ *v* DMD.
‖$P < .05$ *v* healthy.
¶$P < .01$ *v* DMD.
‡‡$P < .05$ *v* DMH.
**$P < .001$ *v* healthy.
††$P < .01$ *v* DMH.

acid (20:4 n6) could be a consequence of enhanced arachidonic acid uptake activity of thrombocytes.[25] Vessby et al[26] reported a high proportion of dihomo-γ-linolenic acid (20:3 n-6) in serum cholesterol esters is an independent predictor for the development of diabetes during a 10-year follow-up in middle-aged men. In contrast with the above studies, low levels of DPUFA n-6[27-29] and high saturated FA[16,28,29] in the serum lipids of DM2 patients were reported by other investigators.

The FA composition in DM2 without clinical evidence of significant atherosclerosis shows a patter similar to the alterations described in nondiabetics with cardiovascular complications.[30] In most studies, the decrease in linoleic acid was associated with an increased incidence of myocardial infarction, angina pectoris, ischemic disease of the lower limb, and cardiovascular death.[31-35] The situation is more complicated regarding metabolites of linoleic acid (DPUFA); both deleterious[32,36] and protective[31,33,34] effects of arachidonic acid regarding atherosclerotic complications have been reported in the relevant literature.

What may be the cause of the above changes in FA composition of S-PL in diabetics? Both genetic and environmental factors contribute in determining the serum and tissue FA pattern. Apart from nutrition, changes in the activities of enzymes involved in the synthesis, metabolism, and utilization of FA may play a role.[37] Insulin has been shown to stimulate FA desaturation[38,39] and despite IR in glucose metabolism, insulin action in FA metabolism could be preserved in DM2.[40] Therefore, the shifts in the n-6 FA pattern (high contents of DPUFA n-6 at the expense of linoleic acid) could be due to overstimulation of FA desaturation and elongation by hyperinsulinemia in diabetics. In the present study, we did not confirm increases in the activities of Δ5 and Δ6 desaturases, but the activities were estimated only indirectly;

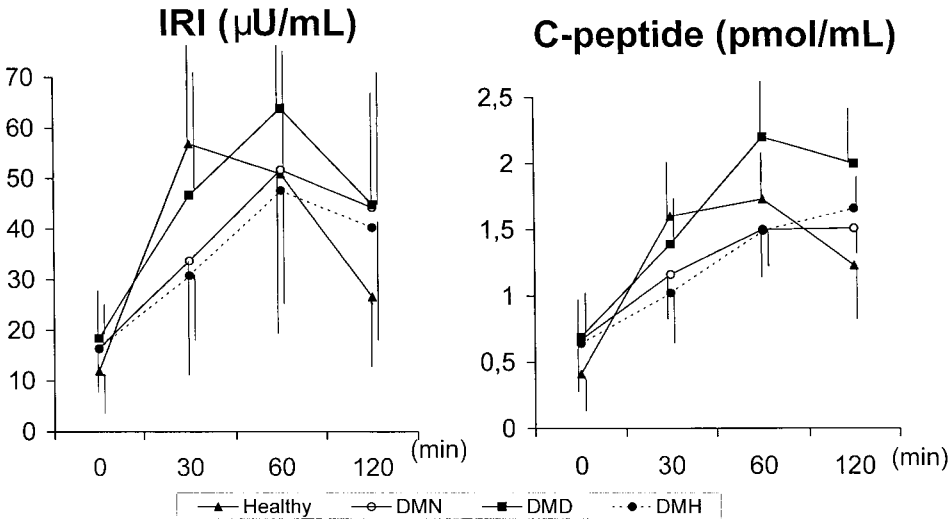

Fig 1. IRI and C-peptide concentrations during OGTT. Statistical significance (ANOVA with repeated measures and grouping factor): Course of IRI (*P* < .001, healthy subjects *v* DMN, DMH; *P* < .01, healthy *v* DMD). Course of C-peptide (*P* < .001, healthy subjects *v* DMN, DMD, and DMH).

moreover, other enzymes and controlling processes may be involved.[37] The metabolic and/or genetic basis for the link between the FA pattern of lipids and diabetes or IR is supported by a study showing an association between the maternal concentrations of serum IRI and triglycerides (metabolic syndrome indicators) and the FA composition of the child's muscle membrane, independent of breast-feeding duration.[41]

The FA composition of serum and tissue lipids is influenced by the dietary fat quality in adults[42] and even by the type of infant feeding in young children.[43] Significant positive associations between the content of different dietary FAs, as estimated from dietary surveys, and the proportion of FA in S-PL have been demonstrated. A change in the quality of diet, while keeping all other nutrients unchanged, is followed by a progressive change in the S-PL FA pattern in the ensuing weeks reaching an apparent new steady state after an approximate 3 weeks.[44] A shift in dietary fat quality, from a diet containing a high proportion of saturated fat to one high in linoleic acid, causes a shift of the serum lipid FA composition with increased proportions of linoleic acid and low levels of 20:3 n-6, while a diet with a high content of milk fat and a small amount of linoleic acid induces high levels of n-6 DPUFA.[45] Therefore, the low concentrations of linoleic and higher proportions of DPUFA n-6 in diabetics may mirror a lower intake of linoleic acid-rich foods. Our rough dietary intake data did not show differences between groups, but the methods used for estimating diet composition among free-living populations are far from perfect. Some nutrients or food components, eg, the amount of dietary fat, may be selectively underestimated.[46] Thus, we could not exclude that the altered dietary intake may be responsible for the differences in the FA pattern between the study groups.

The FA composition of lipids is related to physical activity[47] and to the muscle fiber composition.[48] Ten-week regular exercise has been shown to decrease the proportions of 16:0, 18:2 n-6, and the sum of n-6 FA in muscle phospholipids in humans, while no effect has been found in S-PL.[47] While physical fitness was not estimated in our study, the above trends induced by exercise could not explain our results.

Finally, treatment with glibenclamide should be mentioned as a potential cause of the different FA patterns in DMH compared with the other diet-treated diabetic groups. It has

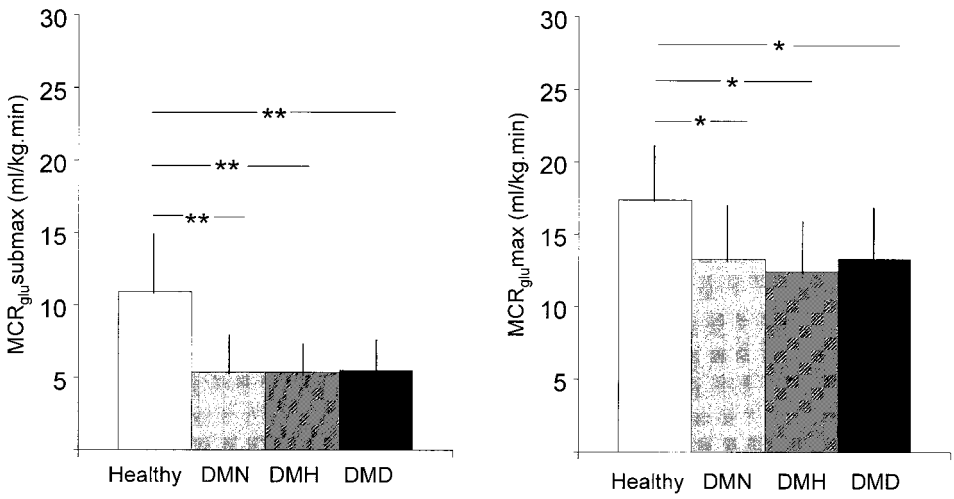

Fig 2. Metabolic clearance rates of glucose during the hyperinsulinemic isoglycemic clamp at insulin infusion rates of 1 U/kg · min (MCR_{glu}submax) and 10 mU/kg · min (MCR_{glu}max) in healthy (n = 24) and DM2 subjects newly diagnosed (DMN; n = 21), treated with diet only (DMD; n = 11), and treated with glibenclamide (DMH; n = 21). Statistical significance: **P* < .05; ***P* < .01.

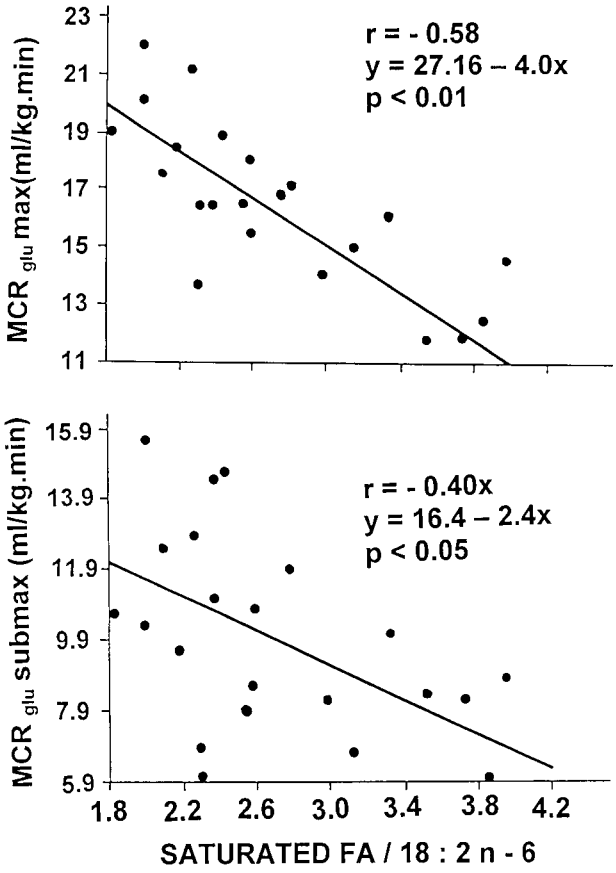

Fig 3. Relationships between saturated FA to 18:2 n-6 ratio and insulin action (MCR_{glu}submax and MCR_{glu}max) in healthy subjects (n = 24).

been shown that improvement of metabolic control in DM2 patients with glyburide[49] or with insulin[50,51] failed to change the FA pattern in serum and tissue lipids.

In our group of HS, insulin sensitivity was associated with low proportions of saturated FA and high proportions of linoleic acid and DPUFA n-6, arachidonic acid, in particular. The significant relationships between insulin action and the FA composition of serum lipids and muscle phospholipids have been found both in experimental animals[4] and humans.[8-10,12,13] The correlations found in our HS subjects are in good agreement with the data reported by Vessby et al.[9,26] They also found a negative association between insulin action and saturated FA and a positive relationship to linoleic acid; furthermore, the high proportion of 20:3 n-6 predicted the development of diabetes. Positive associations between the proportion of arachidonic acid and insulin sensitivity were reported also by other investigators.[11,12,40] In accordance with Vessby at al,[9] we did not observe a significant relationship between the proportion of n-3 FA and insulin sensitivity. By contrast, strong positive relationships were demonstrated in other studies.[12,41,43] The reason for this discrepancy is not clear. It may be due to differences in the FA composition of Scandinavian and Australian populations, presumably reflecting divergent dietary habits. The outcomes of regression analysis do not solve the problem of cause and consequence. It has been shown that modification of the membrane FA composition affects a number of cellular functions,[5,6,39,52] and the hypothesis that alterations in the FA pattern are the primary defect causing IR is supported by intervention studies in experimental animals[4] and humans.[7]

In our study, we have found decreased insulin action at both insulin levels in type 2 diabetic subjects. The degrees of IR were comparable in all groups of diabetics; IR was independent of the duration of disease, long-term blood glucose control, or type of treatment. The finding is consistent with the fact that the differences in the degree of glucose intolerance and progression of diabetes depend mainly on the defect in insulin secretion.[53] In our groups of diabetics, we did not find any significant association between insulin action and the FA composition of S-PL. The absence of relationships supports the hypothesis that the alterations in the FA composition of lipids are not the direct cause of IR in diabetes, which is probably mediated by other factors. The increased proportion of arachidonic acid in DM2 patients could even act as a protective mechanism against IR. A search in the relevant literature did not produce any study designed to test the above relationships in diabetics. The absence of relationships in diabetes together with differences of the FA pattern between DMH and diet-treated patients seems to imply that the causes of FA alterations in diabetics have a secondary character and could be related to impairment of insulin secretion and metabolic disturbances as a consequence.

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