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Abnormal myocardial kinetics of ^{123}I-heptadecanoic acid in subjects with impaired glucose tolerance

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Summary Increased triglyceride accumulation has been observed in the diabetic heart, but it is not known whether the abnormalities in myocardial fatty acid metabolism differ between insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetic patients or whether they are present even prior to overt diabetes. Therefore, we studied myocardial fatty acid kinetics with single-photon emission tomography using ^{123}I-heptadecanoic acid (HDA) in four groups of men: impaired glucose tolerance (IGT) ($n = 13$, age 53 ± 2 years, mean ± SEM), IDDM ($n = 8$, age 43 ± 3 years), NIDDM ($n = 10$, age 51 ± 2 years) and control subjects ($n = 8$, age 45 ± 4 years). Echocardiography and myocardial perfusion scintigraphy (IGT and NIDDM groups) were performed to study cardiac function and flow. In the IGT subjects, myocardial HDA beta-oxidation index was reduced by 53 % (4.6 ± 0.4 vs 9.7 ± 1.0 μmol $\cdot$ min^{-1} $\cdot$ 100 g^{-1}, $p < 0.01$) and HDA uptake by 34 % (3.7 ± 0.2 vs 5.6 ± 0.3 % of injected dose 100 g, $p < 0.01$) compared with the control subjects. The fractional HDA amount used for beta-oxidation was lower in the IGT compared with the control subjects (43 ± 4 vs 61 ± 4 %, $p < 0.05$). NIDDM patients also tended to have a lowered HDA beta-oxidation index, whereas IDDM patients had similar myocardial HDA kinetics compared to the control subjects. Myocardial perfusion imaging during the dipyridamole-handgrip stress was normal both in the IGT and NIDDM groups, indicating that abnormal myocardial perfusion could not explain abnormal fatty acid kinetics. In conclusion, even before clinical diabetes, IGT subjects show abnormalities in myocardial fatty acid uptake and kinetics. These abnormalities may be related to disturbed plasma and cellular lipid metabolism. [Diabetologia (1997) 40: 541–549]

Keywords Impaired glucose tolerance, fatty acids, myocardial metabolism, SPET, heptadecanoic acid, diabetic heart muscle disease.

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; IGT, impaired glucose tolerance; HDA, heptadecanoic acid; SPET, single-photon emission tomography, MIBI, methoxy-isobutyl isonitrile; NEFA, non-esterified fatty acids; S_I, insulin sensitivity; S_G, glucose effectiveness; PET, positron emission tomography; FDG, 18F-2-fluoro-2-deoxy-D-glucose; OGTT, oral glucose tolerance test; IVGTT, intravenous glucose tolerance test.

In addition to increased risk for coronary heart disease and other atherosclerotic vascular diseases [1, 2], diabetic patients may have asymptomatic cardiac dysfunction and even clinical heart failure which are not attributable to any other cardiac disease [3–5]. Furthermore, in experimental diabetes structural changes such as increased triglyceride accumulation and myocardial fibrosis have been observed [4, 6]. One explanation is that chronic hyperglycaemia and abnormalities in lipid and lipoprotein metabolism may lead to altered myocardial energy metabolism, structural changes, and eventually to deterioration of cardiac function [5, 7]. In healthy subjects, myocardial energy metabolism is largely determined by substrate availability, blood and oxygen supply, and

cardiac work, but under most conditions non-esterified fatty acids (NEFA) are the preferred fuel, producing up to 70–80% of energy in the fasting state [8]. In experimental diabetes and patients with insulin-dependent diabetes mellitus (IDDM), myocardial glucose oxidation is decreased, and fatty acid and ketone utilization are increased [9–11].

Loss of pancreatic beta cells leads to insulin deficiency in IDDM patients, whereas non-insulin-dependent diabetes mellitus (NIDDM) is characterized by impaired insulin secretion and insulin resistance. Insulin resistance is related to multiple abnormalities in lipid and lipoprotein metabolism [12]. Thus, myocardial metabolism may be different in these two types of diabetes. With the use of new imaging methods, such as positron emission tomography (PET) and single-photon emission tomography (SPET) it is possible to study substrate utilization of the heart non-invasively. We have previously found evidence of disturbed fatty acid metabolism and increased triglyceride synthesis of the heart in NIDDM patients with the use of ^{123}I-heptadecanoic acid (HDA) and SPET imaging [13]. Using PET and ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG), two studies have shown that NIDDM patients may have decreased myocardial glucose uptake [14, 15]. In contrast, IDDM patients had normal myocardial FDG uptake during the euglycaemic hyperinsulinaemic clamp [16]. The aim of the present study was to examine myocardial fatty acid kinetics with HDA-SPET imaging in IDDM and NIDDM patients and in subjects with impaired glucose tolerance (IGT) to find out whether myocardial NEFA kinetics differ between IDDM and NIDDM and whether possible disturbances could be observed in the pre-diabetic state.

Subjects and methods

Subjects

Patients with IDDM. Eight male patients with IDDM were recruited from the local hospital diabetic clinic. The mean duration of diabetes was 11 ± 4 years. At the time of diagnosis all had the glucagon-stimulated C-peptide concentration less than 0.6 nmol/l. All patients had intensive insulin therapy with multiple daily insulin injections. Two patients had background retinopathy and one had symptoms of neuropathy, but none had microalbuminuria. One patient had cholesterol-lowering medication (lovastatin 20 mg/day) and one took pindolol 5 mg/day for hypertension.

Patients with NIDDM. Ten male patients with NIDDM (duration of diabetes 2 ± 1 years) were studied. Four of them were recruited from the local hospital diabetic clinic. Three of these patients had oral sulphonylurea treatment (glibenclamide 7 mg/day) and one was treated with diet. Furthermore, 1 patient took bezafibrate 400 mg/day. In 6 patients NIDDM had been recently diagnosed with an oral glucose tolerance test (OGTT) according to World Health Organization (WHO) criteria [17]. After the diagnosis they were treated with diet therapy for 3 to 6 months before the SPET study. At the time of the study, one recently diagnosed NIDDM patient was also taking glibenclamide 7 mg/day and thus altogether 4 out of 10 patients had sulphonylurea and 6 were on diet only. None had retinopathy, neuropathy or clinical nephropathy. Microalbuminuria was not determined in these patients.

Subjects with IGT. Thirteen male subjects with stable impaired glucose tolerance were recruited from a larger study programme, in which the prevention of NIDDM in IGT subjects is being studied. IGT was diagnosed according to the WHO criteria [17] with the mean of two consecutive OGTTs. An intravenous glucose tolerance test (IVGTT) [18] was also performed in 12 subjects to determine the glucose tolerance status more precisely. Four subjects were taking antihypertensive medication (patient 1 enalapril 10 mg/day, patient 2 enalapril 20 mg/day + felodipine 5 mg /day, patient 3 verapamil 480 mg/day + lisinopril 20 mg/day and patient 4 metoprolol 95 mg/day + felodipine 10 mg/day, respectively).

Control subjects. Eight healthy men served as control subjects. None of them had family history of diabetes and all had fasting blood glucose within the normal range. In 4 mildly obese control subjects an OGTT was performed and normal glucose tolerance was confirmed. Two of the control subjects had antihypertensive medication (captopril 50 mg/day and enalapril 20 mg/day).

All patients and control subjects were otherwise healthy as assessed by routine liver, thyroid and kidney function tests and were not taking any other medication as stated above. Antihypertensive medication was continued at the time of the study, but lipid-lowering medication was stopped 2 days before the study. None of the subjects had symptoms or signs of ischaemic heart disease and all had a normal ECG. All subjects gave their informed consent and the study was approved by the local hospital ethical committee.

Laboratory measurements and IVGTT. The patients' height and weight were measured and body mass index (BMI) was calculated. Blood glucose was analysed by the glucose oxidase method (Glucose Auto & Stat, model GA-110; Daiici, Kyoto, Japan) and serum lactate concentration by the lactate oxidase method (YSI 2300 Stat, YSI Inc., Yellow Springs, OH, USA). Serum NEFA concentration was measured by turbidometric method and analysed with the specific-analyzer (Kone Ltd., Espoo, Finland). Haemoglobin A_{1c} was analysed with fast liquid chromatography (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Plasma insulin was determined with an RIA method (Phadeseph Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden). To determine high-density lipoprotein (HDL) cholesterol, HDL was separated from very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) by precipitation of apoB-containing lipoproteins with dextran sulfate and magnesium chloride [19]. Enzymatic colorimetric methods were used for the determination of cholesterol and triglycerides (HiCo Cholesterol and Triglycerides GPO-PAP; Boehringer Mannheim GmbH, Mannheim, Germany).

The insulin-modified frequently sampled IVGTT [18, 20] was performed in subjects with IGT. After an overnight fast the baseline blood samples were drawn and a glucose dose of 0.3 g/kg of body weight was administered within 1.5 min through an intravenous forearm cannula and flushed with saline. At 20 min, an insulin dose of 0.03 IU/kg was given intravenously and blood sampling was continued up to 180 min. By using minimal model analysis [21], insulin sensitivity (S_I) and

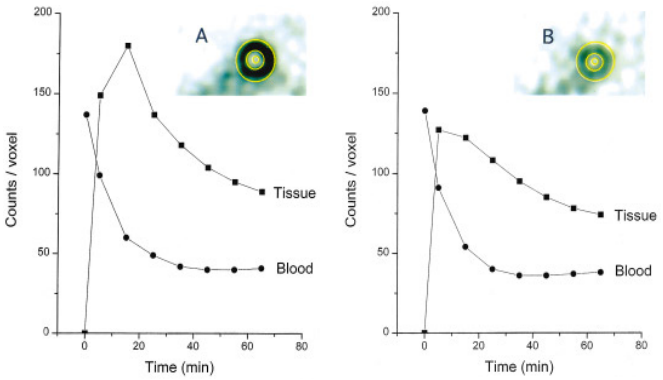

Fig. 1A, B. Regions of interest and time activity curves of the myocardial tissue and left ventricular lumen in a control subject (**A**) and in a subject with IGT (**B**). Note the lower peak and slower washout of the tissue time activity curve in the IGT subject. The results showed that the beta-oxidation index was reduced by 53 % and the fractional amount for back diffusion was increased by 30 % in the IGT subject when compared to the control subject. Their serum NEFA values were similar (0.68 and 0.65 mmol/l, respectively)

glucose effectiveness (S_G) were calculated. First-phase insulin response was calculated by measuring the insulin area under the curve over 0 to 19 min.

Study protocol. The SPET study and echocardiography were performed during the same morning between 08.00 and 13.00 hours (IDDM patients between 08.00 and 10.00 hours). Before the SPET study, the patients had been fasting for at least 12 h. For 3 days prior to fasting they had been on a diet containing a minimum of 250 g of carbohydrates per day. They were advised not to exercise, smoke, drink coffee or alcohol for 1 day before the study. Myocardial perfusion imaging study for the IGT and NIDDM groups was performed afterwards.

Myocardial NEFA kinetics. One hour before, and 12 and 24 h after administration of ^{123}I-HDA (Cygne bv, Eindhoven, The Netherlands), 200 mg potassium perchlorate was given orally to block thyroid ^{123}I-uptake. The patient was lying supine for 10–15 min before tracer injection. Heart rate and systolic and diastolic blood pressure were recorded and the double product was calculated to assess the myocardial work. The left antecubital vein was cannulated for collecting venous blood samples. Just before the dose of 160–185 MBq of ^{123}I-HDA was given intravenously into the right antecubital vein, a blood sample was taken for the determinations of glucose, lactate and NEFA. The specific activity of the tracer was 6×10^{10} Bq/mmol and its radiochemical purity was better than 98 %. Immediately after the injection of the tracer the first SPET scan was started using a dedicated 3-headed gamma camera with high resolution collimators (Siemens MultiSPECT 3; Siemens Gammasonics Inc., Hoffman Estates, Ill., USA). Full 360° rotation was used and 30 views per head (4°), each 18 s, were acquired. This was repeated six times consecutively. Thus, there were seven measurement points: 5, 15, 25, 35, 45, 55 and 65 min (as given in the mid-time of each SPET scan). Venous blood samples of 5 ml were collected at 1.5, 3, 6, 10, 20 and 60 min after the injection of the tracer. A two exponential curve fit was applied to the time activity curve of the blood samples and it served as an input for the heart.

The 7-mm thick transaxial, sagittal and coronal slices were reconstructed using a Butterworth filter (order of 8 and cut-off frequency 0.7 cm^{-1}). Two consecutive coronal slices were added together and the "mid ventricular" slice (14 mm thick) was used for semiquantitative analysis. Two circular regions of interest were drawn, one over the myocardium and one over the middle of the left ventricular lumen (Fig. 1). No spillover or overlapping compartment corrections were applied. The ratio of myocardium-to-lumen was calculated for each of the seven points. The average counts of the left ventricular lumen were related to the counts of the extrapolated time activity curve of the blood samples. Thus, the assumption is that these two blood compartments have equal specific activity. Finally, two time activity curves were derived for further analysis; one for blood pool (input function) and one for myocardium (residue function, Fig. 1).

Data analysis. In kinetic analysis, the modified NEFA model of Schelbert [22] was used which is shown in Figure 2. The first compartment describes the plasma pool, the second is the cell and the third is the mitochondrion. The fourth compartment describes the slow turnover pool of esterified ^{123}I-HDA. Six rate constants between the compartments were used. The first, k_{21}, simply reflects myocardial perfusion and capillary permeability. This perfusion phase is followed by two elimination phases. The first elimination phase is considered to represent beta-oxidation, k_{32}, and it is clinically the most important. The parameter k_{42} mainly reflects esterification of NEFA-CoA into a slow turnover pool. The transfer rate constant k_{24} was fixed to zero by assuming that within first 70 min no backflow from this slow turnover pool happens, since DeGrado et al. [23] showed that k_{24} is 50 times slower than k_{42} in the rat heart. The parameter k_{12} describes back diffusion of unused ^{123}I-HDA into the vascular space and k_{13} its end-products which clear from the myocardium. Thus, there are five transfer constants ($k_{24} = 0$) which were iteratively adjusted to the plasma (input) and myocardium (residue) time activity curves using the SCOP/SCOPFIT program [24].

A mathematical index for beta-oxidation, MR (μmol · min^{-1} · 100 g^{-1} of myocardium) can be directly calculated from the model:

$$MR = \text{s-NEFA} \cdot k_{21} \cdot k_{32}/(k_{12} + k_{32} + k_{42}), \qquad \text{(Eq. 1)}$$

where s-NEFA is serum fatty acid concentration (μmol/ml) and k_{ij} are the transfer rate constants of the model in Figure 1 (min^{-1}). Percentage amounts used for beta-oxidation, for back-diffusion of non-esterified tracer and for esterification into the slow turnover pool were calculated according to DeGrado et al. [23] and Bergman et al. [25]. These calculated estimates are not precisely equivalent to true myocardial fatty acid beta-oxidation, but rather functional parameters or so-called mathematical indices.

Myocardial perfusion imaging. To ensure that no perfusion abnormalities influenced regional fatty acid uptake, patients with IGT and NIDDM underwent myocardial perfusion scintigraphy with ^{99m}Tc-methoxy-isobutyl isonitrile (^{99m}Tc-MIBI). The perfusion study was performed after the HDA-SPET, and only pharmacological stress imaging was performed. Combined dipyridamole infusion (0.56 mg/kg over 4 min) and isometric handgrip (30 % of maximal handgrip force) was used as the mode of stress [26]. The dose of ^{99m}Tc-MIBI was 446–470 MBq. None of the patients showed any symptoms or ECG changes during the stress suggestive of myocardial ischaemia.

SPET imaging with Siemens MultiSPECT3 gamma camera equipped with high resolution collimators was started immediately after completing the stress protocol. A full 360° rotation

Table 1. Clinical characteristics

	Subjects				*P* value (Kruskal-Wallis)
	Control (*n = 8)*	IGT (*n = 13)*	IDDM (*n = 8)*	NIDDM (*n = 10)*	
Age (years)	45 ± 4	53 ± 2	43 ± 3	51 ± 2	0.08
BMI (kg/m^2)	26.0 ± 1.0	31.2 ± 1.3[b]	25.8 ± 0.9	30.2 ± 1.6	0.008
Total cholesterol (mmol/l)	5.4 ± 0.2	5.8 ± 0.3	5.6 ± 0.4	5.5 ± 0.3	NS
HDL cholesterol (mmol/l)	1.08 ± 0.09	1.00 ± 0.05	1.48 ± 0.13[a]	1.14 ± 0.11	0.03
Triglycerides (mmol/l)	1.44 ± 0.20	1.90 ± 0.22	0.99 ± 0.13[d]	1.81 ± 0.33	0.01
Fasting plasma glucose (mmol/l)[e]	4.1 ± 0.1	4.6 ± 0.1[b]	9.6 ± 1.6[b]	7.1 ± 0.5[c]	< 0.001
HbA_{1c} (%)	–	5.9 ± 0.2	7.5 ± 0.5	6.3 ± 0.4	0.05
Lactate (mmol/l)[e]	0.78 ± 0.10	0.79 ± 0.06	0.86 ± 0.10	0.84 ± 0.09	NS
NEFA (mmol/l)[e]	0.61 ± 0.07	0.64 ± 0.07	0.77 ± 0.12	0.55 ± 0.08	NS

Values are mean ± SEM. [a]$p < 0.05$, [b]$p < 0.01$ and [c]$p < 0.001$ compared with the control group; [d]$p = 0.06$ compared with the control group; [e] measured during the SPET study

was used and 30 views per head, each 30 s, were acquired in a 64×64 matrix mode. The 7-mm thick transaxial, sagittal and coronal slices were reconstructed using a Butterworth filter (order of 8 and cut-off frequency 0.7 cm^{-1}). The SPET images were visually evaluated by two experienced readers and classified normal, equivocal or abnormal. In case of discrepancy, the images were re-classified after consensus was reached between the readers.

Echocardiography. M-mode and Doppler echocardiographic examinations were performed by the same observer using Aloka SSD-870 ultrasound system (Aloka Ltd., Tokyo, Japan) with the patient in the left lateral semirecumbent position. All tracings were recorded at 100 mm/s paper speed; the measurements were obtained from 3 to 5 cardiac cycles and averaged [27]. M-mode measurements were performed according to the recommendations of the American Society of Echocardiography [28] and left ventricular mass was calculated according to Devereux and Reichek [29]. Left ventricular diastolic function was determined on the basis of filling indices as previously described [27].

Statistical analysis

Data were analysed using SPSS/PC statistical package (version 4.01; SPSS Inc., Chicago, Ill., USA). Non-parametric Kruskal-Wallis analysis of variance was used in comparisons between the groups, and further comparisons were performed with Mann-Whitney's U-test. Spearman correlation coefficients were used in analysing linear correlations between the variables. *P* values 0.05 or less were considered to be statistically significant.

Results

Clinical characteristics. The mean age of the subjects did not differ significantly among the groups, although subjects with IGT and NIDDM were somewhat older than control subjects. IGT and NIDDM subjects were more obese than the others (Table 1). During the SPET study, systolic blood pressure (control group 129 ± 6, IGT 130 ± 3, IDDM 136 ± 4, and NIDDM 137 ± 4 mmHg, mean ± SEM) and heart rate (control 63 ± 1, IGT 63 ± 2, IDDM 65 ± 5 and NIDDM 67 ± 2 beats/min, respectively) were comparable among the groups. Similarly, double product was equal in the four groups (control 8093 ± 412, IGT 8098 ± 265, IDDM 8710 ± 624 and NIDDM 9280 ± 399 beats. mmHg/min). During the SPET study. IGT, IDDM and NIDDM patients had higher fasting blood glucose compared with the control subjects, but serum NEFA and lactate concentrations did not differ between the groups. Glycaemic control as assessed by HbA_{1c} was good in both diabetic groups. Serum total cholesterol and triglyceride concentrations did not differ among the groups, but HDL cholesterol was the highest in IDDM patients ($p < 0.05$ compared with control subjects, Table 1).

In IGT subjects, fasting plasma glucose during OGTT was 6.3 ± 0.1 mmol/l and 2-h glucose 8.7 ± 0.2 mmol/l. S_I derived from the IVGTT was 2.01 ± 0.24 10^{-4} (min^{-1} /(μU/ml)), S_G 0.0131 ± 0.002 min^{-1}, fasting plasma insulin 78 ± 8 pmol/l and first phase insulin response was 5388 ± 1128 pmol · min (data available only for the IGT group).

Cardiac dimensions and systolic and diastolic function. Left ventricular diastolic diameter was similar in all groups, but IGT subjects had increased septal and posterior wall thicknesses, left ventricular mass and mass index compared with the control subjects (Table 2). Altogether in five patients (three IGT, two NIDDM patients) some of the cardiac dimensions and left ventricular mass could not be determined reliably for technical reasons. Left ventricular mass had a positive correlation with body mass index ($r = 0.69$, $p < 0.001$). In cardiac systolic and diastolic function no significant differences could be observed among the groups (Table 2).

Myocardial NEFA kinetics. Myocardial beta-oxidation index expressed per 100 g of tissue weight was reduced by 53% in the IGT group compared with the control group (Table 3) and also the total beta-oxidation index relative to the total heart weight was decreased in the IGT group (IGT 13.3 ± 1.2, IDDM 23.1 ± 4.1, NIDDM 20.0 ± 2.9 and control subjects 21.7 ± 2.8 μmol/min, $p < 0.05$ between the

Table 2. Cardiac dimensions and function and left ventricular mass

	Subjects				*P* value (Kruskal-Wallis)
	Control ($n = 8$)	IGT ($n = 13$)	IDDM ($n = 8$)	NIDDM ($n = 10$)	
LVEDD (mm)	52 ± 2	52 ± 1	51 ± 1	54 ± 1	NS
Fractional shortening (%)	30 ± 2	35 ± 2	33 ± 2	32 ± 2	NS
Posterior wall (mm)	9.9 ± 0.7	11.6 ± 1.1[b]	10.3 ± 1.5	9.4 ± 1.0	0.003
Septum (mm)	10.1 ± 0.6	12.1 ± 0.9[c]	10.6 ± 1.5	10.7 ± 1.6	0.009
E (cm/s)	58 ± 6	58 ± 2	57 ± 4	59 ± 4	NS
A (cm/s)	50 ± 3	48 ± 3	46 ± 2	55 ± 4	NS
E/A ratio	1.19 ± 0.14	1.25 ± 0.08	1.24 ± 0.11	1.13 ± 0.08	NS
Left ventricular mass (g)	225 ± 13	289 ± 10[b]	236 ± 19	241 ± 17	0.02
Left ventricular mass index (g/m^2)	117 ± 4	140 ± 5[b]	120 ± 7	121 ± 6	0.036

Values are mean ± SEM. [b]$p < 0.01$ and [c]$p < 0.001$ compared with the control group.
LVEDD, left ventricular end-diastolic diameter; E, peak flow velocity of early LV filling; A, peak late (atrial) velocity of LV filling

Table 3. Myocardial fatty acid kinetics

	Subjects				*P* value (Kruskal-Wallis)
	Control ($n = 8$)	IGT ($n = 13$)	IDDM ($n = 8$)	NIDDM ($n = 10$)	
Uptake (%/ID) 100 g	5.6 ± 0.3	3.7 ± 0.2[b]	5.2 ± 0.3	4.7 ± 0.3	0.001
Beta-oxidation (μmol · min^{-1} · 100 g^{-1})	9.7 ± 1.0	4.6 ± 0.4[b]	10.0 ± 1.7	7.3 ± 1.0	0.001
k21 (1/min)	0.265 ± 0.022	0.179 ± 0.013[b]	0.251 ± 0.014	0.225 ± 0.018	0.005
k12 (1/min)	0.051 ± 0.015	0.061 ± 0.006	0.061 ± 0.015	0.051 ± 0.012	NS
k32 (1/min)	0.106 ± 0.029	0.053 ± 0.006[a]	0.071 ± 0.007	0.083 ± 0.010	0.027
k13 (1/min)	0.258 ± 0.047	0.292 ± 0.047	0.241 ± 0.050	0.262 ± 0.043	NS
k42 (1/min)	0.013 ± 0.002	0.009 ± 0.002	0.011 ± 0.001	0.014 ± 0.001	0.079

Values are mean ± SEM. [a]$p < 0.05$ and [b]$p < 0.01$ compared with the control group.
ID, Injected dose; transfer constant k_{21}, myocardial perfusion and capillary permeability; k_{12}, backdiffusion of unused ^{123}I-HDA into the vascular space; k_{32}, beta-oxidation; k_{13}, beta-oxidation end-products clearing from the myocardium; k_{42}, esterification of NEFA-CoA into a slow turnover pool. See also Fig. 2.

control and IGT group). Furthermore, the initial myocardial HDA uptake was reduced by 34% in subjects with IGT (Table 3). In subjects with IGT, HDA beta-oxidation index differed significantly from that of IDDM patients ($p < 0.01$) and NIDDM patients ($p < 0.05$). Patients with NIDDM also tended to have decreased HDA uptake and beta-oxidation index, but these differences did not reach statistical significance (for HDA uptake $p = 0.08$ and for HDA beta-oxidation index $p = 0.11$ compared with control subjects, respectively). Patients on antihypertensive medication ($n = 7$) had similar HDA uptake and beta-oxidation compared with normotensive patients ($n = 32$) (uptake 4.5 ± 0.5 vs 4.7 ± 0.2 %/ID/100 g, beta-oxidation 6.5 ± 1.5 vs 7.6 ± 0.7 μmol · min^{-1} · 100 g^{-1}).

In the IGT group, the transfer rate constant k_{32} indicating beta-oxidation was 50% less than in the control group, and also, the rate constant k_{21} was lower. Other transfer rate constants did not differ significantly among the groups (Table 3). Fractional HDA amount used for beta-oxidation was significantly reduced in the IGT group (IGT 43 ± 4, IDDM 53 ± 5, NIDDM 58 ± 4 and control subjects 61 ± 4%, $p < 0.05$ between the IGT and control groups), and consequently, the fractional amount of back diffusion was increased (IGT 50 ± 4, IDDM 39 ± 5, NIDDM 32 ± 4 and control subjects 30 ± 4, $p < 0.01$ between the IGT and control groups). HDA esterification into the slow turnover pool was similar in all groups (IGT 7 ± 1, IDDM 8 ± 1, NIDDM 10 ±1, and control subjects 9 ± 1%).

In the whole group serum triglyceride concentration had a weak inverse correlation with the HDA beta-oxidation index ($r = –0.39$, $p < 0.01$). Left ventricular mass correlated inversely with the HDA uptake ($r = –0.50$, $p = 0.001$) and HDA beta-oxidation index expressed by 100 g of tissue weight ($r = –0.42$, $p < 0.01$). BMI correlated inversely with HDA uptake ($r = –0.52$, $p < 0.001$) and HDA beta-oxidation index ($r = –0.41$, $p < 0.01$). Double product did not correlate with HDA uptake or beta-oxidation. Age made no significant contribution to these kinetic variables. In subjects with IGT, 2-h glucose concentration was positively correlated with the constant k_{42} (HDA esterification into the slow turnover pool) ($r = 0.74$, $p < 0.01$), whereas S_I had an inverse correlation with k_{42} ($r = –0.67$, $p < 0.01$).

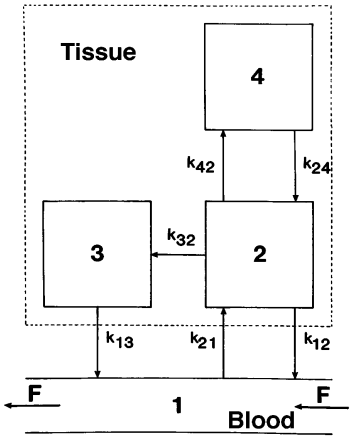

Fig. 2. Modified Schelbert's model for myocardial NEFA kinetics

Myocardial perfusion. After the HDA-SPET study, myocardial perfusion imaging was performed for the IGT and NIDDM groups, since these groups had abnormal HDA kinetics. The average time between HDA-SPET and perfusion imaging was 20 months (range 18–22) in the IGT group and 13 months (11–15) in the NIDDM group. Perfusion data are available for 11 out of 13 IGT and 8 out of 10 NIDDM subjects. All patients showed homogeneous myocardial perfusion during the stress. Furthermore, one NIDDM patient underwent coronary angiography which showed normal coronary arteries. Thus, in 11 of 13 IGT subjects and in 9 of 10 NIDDM subjects the possibility of myocardial ischaemia or scarring could be confidently excluded. The two remaining IGT subjects and one NIDDM subject were either not willing or were lost for the perfusion study.

Thus, IGT subjects showed decreased myocardial HDA uptake and beta-oxidation, and in turn, HDA back diffusion was increased compared with the control group. Patients with NIDDM also tended to have reduced HDA uptake and beta-oxidation index, whereas IDDM patients had similar myocardial HDA kinetics to the control subjects.

Discussion

By using HDA-SPET imaging and mathematical modelling, abnormal myocardial NEFA kinetics were observed in subjects with IGT. HDA uptake and beta-oxidation index were decreased compared with the control subjects, and indirectly, these results may suggest an enhanced use of myocardial endogenous fat storage or circulating triglyceride-rich lipoproteins for energy. No previous studies applying this methodology for the evaluation of myocardial metabolism in IGT subjects are available. These results show that abnormalities in myocardial fatty acid uptake can be observed even before the

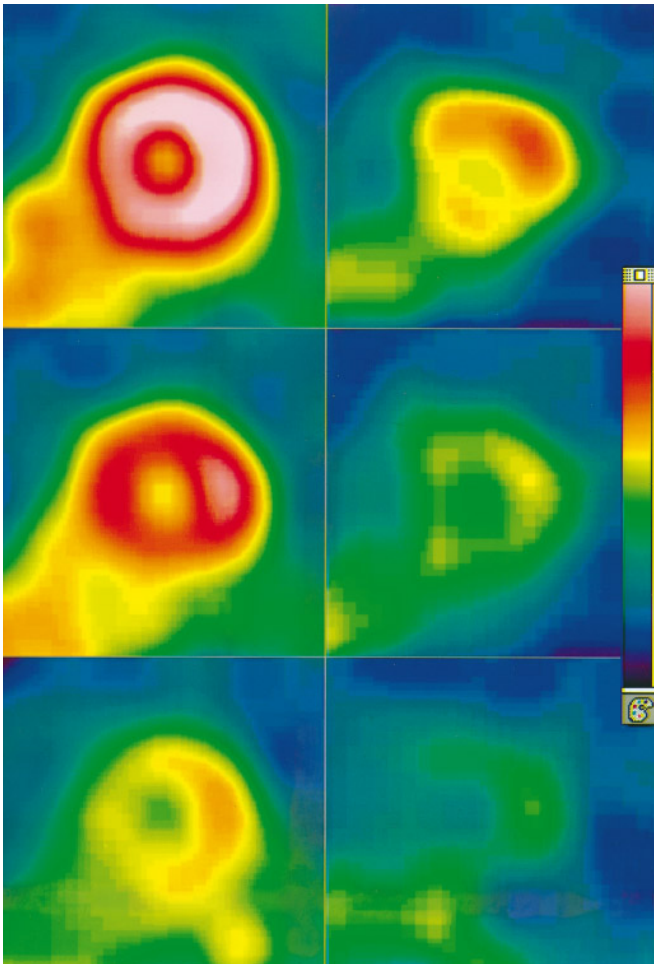

Fig. 3. Myocardial HDA distribution as shown by transaxial SPET slices at 10, 40 and 70 min. Left panel: control subject; right panel: IGT subject. Note the heterogeneous HDA uptake and rapid wash-out in the IGT subject. Colour scale on the right side

diagnosis of clinical diabetes and they are related to indices of insulin resistance, e. g. S_I and 2-h glucose level. Importantly, myocardial perfusion was normal in IGT and NIDDM groups and thus defective fatty acid metabolism in these conditions cannot be ascribed to silent coronary flow abnormalities.

Heart failure and death from acute myocardial infarction as well as chronic pump failure are more common in diabetic patients, and a specific diabetic heart muscle disease has been suggested to contribute to increased cardiovascular morbidity and mortality [3–5]. The pathophysiology of diabetic heart muscle disease is still largely unknown, but in addition to metabolic abnormalities in the myocardium, increased fibrosis, small vessel disease, disturbed intracellular calcium transport and metabolism, and autonomic neuropathy have been observed in this condition [4–7]. Most studies concerning metabolism in the diabetic myocardium have focused on the effects of insulin-deficient diabetes. In experimental studies performed

with insulin-deficient animals, decreased myocardial glucose oxidation has been observed, whereas NEFA oxidation was increased [9, 10]. Moreover, increased exo- and endogenous NEFA oxidation and fat accumulation are associated with depressed cardiac function observed in insulin-deficient animals [6, 9]. Similarly, in patients with IDDM myocardial glucose uptake is depressed, and NEFA and ketone utilization are increased [11]. However, during hyperinsulinaemia myocardial glucose uptake as assessed with PET is normal in young IDDM patients [16]. Also, in this study IDDM patients had similar myocardial HDA kinetics to the control subjects. Thus, our results support the view that in otherwise healthy IDDM patients with intensive insulin therapy and good metabolic control, myocardial substrate utilization is normal.

IGT is frequently characterized by insulin resistance and hyperinsulinaemia, and most subjects with IGT are obese. When pancreatic beta cells can no longer maintain a state of hyperinsulinaemia, hyperglycaemia and NIDDM develop [30]. In addition, IGT and NIDDM share similar abnormalities in lipid and lipoprotein metabolism. Increased lipid turnover rate and elevated NEFA concentration lead to increased triglyceride accumulation, enhanced lipid oxidation and further to inhibition of glycolysis and glucose oxidation [12, 30]. The glucose-fatty acid cycle initially described by Randle et al. [31] has been shown to be present also in the human heart: increased fatty acid oxidation inhibits glucose oxidation delete [32]. However, data concerning myocardial metabolism in NIDDM and IGT patients are scarce, and it is not known whether myocardial substrate utilization is altered in these conditions.

^{123}I-heptadecanoic acid (HDA) is a radioiodinated fatty acid analogue, which is largely metabolized through beta-oxidation with concomitant release of free iodide in the myocardium [33, 34]. We have previously demonstrated disturbed myocardial fatty acid metabolism in NIDDM patients with HDA-SPET-imaging, suggesting increased triglyceride synthesis in the heart [13]. In contrast to our expectations, myocardial HDA kinetics were even more abnormal in IGT subjects than in NIDDM patients. The most plausible explanation for this is that some of the NIDDM patients were lean and might have had a relative insulin deficiency. Instead, all subjects with IGT were obese, their glucose tolerance status was confirmed with two consecutive OGTTs and furthermore, insulin sensitivity index (S_I) based on the IVGTT was found to be moderately reduced [18, 20]. Thus, the discrepancy between the results of the IGT and NIDDM patients can be explained by the heterogeneity of the disease; IGT subjects probably were more insulin-resistant than treated NIDDM patients in the present study.

According to the glucose-fatty acid cycle [31, 32], hyperglycaemia could result in depression of myocardial NEFA utilization. However, even though IGT subjects were normoglycaemic during the study, they still had the most abnormal HDA kinetics. In contrast, hyperglycaemic IDDM patients had similar fatty acid kinetics to the control subjects. Thus, our results do not completely support the concept of the glucose-fatty acid cycle in vivo among these subjects. Rather, it seems that other factors, e. g. insulin resistance and disturbed lipid metabolism, also significantly contribute to myocardial substrate utilization especially in patients with IGT and NIDDM. It must be emphasized that differences in serum NEFA concentration or myocardial work-load do not explain our results, since these variables were similar among the four groups.

Assuming that subjects with IGT are insulin-resistant and might have a decreased myocardial glucose uptake, the question arises as to what is the principal energy source of human myocardium in this condition. According to the results of the present study, uptake of circulating NEFA by the myocardium was also reduced in the IGT group. Interestingly, in the skeletal muscle, impaired NEFA utilization not due to glucose-fatty acid competition has been observed in NIDDM [35]. We hypothesize that intramyocardial triglyceride stores or plasma triglycerides, which are increased in availability in IGT and NIDDM could have a more important role than believed. The existence of a VLDL receptor [36], highly abundant in the heart and capable of mediating uptake of apolipoprotein E-containing triglyceride-rich particles, fits well with this hypothesis. Furthermore, a recent study suggested that intramuscular lipolysis in the skeletal muscle may be unexpectedly high and regulate fuel metabolism [37]. In the present study S_I and 2-h glucose determined in the IGT group correlated with the kinetic abnormalities of HDA, suggesting that insulin resistance per se or the underlying factors are directly responsible for the observed disturbances in fatty acid metabolism. Note also that insulin resistance has been shown to correlate with the muscle triglyceride content [38].

The question of coronary heart disease and silent myocardial ischaemia in IGT and NIDDM patients is crucial, because reduced myocardial flow could lead to impaired fatty acid uptake and beta-oxidation. Since myocardial perfusion was normal even during pharmacological stress in IGT and NIDDM groups, it is clear that myocardial ischaemia did not explain our results. In addition, those patients with antihypertensive medication had similar myocardial HDA kinetics compared with normotensive patients. One might also argue that increased left ventricular mass observed in IGT subjects could in part contribute to disturbed fatty acid metabolism, since in hypertrophic myocardium increased glucose oxidation and

decreased NEFA utilization have been observed [39]. On the other hand, in some studies left ventricular hypertrophy has been related to insulin resistance [40, 41]. In the present study, lowered HDA beta-oxidation index in IGT subjects was observed even if the results were normalized for myocardial mass.

We must emphasize that SPET imaging with the use of a fatty acid analogue has limitations and gives only an estimate of NEFA metabolism of the heart. However, this study was performed in the fasting state under physiological glucose and insulin concentrations. Thus, these preliminary data give valuable new information concerning myocardial fatty acid utilization across the spectrum of glucose tolerance.

In conclusion, abnormal myocardial HDA uptake and beta-oxidation index not attributable to myocardial ischaemia could be observed in patients with IGT. NIDDM patients also tended to have lowered HDA uptake and beta-oxidation, whereas in IDDM patients HDA kinetics were similar to normal subjects. These data suggest that insulin resistance per se or the underlying factors, possibly related to an altered plasma or cellular triglyceride metabolism are responsible for the abnormal myocardial fatty acid metabolism observed in IGT patients.

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