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Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity

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Summary

Abnormalities in endothelial function may be associated with increased cardiovascular risk in diabetic patients. We examined the effect of an oleic-acid-rich diet on insulin resistance and endothelium-dependent vasoreactivity in type 2 diabetes. Eleven type 2 diabetic patients were changed from their usual linoleic-acid-rich diet and treated for 2 months with an oleic-acid-rich diet. Insulin-mediated glucose transport was measured in isolated adipocytes. Fatty acid composition of the adipocyte membranes was determined by gas-liquid chromatography and flow-mediated endothelium-dependent and -independent vasodilatation were measured in the superficial femoral artery at the end of each dietary period. There was a significant increase in oleic acid and a decrease in linoleic acid on the oleic-acid-rich diet ($p < 0.0001$). Diabetic control was not different between the diets, but there was a small but significant decrease in fasting glucose/insulin on the oleic-acid-rich diet. Insulin-stimulated (1 ng/ml) glucose transport was significantly greater on the oleic-acid-rich diet (0.56 ± 0.17 vs. 0.29 ± 0.14 nmol/10^5 cells/3 min, $p < 0.0001$). Endothelium-dependent flow-mediated vasodilatation (FMD) was significantly greater on the oleic-acid-rich diet ($3.90 \pm 0.97\%$ vs. $6.12 \pm 1.36\%$ $p < 0.0001$). There was a significant correlation between adipocyte membrane oleic/linoleic acid and insulin-mediated glucose transport ($p < 0.001$) but no relationship between insulin-stimulated glucose transport and change in endothelium-dependent FMD. There was a significant positive correlation between adipocyte membrane oleic/linoleic acid and endothelium-dependent FMD ($r = 0.61$, $p < 0.001$). Change from polyunsaturated to monounsaturated diet in type 2 diabetes reduced insulin resistance and restored endothelium-dependent vasodilatation, suggesting an explanation for the anti-atherogenic benefits of a Mediterranean-type diet.

Introduction

Diabetes is associated with an increased risk (up to fourfold) of developing atherosclerosis. Hypercholesterolaemia does not usually explain this increased risk. Recent studies have suggested abnormalities in the vascular wall, and particularly in endothelial function, which may be associated with the increased cardiovascular risk in diabetes.[1,2] The relationship between subtle changes in lipoproteins

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found in diabetes and vascular reactivity has become a focus of interest. Low-density lipoprotein (LDL) in diabetes has an increased ability to be oxidized. Oxidized and, in particular, glycoxidized LDL has been associated with impairment in vascular reactivity.[3] Reduction in cholesterol with statins improves vascular reactivity, at least in non-diabetic patients.[4] The role of insulin resistance in vascular reactivity is uncertain. A recent study[5] demonstrated an inverse relationship between blood pressure and insulin sensitivity, with a physiological association between skin microvascular function on the one hand and insulin sensitivity and blood pressure on the other. Acetylcholine-mediated vasodilatation was strongly related to insulin sensitivity in that group of normal individuals. Other studies have shown that insulin resistance is associated with a decreased vasodilatory response to insulin.[6] However, a study in insulin-resistant obese subjects found vascular function to be normal in spite of the impaired response to insulin.[7] In that study, troglitazone, which improved insulin sensitivity, had no effect on vascular responses.

The major lipoprotein abnormality in diabetes occurs in the post-prandial state, with significant abnormalities in both the intestinally-derived chylomicron and hepatic very-low-density lipoprotein (VLDL).[8,9] The chylomicron remnant is particularly atherogenic[10,11] and has been shown to impair endothelium-dependent vasorelaxation *in vitro*.[12] We have recently shown that dietary intervention can significantly alter chylomicron remnant levels as well as reducing VLDL particles.[13] Fatty acids are involved in gene regulation, and in particular have been shown to activate peroxisome proliferator-activated receptors (PPARs), nuclear receptors which regulate gene transcription.[14,15] PPARs are expressed in hepatocytes and adipocytes, and PPAR activators such as the thiazolidinediones have major effects on insulin action,[16] however, little is known about the effect of different fatty acids on PPAR activation. Cell membrane fatty-acid composition may be another important factor determining cell function, due to its effect on the physical properties of the membrane.[17] The purpose of this study was to examine the relationship between membrane fatty acid composition, insulin-dependent glucose transport and vasoreactivity.

Methods

Patients

Eleven male Irish Caucasian patients with type 2 diabetes between the ages of 40 and 65 years (mean 55 ± 4.6 years) were asked to take part in the study. Patients with hypertension, hepatic or renal failure, peripheral vascular disease or neuropathy, were excluded, as were smokers. Patients were treated with diet only ($n = 2$) or diet and sulphonylurea ($n = 9$), and treatment had not been changed in the previous 8 weeks. No patient was on lipid-lowering agents or drugs which might effect vascular reactivity. All patients gave informed consent and the studies were approved by our ethics committee.

Study design

Patients were seen by a qualified dietician to reinforce advice on the usual high-polyunsaturated-fatty-acid diet, using spreads and cooking oils that were polyunsaturate-rich. At the end of a 2-month run-in period, fasting blood was taken and abdominal adipose tissue was biopsied using subcutaneous suction. Femoral artery vasoreactivity studies were done on a separate occasion. Patients were again seen by the dietician and changed to an isocaloric mono-unsaturated, olive-oil-rich diet. Following 2 months treatment with this diet, blood samples and adipose tissue biopsies were again taken, and vasoreactivity measurements were repeated.

Plasma lipoproteins and glycaemic control

Plasma lipoproteins were determined using enzymic colorimetric assays (Boehringer Mannheim). Plasma high-density lipoprotein (HDL) cholesterol was determined after precipitation of apoB-containing lipoproteins, and plasma LDL cholesterol was calculated using the Friedewald equation. Blood glucose was measured by an enzymic method (Boehringer Mannheim). HbA_{1c} was determined using an enzyme immunoassay (Novo-Nordisk) (normal value $< 4.9\%$). Serum total insulin was measured using a microparticle enzyme immunoassay which does not cross-react with pro-insulin (Abbott), and the ratio of fasting glucose/fasting insulin was measured by the method of Phillips *et al.*[18]

Flow-mediated endothelium-dependent vasodilation

Flow mediated, endothelium-dependent vasodilation (FMD) was measured following reactive hyperaemia[19] (induced by inflation of a pneumatic tourniquet to a pressure of 300 mmHg for 4.5 min). Endothelium-independent vasodilation was examined following sublingual administration of glyceryltrinitrate (GTN) (400 μg). Measurements were made in the superficial femoral artery at a point 3 cm distal to the bifurcation of the common femoral artery using high-resolution ultrasound (Toshiba Sonolayer Scanner SSH-14OA with a high resolution 7.5 MHz linear scan head) with a theoretical limit of > 0.1 mm in the near field. A mark was made on the skin for repeat examination. Arterial diameter measurements

were made on longitudinal scanning from the anterior to the posterior intimal margins. Vasodilation was calculated as the percent change in the diameter compared to baseline. To verify that reactive hyperaemia caused similar increase in blood flow in patients at both visits, maximum flow velocity was measured at rest and within 15 s after cuff deflation. A flow index was calculated by multiplying the maximum flow velocity by the vessel cross sectional area ($3.14 \times D^2/4$). Reactive hyperaemia was calculated as percent change in flow during hyperaemia compared to baseline. Baseline flow was calculated by multiplying the velocity time integral of the flow signal by the heart rate and the vessel cross sectional area.

Adipocyte biopsy

Adipose tissue was obtained from all patients immediately before and after the 2-month oleic acid dietary period. A solution (200 ml) containing 0.9% normal saline, 0.2% lignocaine, adrenaline (1 mg) and 0.084% sodium bicarbonate was injected using aseptic techniques into the adipocyte layer in the abdominal wall. Adipose tissue was collected by suction using a 20 ml syringe.

Adipocyte isolation

Adipocytes were isolated by the method of Rodbell.[20] Adipose tissue was weighed, and adipocytes were released by incubation with collagenase (1 mg/ml) (Sigma-Aldrich) at 37 °C for 1 h in Dulbecco's MEM containing 10 mM HEPES, 2% fetal calf serum, 1% bovine serum albumin, penicillin (20 units/ml), streptomycin (20 mg/ml) (all from Gibco) and in a sterile polypropylene container. The adipocytes were removed and filtered through nylon mesh and washed three times with glucose-free buffer.

Glucose transport

Adipocytes were re-suspended in glucose-free buffer and incubated for 40 min at 37 °C to dissociate any remaining receptor-bound insulin.[21] These washing procedures effectively removed all extracellular and receptor-bound insulin and allowed the glucose transport system to deactivate to basal levels. Adipocytes were stained with acridine orange, counted by microscopy, and resuspended at a concentration of 2×10^5 cells/ml in glucose-free buffer in Eppendorf tubes. Adipocytes were incubated in a shaking water bath for 1 h at 37 °C with or without insulin at various concentrations (0, 1 and 5 ng/ml). [^{3}H]-2-deoxyglucose (0.1 mmol/l, 0.2 μCi 2-deoxy(1-^{3}H) glucose was added, the reaction was terminated after 3 min by centrifugation through silicone oil, and [^{3}H]-2-deoxyglucose uptake was determined by liquid scintilation counting.

Adipocyte membrane fatty acids

Isolated adipocytes (2×10^5) were suspended in 20 ml cold Tris buffer (10 nmol/l, pH 8) and homogenized using a Potter glass homogenizer.[22] The cell suspension was centrifuged for 10 min at 1000 g and the pellet was discarded. The supernatant was centrifuged at 33 000 rpm at 4 °C for 30 min. The resulting cell membrane pellet was stored at −70 °C for fatty acid determination.

Fatty acid determination

Heptadecanoic acid (100 μg) was added as an internal standard to the adipocyte membrane preparations, and lipids were extracted by a modification of the method of Folch *et al.*[23] The organic phase was dried under nitrogen and transmethylated as previously described.[24] Fatty acid methyl esters were extracted into hexane, dried under nitrogen and reconstituted in iso-octane immediately prior to chromatography. The fatty acids were analysed using a Shimadzu GC-14A gas chromatograph and expressed in relation to the internal standard as μg fatty acid/10^5 adipocytes. Intra- and interassay variations of the method were 1.8 and 2.6%, respectively.

Statistical analysis

Results are expressed as means ± SD. Statistical analysis was performed using the Student t-test or paired Student's t-test. Correlation coefficients were calculated for regression analysis. Inter- and intra-assay variation are expressed as SD/mean × 100. A p value of <0.05 was regarded as statistically significant.

Results

There was no change in medication or diabetic control throughout the study. There was no difference in patient weight between the two diets. Fasting plasma insulin was significantly lower on the oleic acid diet (0.44 ± 0.07 vs. 0.51 ± 0.07 ng/ml, $p < 0.02$), and the fasting insulin/glucose ratio was also significantly lower on the oleic acid diet 14.0 ± 1.6 vs. 16.25 ± 2.4 ($p < 0.002$). There was no significant difference in any of the lipid parameters with either of the diets (Table 1).

There was no significant difference in the adipocyte membrane total fatty acids between the diets (1743 ± 209 vs. 1755 ± 238 μg/ml). There was a significant change in the fatty acid composition of the adipocyte membrane when the linoleic acid diet was compared to the oleic acid diet (Figure 1). On

Table 1 Patient characteristics

	Diet	
	Linoleic-acid-rich	Oleic-acid-rich
BMI kg/m^2	28 ± 4.4	27.8 ± 4.4
Blood glucose (mmol/l)	7.2 ± 0.8	7.1 ± 1.0
HbA_{1c} (%)	6.2 ± 0.8	6.1 ± 0.6
Plasma fasting insulin	0.5 ± 0.1	0.4 ± 0.1*
Fasting glucose/fasting insulin	16.2 ± 2.4	14.0 ± 1.6**
Plasma triglycerides (mmol/l)		
Mean	1.5 ± 0.5	1.5 ± 0.5
Range	1.0–2.3	1.0–2.4
Median	1.3	1.2
Plasma cholesterol (mmol/l)	5.1 ± 0.5	5.1 ± 0.5
LDL cholesterol (mmol/l)	3.0 ± 0.5	3.0 ± 0.5
HDL cholesterol (mmol/l)	1.0 ± 0.3	1.0 ± 0.2

Data are means ± SD. * $p < 0.02$; ** $p < 0.002$ (paired t-test).

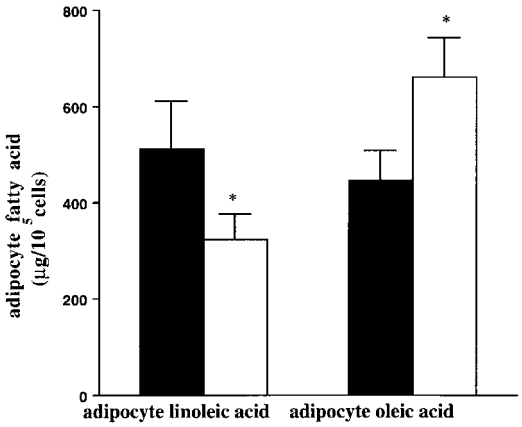

Figure 1. Amount of linoleic and oleic acid (μg/10^5 cells) in adipocyte membrane fatty acids on linoleic-acid-rich (■) and oleic-acid-rich (□) diets. Linoleic acid was significantly reduced ($p < 0.0001$) and oleic acid was significantly increased ($p < 0.0001$) when patients ($n = 11$) were changed from a linoleic-acid-rich to an oleic-acid-rich diet (paired t-test).

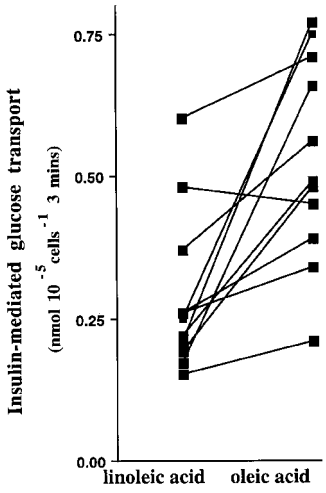

Figure 2. Insulin-mediated (1 ng/ml insulin) glucose transport (nmol/10^5 cells/3 min) in the 11 patients on linoleic-acid-rich diets and oleic-acid-rich diets. Insulin-mediated glucose transport was significantly greater on the oleic-acid-rich diet. *$p < 0.00001$, paired t-test.

the linoleic acid diet, linoleic acid was significantly greater than it was on the oleic acid diet (513 ± 100 μg/10^5 cells vs. 325 ± 52 μg/10^5 cells $p < 0.0001$). On the linoleic acid diet, oleic acid was significantly less than on the oleic acid diet (447 ± 61 μg/10^5 cells vs. 661 ± 81 μg/10^5 cells, $p < 0.0001$). There was no significant difference in palmitic acid on the two diets, but stearic acid was significantly lower on the oleic acid diet ($p < 0.01$).

Insulin-mediated glucose transport

Changes in insulin-mediated glucose transport (1 ng/ml insulin) with changes in diet for the individual patients are shown in Figure 2. Mean insulin-mediated glucose transport on the linoleic acid diet was 0.29 ± 0.14 and 0.56 ± 0.17 nmol/10^5 cells/3 min at 1 ng/ml and 5 ng/ml insulin, and these values increased to 0.53 ± 0.18 and 0.79 ± 0.28 nmol/10^5 cells/3 min, respectively, on the oleic acid diet ($p < 0.0001$ different from linoleic acid).

Flow-mediated vasodilatation (FMD)

There was a significant increase in endothelium-dependent FMD in the reactive hyperaemia phase when the linoleic acid was changed to oleic acid (3.903 ± 0.97% vs. 6.12 ± 1.36%, $p < 0.0001$) (Figure 3). There was a small, but significant change in the glyceryl trinitrate (GTN)-induced FMD

Table 2 Effect of an oleic-acid-rich diet on vascular reactivity in type 2 diabetes

	Diet	
	Linoleic-acid-rich	Oleic-acid-rich
Baseline flow ml/min)	783.9 ± 140.4	786.1 ± 143.5
Flow-mediated vasodilatation (%)	3.9 ± 1.0**	6.1 ± 1.4**
Baseline diameter (mm)	6.5 ± 0.5	6.5 ± 0.5
Hyperaemia diameter (mm)	6.8 ± 0.5	6.9 ± 0.5
GTN diameter (mm)	6.9 ± 0.5	6.9 ± 0.5
GTN-induced FMD (%)	6.0 ± 1.1	6.6 ± 1.5**
FMD/GTN	0.6 ± 0.1	0.9 ± 0.1***
SystolicBlood pressure (mmhg)	142.3 ± 12.7	137.7 ± 13.6**
Diastolic BP (mmHg)	76.4 ± 7.1	73.6 ± 5.5*

Data are means ± SD. * $p<0.05$; ** $p<0.001$; *** $p<0.00001$ different from linoleic acid.

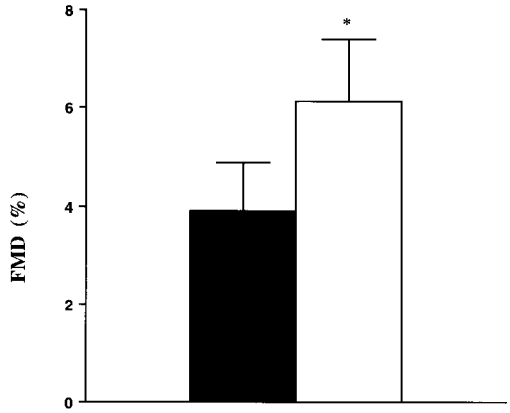

Figure 3. Mean endothelium-dependent flow-mediated (FMD) vasodilatation in patients ($n=11$) on linoleic-acid-rich (■) and oleic-acid-rich (□) diets. FMD(%) was significantly higher on the oleic-acid-rich diet. *$p<0.00001$, paired t-test.

(6.03 ± 1.13% vs. 6.63 ± 1.511%, $p<0.05$) (Table 2).

There was a significant correlation between the ratio of adipocyte membrane oleic/linoleic acid and insulin-mediated glucose transport at 1 μg/ml insulin ($r=0.76$, $p<0.001$) and at 5 ng/ml insulin ($r=0.40$, $p<0.05$). There was no significant correlation between the change in insulin-stimulated glucose transport and change in endothelium-dependent FMD. There was a significant positive correlation between the adipocyte membrane oleic/linoleic acid ratio and the endothelium-dependent FMD ($r=0.61$, $p<0.001$) but not with endothelium-independent (GTN) FMD.

Discussion

Some studies have demonstrated an improvement in insulin sensitivity in type 2 diabetic patients when diet is changed from a high carbohydrate diet to an isocaloric high monounsaturated fatty acid diet.[25] We have been unable to find studies where a high polyunsaturated diet has been changed to a high mono-unsaturated diet. In a previous study in which we replaced both saturated and polyunsaturated fats and spreads with mono-unsaturated fats and spreads we found a small, but insignificant, reduction in HbA_{1c}.[26] In a meta-analysis, Garg[27] found that high mono-unsaturated fat diets improve glycaemia control, but again most of these studies compare a high mono-unsaturated diet with a high carbohydrate diet.[27] In the present study, in which we replaced polyunsaturated by mono-unsaturated fatty acids, there was a reduction in the fasting insulin and in glucose/insulin which is an index of insulin resistance.[18] The benefit of improvement in insulin resistance is most easily seen in the postprandial state, thus HbA_1 and fasting blood sugar, in our small group of patients over a relatively short period of time, may not be a sufficiently sensitive test of glycaemic improvement. Another major site of insulin resistance is smooth muscle, and it is possible that the changes that we found in the adipocyte might not be reflected in muscle to a similar extent. However the large reduction in insulin resistance so often seen with very modest weight reduction would not seem to support this suggestion.

We did not use a cross-over design, since the purpose of the study was to examine the relationship between change in membrane fatty acid composition, glucose transport as an index of insulin sensitivity and endothelial function. It is unlikely that any bias in study design could have changed the membrane fatty acid composition. Our studies on insulin-stimulated glucose transport in the adipocyte are confirmatory evidence of the reduction in insulin resistance when a linoleic-acid-rich diet is changed to an oleic-acid-rich diet. We have shown that adipocytes with oleic-acid-rich membranes have a higher insulin-stimulated glucose transport rate. The

improvement in glucose transport in these adipocytes was clearly related to the change in the oleic/linoleic acid, and could be explained by the changes in membrane fluidity. Tong *et al.*[17] have suggested that high fluidity of adipocyte membranes may hinder conformational changes and aggregation of insulin receptors, resulting in impaired action of insulin. Oleic acid has only one double bond therefore an oleic-acid-rich membrane would be less fluid than a membrane rich in linoleic acid which has two double bonds.

An alternative explanation may be the effect of oleic acid on nuclear receptors and thus the stimulation of gene expression. For example, it has recently been shown that there is considerable difference in the ability of various fatty acids to stimulate release of insulin from the pancreas in response to glucose.[28] The study showed, in perfused rat pancreas, that the insulinotropic effect of different fatty acids is related to their chain length and degree of saturation. Little is known about the effect of different fatty acids on peripheral insulin sensitivity, although free fatty acids have been clearly demonstrated to induce insulin resistance in humans through inhibition of glucose transport.[29] The regulation of insulin resistance has been defined at least to some extent by the discovery of PPARs, their role in regulation of adipocyte differentiation and gene expression and the discovery of chemicals which activate PPARs and reduce insulin resistance. Fatty acids are regulators of PPARs, but the mechanism by which fatty acids regulate insulin resistance is unknown. Our results suggest that the individual fatty acids have specific effects on mediators of insulin resistance. Indeed, a clinical study in Finland found a highly significant correlation between fatty acid composition of serum cholesteryl esters in healthy men and risk of developing type 2 diabetes 10 years later.[30]

Endothelium-dependent vasodilatation is abnormal in atherosclerosis[31] and in diabetes.[1] Mechanisms accounting for the abnormalities have been related to the precursors of nitric oxide and the increase in metabolism of nitric oxide. A report that troglitazone, a drug which stimulates PPARS and reduces insulin resistance, did not effect vasodilatation in insulin resistant subjects, suggests that insulin resistance *per se* may not be the cause of the disturbance of endothelium-dependent vasodilatation in diabetes.[7] The role of PPARγ in atherosclerosis is in its infancy, and to date there is no convincing evidence linking oleic acid, rather than other fatty acids, to PPARγ stimulation. However, it should be noted that there are reports that the ω-3-fatty acids and other eicosanoids bind and activate PPARγ and in particular, two separate eicosanoids present in oxidized low-density lipoproteins have been shown to be potent PPARγ ligands.[32] It is likely that the alteration in the ratio of mono/polyunsaturated fatty acids in LDL would have an effect on LDL oxidizability and therefore on PPARγ activation. Although we had an improvement in the fasting glucose/insulin ratio on the oleic acid diet, there was no relationship between change in glucose/insulin and change in endothelium-dependent FMD. Neither did we find any relationship between insulin-mediated glucose transport in adipocytes and change in endothelium-dependent FMD with change in diet. There was however a strong correlation between the change in the oleic/linoleic acid ratio in the adipocyte membrane and the change in endothelium-dependent vasodilatation. The results suggest a significant regulatory effect of individual fatty acids on endothelial function which is independent of insulin resistance. It has been suggested that increased oxidative stress plays a part in the development of endothelial dysfunction in NIDDM.[33] We have previously shown that oxidation of LDL in NIDDM is related to fatty acid composition,[24,26] thus, it is possible that the changes in vasodilatation that we have observed might be related to a decrease in the oxidizability of the LDL on the oleic acid diet. This could explain the lack of correlation between insulin mediated glucose transport and endothelial dependent vasodilatation.

The role of insulin in vasodilatation has received much attention. Further studies are needed to determine the roles of physical change in the endothelium as compared to regulation of gene expression, but probably both mechanisms play a part. These studies suggest that the anti-atherogenic benefits from the Mediterranean diet in type 2 diabetes might be explained by the independent effect of oleic acid on insulin resistance and on endothelium-dependent vascular reactivity.

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