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ORIGINAL ARTICLE

Glucose turnover and intima media thickness of internal carotid artery in type 2 diabetes offspring

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ABSTRACT

Background First-degree offspring (OFF) of type 2 diabetic (T2DM) patients bear a ~40% lifetime risk of developing T2DM. They are insulin resistant and carry a risk of premature atherosclerosis, the extent of which can be estimated by intima media thickness (IMT) of the carotid artery (CA). Thus, this study examines parameters of glucose and lipid metabolism, insulin sensitivity, beta cell function (BCF) and IMT with their interrelationships in middle-aged OFF.

Materials and methods T2DM-OFF (n = 18, 14f/4m, 45·6 ± 2·1 years, BMI: 26 ± 1 kg m^{-2}) were compared with 18 matching humans without a family history of diabetes (CON; 14f/4m, 44·5 ± 2·1 years, BMI: 24 ± 1 kg m^{-2}; each $P > 0{\cdot}30$), all with normal glucose tolerance as tested by three-hour (75 g) oral glucose tolerance tests (OGTT). Two-hour hyperinsulinaemic (40 mU $min^{-1}{\cdot}m^{-2}$)isoglycaemic clamp tests were performed with simultaneous measurement of endogenous glucose (D-[6,6-$^2H_2$]glucose) production (EGP). IMT [internal (ICA), common CA, and bulb] were measured sonographically. BCF was assessed by Adaptation Index (AI).

Results Before and during OGTT, both groups were similar in plasma glucose, insulin, C-peptide and free fatty acids (FFA), whereas OFF showed ~30% lower ($P < 0{\cdot}03$) fasting plasma triglycerides before OGTT. During hyperinsulinaemic clamps, insulin sensitivity was ~38% lower ($P < 0{\cdot}03$) in OFF who showed higher plasma FFA (44 ± 9 μmol L^{-1}) than CON (26 ± 3 μmol L^{-1}, $P < 0{\cdot}05$) after 90 min. EGP was similar in both groups. OFF had 38% ($P < 0{\cdot}007$) reduced AI. ICA-IMT was ~18% higher in OFF ($P < 0{\cdot}002$), but did not correlate with insulin sensitivity.

Conclusion The data obtained show middle-aged T2DM-OFF with normal glucose tolerance displaying reduced total insulin sensitivity and impaired beta cell function, which relates to impaired insulin-dependent suppression of plasma FFA and increased ICA-IMT.

Keywords Free fatty acids, insulin resistance, intima-media-thickness, type 2 diabetic offspring.

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Introduction

Type 2 diabetes mellitus (T2DM) becomes overt once insulin resistance, which is seen in nearly all patients with T2DM, cannot be overcome by augmented insulin secretion [1]. The impairment of insulin sensitivity in the offspring (OFF) of parents with T2DM, but not in humans without a family history of T2DM, can be used as a predictor for the later onset of the disease [2,3].

Perseghin *et al*. have demonstrated the relationship of skeletal muscle insulin resistance with increased intramyocellular lipid content in OFF [4], an association also seen in patients with overt T2DM [1]. Thus, humans with a family history of T2DM bear a markedly increased lifetime risk of developing diabetes, which can even be regarded a pre-diabetic state. Since T2DM goes along with endothelial dysfunction, associated premature central and peripheral macrovascular disease can be regarded, among others, also as a late result of insulin resistance [5–8].

In order to study glucose and lipid parameters and their interference with atherosclerosis in the very early state of pre-diabetes, we initiated our investigations in healthy, normotensive, middle-aged OFF with normal glucose tolerance. We hypothesised that in healthy middle-aged first degree relatives of T2DM with normal glucose tolerance, insulin insensitivity in insulin sensitive tissues (i.e. skeletal muscle, liver and fat) (i) will be associated with abnormal β-cell function, (ii) can furthermore be linked to accelerated atherosclerosis that can be assessed by the measurement of

intima media thickness (IMT) of the carotid artery [9], and (iii) will lead to peripheral arterial disease [6] that can be clinically proven by reduced leg perfusion.

We therefore examined (i) glucose tolerance and frequently sampled time course of plasma insulin, C-peptide and free fatty acids (FFA) during a three-hour oral glucose tolerance test (OGTT) for calculation of β-cell function indices, (ii) whole body insulin sensitivity during a two-hour hyperinsulinaemic isoglycaemic clamp test with simultaneous measurements of endogenous glucose production (EGP) by tracer dilution technique and insulin-mediated suppression of plasma FFA, (iii) IMT of three carotid artery sections (the common trunk, the bulb and the inner branch), (iv) ankle-brachial index (ABI), and (v) physical activity in the offspring (OFF) of T2DM patients with normal glucose tolerance as well as in humans without a family history of T2DM (CON). These two groups were matched for major anthropometrical characteristics.

Materials and methods

Study participants

All participants were recruited by means of local advertising between 2003 and 2004. They were pre-screened for a family history of T2DM and confirmation of excellent health and the absence of any regular drug intake. Modified versions of the *Rose* and the *Edinburgh* claudication questionnaires were used to exclude humans with cardiovascular disease (CVD) and peripheral arterial disease (PAD), respectively [10–12]. The subjects had been instructed to refrain from excessive physical exercise and to ingest an isocaloric carbohydrate-rich diet for three days before baseline examination (Study Day 1) and the clamp test (Study Day 2). All participants gave informed consent to the protocol, which was approved by the Institutional Ethics Board.

Study Day 1

After an overnight fast for at least 12 h, the participants underwent a complete medical history and a thorough clinical examination to confirm their health was good, followed by anthropometrical measurements, a resting electrocardiogram (ECG) recording and a routine laboratory check. Sitting blood pressure was measured three times using the Omron 705 cp (Omron Healthcare Europe, Hoofddorp, The Netherlands). Body weight and fat mass were measured by the Tanita Bioimpedance Balance (TBF-300 body composition analyzer, Tanita International Division, Yiewsley, UK); waist and hip circumferences by tape measure according to a standardized written protocol [10].

Anthropometrical characteristics (Table 1)

The two groups, T2DM-OFF and controls (CON), were matched for gender, age and body mass index (BMI) (each $P > 0{\cdot}30$), and they also did not differ in body weight, height and fat mass. Waist to hip ratio in OFF was slightly, but significantly higher by ~7% ($P < 0{\cdot}05$ vs. CON).

Oral glucose tolerance test (OGTT)

The participants drank 75 g of glucose (Gluco-Drink 75®, Roche Diagnostics, Vienna, Austria). Blood samples for the determination of plasma glucose, insulin, C-peptide and FFA were obtained at 0, 10, 20, 30, 40, 60, 90, 120, 150 and 180 min, immediately kept on ice for 10 min and then centrifuged (10 min, 4 °C, 3634 *g*). The supernatant was separated to be stored at –80 °C until further analysis [13–16]. The participants remained in the sitting or supine position throughout the entire OGTT.

All study participants showed normal fasting glucose values and normal glucose tolerance.

Physical activity

Immediately after the OGTT, daily physical activity was estimated in 15 subjects (7 CON and 8 OFF) by actigraph, an accelerometer that employed a piezoelectric transducer to sense body movements. These subgroups studied with the actigraph were representative of the entire two groups, since they did not differ in major anthropometrical characteristics, such as body weight, height, BMI or gender (each $P > 0{\cdot}42$). The actigraph used in this study (Computer Science Application, model AM7164, Manufacturing Technology, Fort Walton Beach, FL, USA) was attached to a waist belt for between three and seven days (CON: 5·8 ± 0·6 days, OFF: 5·7 ± 0·5 days). Acceleration signals were digitized, summed over a 1-min period and saved in a memory. Saved data were electronically processed to evaluate energy expenditure (in kcal day^{-1}) over the recording period [17].

Study Day 2

In CON and OFF, Study Day 2 was performed 33 ± 4 and 32 ± 3 days, respectively, after the Study Day 1. After a further overnight fast for at least 12-h, two catheters (Vasofix; Braun, Melsungen, Germany) were inserted into one antecubital vein of the left and right arm for blood sampling and infusions. A primed-continuous infusion [0–5 min: 4 mg × kg lean body weight (obtained from Tanita measure); thereafter 0·04 mg min^{-1} × lean body weight] of D-[6,6-$^2H_2$]glucose (98% enriched; Cambridge Isotope Laboratories, Andover, MA, USA) was given to 21 participants (14 OFF/7 CON) for 120 min before and until 15 min after the start of the clamp to determine EGP [1,18–20]. These subgroups were also representative for all the groups, since they did not differ in major anthropometrical characteristics (each $P > 0{\cdot}57$).

The isoglycaemic clamp glucose target was determined from the mean value of three fasting plasma glucose measurements.

Table 1 Anthropometrical characteristics (serum) routine laboratory measurements, ankle brachial index of the right/left dorsal pedal and posterior tibial artery, and physical activity, measured by the CSA Actigraph as described [17] as well as markers of β-cell function calculated from OGTT plasma insulin, C-peptide and glucose in controls (CON) (n = 18) and type 2 diabetes offspring (OFF) (n = 18). All data are given as means ± SE. Student's t-test CON vs. OFF.

	CON	OFF	*P*
n (f/m)	18 (14/4)	18 (14/4)	1·000
Age (years)	45·6 ± 2·1	44·5 ± 2·1	0·713
Body weight (kg)	69·0 ± 2·3	73·5 ± 3·2	0·257
Height (cm)	168·9 ± 1·1	169·8 ± 1·7	0·656
BMI (kg m^{-2})	24·2 ± 0·7	25·6 ± 1·1	0·298
Fat mass (kg)	18·9 ± 1·9.	23·6 ± 2·2	0·144
Waist-to-hip ratio	0·84 ± 0·01	0·91 ± 0·03	**0·041**
Waist circumference (cm)	84 ± 2	91 ± 4	0·125
RR sys/dia (mmHg)	115 ± 3/76 ± 2	119 ± 3/75 ± 2	0·427
Serum creatinine (mg dL^{-1})	0·85 ± 0·03	0·79 ± 0·03	0·210
Serum uric acid (mg dL^{-1})	4·9 ± 0·5	4·3 ± 0·2	0·270
HbA1c (%)	5·5 ± 0·1	5·4 ± 0·1	0·554
Serum triglycerides (mg dL^{-1})	99 ± 11	69 ± 6	**0·028**
Serum total cholesterol (mg dL^{-1})	211 ± 9	197 ± 10	0·281
Serum HDL cholesterol (mg dL^{-1})	61 ± 3	57 ± 3	0·371
Serum LDL cholesterol (mg dL^{-1})	130 ± 9	126 ± 8	0·692
Serum ASAT (GOT) (U L^{-1})	24 ± 3	25 ± 2	0·806
Serum ALAT (GPT) (U L^{-1})	20 ± 2	21 ± 2	0·809
Right ABI (dors. pedal artery)	1·21 ± 0·03	1·23 ± 0·04	0·752
Right ABI (post. tib. artery)	1·23 ± 0·03	1·27 ± 0·04	0·431
Left ABI (dors. pedal artery)	1·19 ± 0·04	1·22 ± 0·05	0·685
Left ABI (post. tib. artery)	1·22 ± 0·03	1·23 ± 0·03	0·834
Physical activity (kcal d^{-1})	431 ± 56	538 ± 116	0·402
Dynamic AUC_{gluc} (mmol·L^{-1}·min^{-1})	3447 ± 603	5277 ± 602	**0·040**
Dynamic AUC_{ins} (nmol·L^{-1}·min^{-1})	4816 ± 481	7042 ± 1313	0·216
Dynamic $AUC_{C\text{-}pep}$ (nmol·L^{-1}·min^{-1})	712 ± 47	839 ± 75	0·107
HOMA2%B	98 ± 7	114 ± 6	0·086
Adaptation Index (mg·kg^{-1}·ng·mL^{-1})	6568 ± 628	4766 ± 364	**0·007**

f, female; m, male; ABI, ankle brachial index; ALAT, alanine aminotransaminase; ASAT, aspartate aminotransaminase; AUC, area under the curve; BMI, body mass index; dors., dorsal; HbA1c, glycated haemoglobin A1c; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; RR sys/dia, systolic and diastolic blood pressure; tib., tibial.

However, in case of a value lower than 80 mg dL^{-1}, the glucose clamp target was set to 80 mg dL^{-1}, and in case of a value higher than 100 mg dL^{-1}, the clamp goal was then 100 mg dL^{-1}. Hyperinsulinaemic-isoglycaemic clamps were performed for 120 min, with primed (0–4 min: 4 fold, 5–7 min: 2-fold rate) continuous insulin (Actrapid; NovoNordisk, Bagsvaerd, Denmark) infusion (40 mU insulin·min^{-1}·m^{-2} body surface area) [15,16,19]. In order to measure EGP during the clamp tests, the 20% glucose infusions were enriched with D-[6,6-$^2H_2$]-glucose to ~2% mole percent excess (MPE) as previously described [1,19].

Intima media thickness

High-resolution B-mode ultrasound system (Acuson XP, 5 MHz linear transducer, Siemens, Mountaintop, PA, USA) was utilized to obtain longitudinal images of the right and left extracranial carotid arteries from the anterior, lateral and posterior angles. The images were recorded on videotape and evaluated in the

reading centre of the 'Relationship between Insulin Sensitivity and Cardiovascular disease risk'('RISC' Study, Institute of Clinical Physiology, Pisa, Italy) by a single reader blinded to the identity of the participant and using a high-resolution video recorder (Panasonic AG-MD830, Panasonic System Engineering Italia S.P.A., Milan, Italy) coupled with the computer-driven image analysis system developed by the reading centre [21]. End-diastolic frames of common carotid artery (CCA), the carotid bulb (CB) and the internal carotid artery (ICA) were selected as recommended [22], and digitized. In each carotid segment, near- and far-wall IMTs were measured bilaterally for a single CCA view and for three different views of the CB and ICA. Each measure of IMT represented an average of three to five measurement points. CCA, CB and ICA IMTs used for statistical analysis were calculated as the overall mean of the available IMT measurements (up to four for CCA-IMT and up to 12 for CB- and ICA-IMTs). Intra- and interindividual variability of IMT measurements in the reading lab was 3·9 and 4·8% respectively.

Ankle brachial index (ABI)

Resting ankle and brachial systolic blood pressures were measured as recommended [23] using the bidirectional MultiDopplex® II (Huntleigh Diagnostics, Cardiff, UK) handheld Doppler.

Plasma metabolites and hormones

Plasma glucose concentrations were measured using the glucose oxidase method (Glucose Analyzer II; Beckman, Fullerton, CA, USA). Plasma insulin and C-peptide were analyzed by commercially available radioimmunoassays from Linco Research (St. Charles, MO, USA) and plasma FFA concentrations with a microfluorimetric assay (Wako, Richmond, VA, USA) [15,16,24].

MPE of plasma and infusate D-[6,6-$^2H_2$] glucose was measured on a Hewlett-Packard 5890 gas chromatograph equipped with a CP-Sil5 25 m × 0·25 mm × 0·12 μm capillary column (Chrompack, Middelburg, The Netherlands) and interfaced to a Hewlett-Packard 5971 A Mass Selective Detector as described [1,19].

Calculations

Baseline rates of EGP were calculated by dividing the tracer (D-[6,6-$^2H_2$]glucose) infusion rate times tracer enrichment by the tracer enrichment in plasma and subtracting the tracer infusion rate [1,19,25]. During the clamps, EGP was assessed using the formula: EGP = glucose infusion rate $(GIR)_{mean} \times [(enrichment_{inf}/enrichment_{plasma}) - 1]$, where GIR_{mean} is the mean GIR during the preceding 30 min, $enrichment_{inf}$ is the MPE of glucose in infusate, and $enrichment_{plasma}$ is the MPE of plasma glucose during steady-state conditions of the clamp. All EGP results are given in mg glucose·min^{-1}·kg^{-1} total body weight. Whole body insulin sensitivity was calculated as the mean GIR (mg glucose·min^{-1}·kg^{-1} total body weight) during 20 min intervals of the clamp test [1,14–16,26].

Concentration areas under the curve (AUC) during the OGTT were calculated by the trapezoidal rule. Dynamic concentrations AUC (dyn AUC) were calculated as total AUC-(180 × basal concentration). The Adaptation Index (AI, nmol·m^{-2}), firstly introduced by *Ahren* and *Pacini* [27], is a measure of the capacity of the β-cells to adapt to changes in surrounding insulin sensitivity, and was calculated as the product between clamp insulin sensitivity (clamp GIR between 100 and 120 min) and $AUC_{C\text{-}pep}$ from oGTT. This avoids biases possibly encountered with composite indices when the parameters arise from the same experimental procedure [28,29]. A marker of fasting β-cell function [homeostasis model assessment-2%B (HOMA2%B)] was calculated as the ratio of fasting C-peptide concentration to fasting glucose, since C-peptide is not cleared by the liver [13,15].

Statistics

Before further analysis, normal distribution of the variables was tested by applying the *Kolmogorov-Smirnov* test for the entire study population and each sub-group separately [16]. This test showed that all of the continuous variables except for ASAT, OGTT insulin at 60 and 90 min, OGTT FFA at 150 and 180 min as well as clamp FFA 120 min were normally distributed. Therefore, those listed variables data were logarithmically transformed to achieve normal distributions, and statistical tests were applied to the transformed variables [16]. Comparisons between the groups were performed by the two-sided *Student*'s *t*-test for unpaired data and the data are given as means ± SE. *Pearson*'s product moment correlation was used to estimate linear relationships between variables. Differences were considered statistically significant at $P < 0·05$.

Multiple linear regression analysis, based on the data of all participants using ICA-IMT as a dependent variable was applied. Variables correlating with ICA-IMT on a level of $P < 0·05$ were considered for the first model (1 covariate per 10 participants) to find possible predictors for ICA-IMT. Predictors of ICA-IMT at a significance level of $P < 0·1$ remained in the model, as described in detail elsewhere [15,30]. The final model was verified by backward stepwise linear multiple regression analysis.

Results

Fasting routine lab

The results of the fasting routine lab check are shown in Table 1, confirming the excellent health of CON and OFF. In particular, OFF showed 30% lower fasting serum triglyceride concentrations ($P < 0·03$). Glycated haemoglobin A1c (HbA1c) was similar in both groups.

Oral (75 g) glucose tolerance test

In both groups, the plasma concentrations of glucose, insulin, C-peptide and FFA were not different before or during the entire OGTT (Fig. 1). Total AUC of plasma glucose, insulin, C-peptide and FFA during the OGTT were also similar in both groups (data not shown), whereas dynamic AUC of glucose during the OGTT was higher by 53% in the OFF ($P < 0{\cdot}04$) (Table 1).

Physical activity

No difference between both groups was found in physical activity, as measured by the movement sensor (Table 1).

β-cell function (Table 1)

No differences were found regarding fasting β-cell function (HOMA2%B). The Adaptation Index (AI), reflecting the dynamic ability of β-cells to adapt to ambient insulin sensitivity changes under the dynamic conditions of OGTT, was 38% ($P < 0{\cdot}007$) lower in OFF.

Clamp results (Fig. 2)

Plasma glucose was slightly, but significantly different at the start of the clamp, and at 60, 100, 110 and 115 min (each $P < 0{\cdot}05$) (Fig. 2a), whereas the average clamp glucose levels were similar between both groups (CON: 88 ± 2 vs. OFF: 85 ± 3 mg dL^{-1}). The plasma concentrations of insulin and C-peptide before and during the course of the clamp were comparable (Fig. 2b,c). Again, fasting plasma FFA were not different between CON and OFF, but in the latter, plasma FFA were elevated by 70% after 90 min of insulin infusion ($P < 0{\cdot}05$ vs. CON) (Fig. 2d). AUC of clamp FFA plasma was higher in OFF (24·7 ± 1·6 mmol L^{-1}·min) than in CON (19·5 ± 1·7 mmol L^{-1}·min, $P < 0{\cdot}04$) (Fig. 2d inset). Between 60 and 120 min, OFF showed overall ~40% (each $P < 0{\cdot}05$ vs. CON) lower GIR (calculated in 20 min intervals; 100–120 min: OFF: 6·9 ± 0·7 mg glucose·min^{-1} kg^{-1} vs. CON: 9·5 ± 0·8 mg min^{-1}·kg^{-1}) (Fig. 2e). Whereas EGP in absolute terms (mg glucose·min^{-1}·kg^{-1}) was comparable at fasting and during the clamp test (Fig. 2f), insulin mediated EGP suppression relative to fasting values was impaired in OFF after 120 min insulin infusion (–72 ± 4% vs. CON: –86 ± 4%, $P < 0{\cdot}05$) (Fig. 2g).

Vascular alterations

Intima media thickness

While IMT in the CCA and the CB tended to be higher in OFF, we found a significant ($P < 0{\cdot}002$) IMT elevation by 18% in the internal carotid artery of the OFF group (Fig. 3).

Ankle brachial index (Table 1)

Both groups were similar in right and left ABI (between 1·2 and 1·3), which were obtained from measurements of dorsal pedal and posterior tibial arteries of both legs.

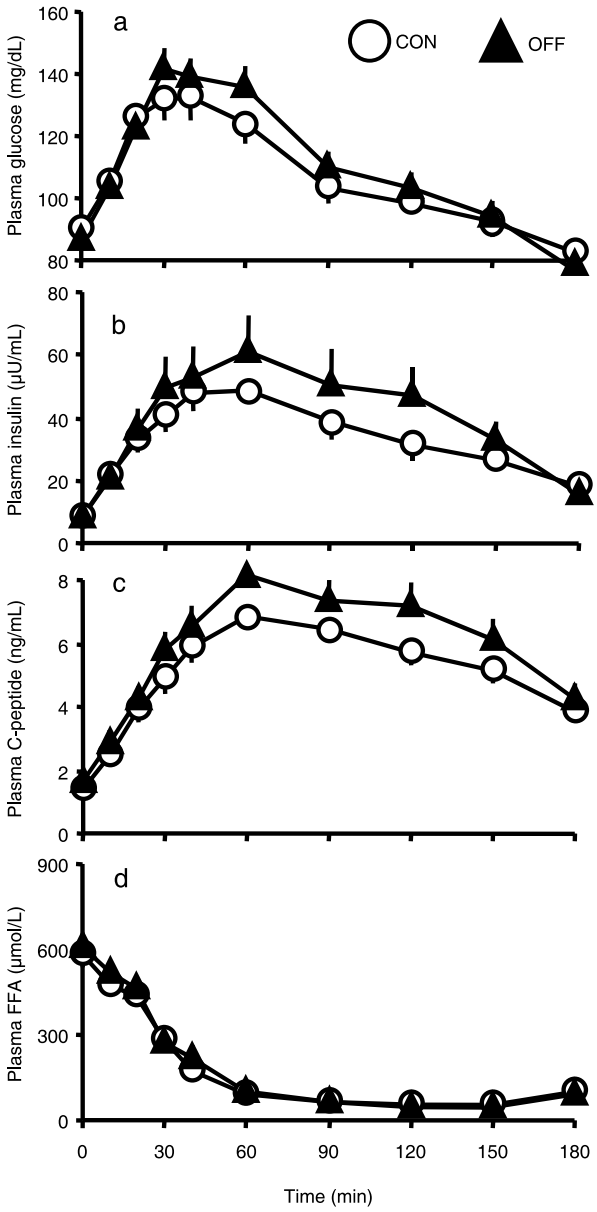

Figure 1 Plasma concentrations (means ± SE) of (a) glucose, (b) insulin, (c) C-peptide and (d) free fatty acids (FFA) in OFF ($n = 18$, ▲) and CON ($n = 18$, ○) during the OGTT.

Correlation analyses

Intima media thickness (Table 2)

IMT of CCA was positively correlated with age, fat mass, HbA1c as well as total and serum low-density lipoprotein (LDL) cholesterol in all study participants and the CON; with systolic blood pressure in all study participants and OFF;

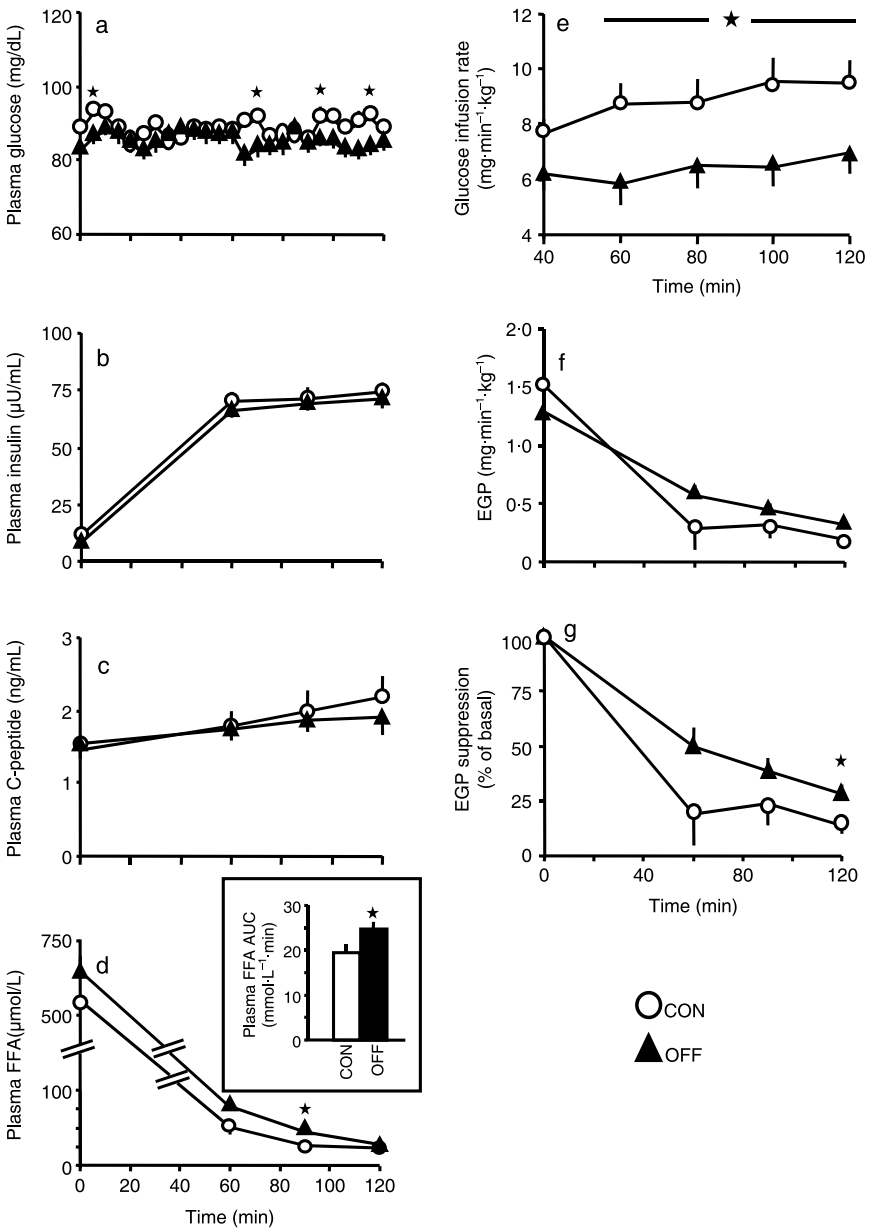

Figure 2 Plasma concentrations of (a) glucose, (b) insulin, (c) C-peptide and (d) free fatty acids (FFA), with inset, FFA area under the curve (AUC), as well as (e) glucose infusion rates (GIR), (f) endogenous glucose production (EGP), and (g) EGP in percentage of the basal value during the isoglycaemic-hyperinsulinaemic (40 mU $min^{-1}·m^{-2}$) clamp-test in OFF ($n = 18$, ▲) and CON ($n = 18$, ○). All data are given as means ± SE. *Student*'s *t*-test: $^*P < 0·05$ CON vs. OFF.

and with body weight in all study participants. IMT of carotid bulb was directly related to age in all participants; to fat mass in CON; and to systolic blood pressure in all participants and OFF. The IMT of ICA was positively associated with age in all participants and the CON; with fat mass and HbA1c in CON; and with fasting insulin concentrations in all study participants.

The AUC of clamp FFA was inversely related to clamp GIR during the 40–60 min interval ($r = -0·436$, $P < 0·008$).

Multiple regression analysis

Age, fasting plasma insulin and the factor OFF/CON (0/1, respectively) correlated with ICA-IMT (Table 2) and were therefore included in the first model. The stepwise backward regression performed revealed the factors OFF/CON ($\beta = 0·095 \pm 0·025$, $P = 0·01$), age ($\beta = 0·003 \pm 0·002$, $P = 0·08$) and fasting plasma insulin ($\beta = 0·005 \pm 0·003$, $P = 0·08$) to be predictors of ICA-IMT ($R^2 = 0·52$). After removal of the other predictors, the estimates of ICA-IMT remained almost the

Table 2 Correlation coefficients (*Pearson* moment products) in all study participants, CON ($n = 18$) and OFF ($n = 18$), based on analyses of intima media thickness (IMT) in the common carotid artery (CCA), carotid bulb (CB), and the internal carotid artery (ICA) (mm) with anthropometrical characteristics, systolic blood pressure, glycated haemoglobin A1c (HbA1c) and fasting concentrations of total and low-density lipoprotein (LDL) serum cholesterol, and plasma insulin as well as glucose infusion rates (100–120 min interval) during the clamp test

	IMT-CCA (mm)	IMT-CB (mm)	IMT-ICA (mm)
Age (years)			
All participants	**$r = 0{\cdot}509$, $P = 0{\cdot}003$**	**$r = 0{\cdot}439$, $P = 0{\cdot}011$**	**$r = 0{\cdot}407$, $P = 0{\cdot}032$**
CON	**$r = 0{\cdot}576$, $P = 0{\cdot}012$**	$r = 0{\cdot}459$, $P = 0{\cdot}055$	**$r = 0{\cdot}568$, $P = 0{\cdot}014$**
OFF	$r = 0{\cdot}396$, $P = 0{\cdot}143$	$r = 0{\cdot}445$, $P = 0{\cdot}096$	$r = 0{\cdot}270$, $P = 0{\cdot}450$
Body weight (kg)			
All participants	**$r = 0{\cdot}440$, $P = 0{\cdot}010$**	$r = 0{\cdot}185$, $P = 0{\cdot}302$	$r = 0{\cdot}254$, $P = 0{\cdot}192$
CON	$r = 0{\cdot}445$, $P = 0{\cdot}064$	$r = 0{\cdot}074$, $P = 0{\cdot}771$	$r = 0{\cdot}137$, $P = 0{\cdot}588$
OFF	$r = 0{\cdot}405$, $P = 0{\cdot}135$	$r = 0{\cdot}180$, $P = 0{\cdot}521$	$r = 0{\cdot}345$, $P = 0{\cdot}329$
Fat mass (kg)			
All participants	**$r = 0{\cdot}524$, $P = 0{\cdot}002$**	$r = 0{\cdot}179$, $P = 0{\cdot}318$	$r = 0{\cdot}182$, $P = 0{\cdot}354$
CON	**$r = 0{\cdot}805$, $P = 0{\cdot}0001$**	**$r = 0{\cdot}507$, $P = 0{\cdot}032$**	**$r = 0{\cdot}501$, $P = 0{\cdot}034$**
OFF	$r = 0{\cdot}076$, $P = 0{\cdot}788$	$r = -0{\cdot}147$, $P = 0{\cdot}600$	$r = 0{\cdot}370$, $P = 0{\cdot}292$
Systolic blood pressure (mmHg)			
All participants	**$r = 0{\cdot}357$, $P = 0{\cdot}041$**	**$r = 0{\cdot}492$, $P = 0{\cdot}004$**	$r = 0{\cdot}152$, $P = 0{\cdot}440$
CON	$r = 0{\cdot}175$, $P = 0{\cdot}489$	$r = 0{\cdot}366$, $P = 0{\cdot}136$	$r = 0{\cdot}066$, $P = 0{\cdot}793$
OFF	**$r = 0{\cdot}548$, $P = 0{\cdot}034$**	**$r = 0{\cdot}565$, $P = 0{\cdot}028$**	$r = 0{\cdot}134$, $P = 0{\cdot}712$
HbA1c (%)			
All participants	**$r = 0{\cdot}410$, $P = 0{\cdot}018$**	$r = 0{\cdot}142$, $P = 0{\cdot}429$	$r = 0{\cdot}248$, $P = 0{\cdot}203$
CON	**$r = 0{\cdot}666$, $P = 0{\cdot}003$**	$r = 0{\cdot}301$, $P = 0{\cdot}225$	**$r = 0{\cdot}469$, $P = 0{\cdot}049$**
OFF	$r = 0{\cdot}236$, $P = 0{\cdot}397$	$r = 0{\cdot}092$, $P = 0{\cdot}745$	$r = 0{\cdot}472$, $P = 0{\cdot}169$
Total serum cholesterol (mg dL^{-1})			
All participants	**$r = 0{\cdot}384$, $P = 0{\cdot}027$**	$r = 0{\cdot}046$, $P = 0{\cdot}798$	$r = 0{\cdot}059$, $P = 0{\cdot}766$
CON	**$r = 0{\cdot}583$, $P = 0{\cdot}011$**	$r = 0{\cdot}096$, $P = 0{\cdot}705$	$r = 0{\cdot}342$, $P = 0{\cdot}165$
OFF	$r = 0{\cdot}217$, $P = 0{\cdot}436$	$r = 0{\cdot}077$, $P = 0{\cdot}785$	$r = 0{\cdot}133$, $P = 0{\cdot}715$
Serum LDL cholesterol (mg dL^{-1})			
All participants	**$r = 0{\cdot}486$, $P = 0{\cdot}004$**	$r = 0{\cdot}113$, $P = 0{\cdot}532$	$r = 0{\cdot}162$, $P = 0{\cdot}410$
CON	**$r = 0{\cdot}642$, $P = 0{\cdot}004$**	$r = 0{\cdot}159$, $P = 0{\cdot}528$	$r = 0{\cdot}326$, $P = 0{\cdot}187$
OFF	$r = 0{\cdot}280$, $P = 0{\cdot}312$	$r = 0{\cdot}083$, $P = 0{\cdot}769$	$r = 0{\cdot}223$, $P = 0{\cdot}535$
Fasting plasma insulin (μU mL^{-1})			
All participants	$r = 0{\cdot}241$, $P = 0{\cdot}176$	$r = -0{\cdot}027$, $P = 0{\cdot}884$	**$r = 0{\cdot}381$, $P = 0{\cdot}045$**
CON	$r = 0{\cdot}353$, $P = 0{\cdot}151$	$r = 0{\cdot}075$, $P = 0{\cdot}768$	$r = 0{\cdot}368$, $P = 0{\cdot}133$
OFF	$r = 0{\cdot}145$, $P = 0{\cdot}607$	$r = -0{\cdot}055$, $P = 0{\cdot}847$	$r = 0{\cdot}497$, $P = 0{\cdot}144$
Clamp glucose infusion rates (mg glucose·min^{-1}·kg^{-1})			
All participants	$r = -0{\cdot}021$, $P = 0{\cdot}909$	$r = -0{\cdot}025$, $P = 0{\cdot}890$	$r = 0{\cdot}030$, $P = 0{\cdot}880$
CON	$r = 0{\cdot}134$, $P = 0{\cdot}597$	$r = -0{\cdot}053$, $P = 0{\cdot}834$	$r = 0{\cdot}409$, $P = 0{\cdot}092$
OFF	$r = 0{\cdot}011$, $P = 0{\cdot}969$	$r = 0{\cdot}199$, $P = 0{\cdot}477$	$r = -0{\cdot}196$, $P = 0{\cdot}588$

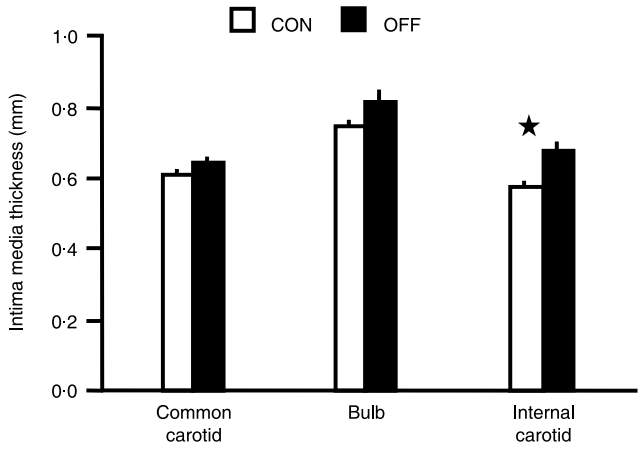

Figure 3 Intima media thickness of common carotid artery, carotid bulb, and the internal carotid artery in OFF ($n = 18$, ■) and CON ($n = 18$, □). All data are given as means ± SE. Student's *t*-test: $*P < 0{\cdot}002$ CON vs. OFF.

same as in the first model, suggesting that the factors OFF/CON, age and fasting plasma insulin are independent predictors of ICA-IMT.

Discussion

In this study, we show that healthy, middle-aged T2DM-OFF with normal glucose tolerance, matched to humans without diabetes in their family history, display marked skeletal muscle insulin resistance in the hyperinsulinaemic clamp test. We ultrasonographically measured intima-media-thickness of the carotid artery in three different sections (common trunk, bulb and inner branch) and found increased IMT of the internal carotid artery only in the OFF. In addition, the insulin resistant OFF showed reduced dynamic β-cell function (reduced AI), impaired insulin mediated suppression of FFA, and discrete hepatic insulin resistance. Regression analysis revealed that a family history of T2DM, higher age and fasting plasma insulin were predictors of increased IMT of the ICA.

Oral glucose tolerance test

Comparisons of plasma glucose, insulin, C-peptide and FFA between OFF and CON before and during the OGTT did not yield any difference. However, dynamic AUC of glucose was higher in the OFF, which indicates decreased glucose disappearance from the blood. This observation is well reflected by the clamp test, in which the OFF displayed 38% lower insulin sensitivity than CON.

During the OGTT, which is a good tool to study β-cell function, OFF showed similar β-cell function at fasting. However, among the dynamic β-cell parameters, the AI was reduced in OFF, indicating impaired pancreatic release of insulin and C-peptide upon stimulation by circulating glucose elevation. Other studies also reported β-cell dysfunction in OFF, though using different methods [31,32]. This suggests that the worst dynamic β-cell function in OFF, combined with insulin resistance, is even evident long before the onset of T2DM.

Whole body insulin sensitivity

Glucose infusion rates to measure whole body insulin sensitivity were lower by 38% in the T2DM-OFF. Such insulin resistance may be of genetical or environmental origin, due to an excess of plasma FFA [33] or the lack of exercise, because exercise training was highly effective in increasing whole body insulin-stimulated glucose uptake in both T2DM-OFF and humans without a family history of T2DM [34]. In order to measure the impact of lifestyle on insulin resistance in the OFF, we examined physical activity by a movement sensor in our participants for several days and found comparable energy expenditure in both groups, indicating that the observed insulin resistance in the first degree relatives of T2DM is not the result of too low physical activity.
It is noteworthy that our results are in line with a previous study with a similar outcome on T2DM-OFF which, however, estimated physical activity by a questionnaire only [4].
Other impacts that influence insulin sensitivity, such as fat rich nutrition, have not been examined, but appear rather unlikely because the fasting circulating lipids (triglycerides and FFA) were not higher in OFF. Taken together, all the OFF led a rather healthy lifestyle and were neither lean nor obese, but clearly insulin resistant which is rather of primary origin. Whereas some metabolic syndrome criteria are on the average present in the OFF group (Table 1), all OFF showed normal fasting glucose and glucose tolerance. On the other hand, a less optimal lifestyle in a normal population (without any genetical background favouring T2DM development) could gradually induce hyperglycaemia, hyperlipidaemia, arterial hypertension and other features of the metabolic syndrome, which is associated with an increased risk for T2DM development [35].

The pathophysiological mechanisms of insulin resistance in OFF have been elucidated only in part, though in young, lean T2DM-OFF, providing evidence for higher intramyocellular lipid content combined with reduced mitochondrial oxidative phosphorylation activity and oxygen uptake in skeletal muscle [4,32,36]. In overweight T2DM-OFF, insulin signal transduction studies in skeletal muscle biopsies revealed unchanged insulin receptor phosphorylation, but reduced insulin-mediated tyrosine phosphorylation of insulin receptor substrate-1, and decreased phosphoinositol-3-kinase activity [37].

Endogenous glucose production

When calculated in absolute terms, EGP was similar between both groups. However, EGP in relation to fasting values (i.e. relative

EGP suppression by insulin) was lower at the end of the clamp test in OFF when compared to CON. Insulin-mediated EGP suppression is important in postprandial glucose regulation, commonly impaired in patients with T2DM [1,20,24] and indicates hepatic insulin resistance [38] which was also detectable, but less pronounced (–14%), in our OFF.

Plasma lipids

Fasting serum triglycerides were lower in the insulin resistant OFF, which was surprising because insulin resistance was described to be associated with hypertriglyceridaemia in overweight and obese, insulin-resistant humans [39]. Of note, lipoprotein lipase activity, the enzyme responsible for triglyceride hydrolysis and subsequent uptake into the cell, is decreased in insulin resistance due to overweight and obesity leading to higher circulating triglycerides, but remains unaltered in insulin resistance rather based on a genetical background, such as in African Americans who were normotriglyceridaemic [40,41]. Thus, it appears conceivable that inherited insulin resistance, as also seen in our OFF, is not associated with increased circulating triglycerides, most likely because of unchanged lipoprotein lipase activity. Fasting plasma FFA were similar in both groups, although insulin mediated suppression of plasma FFA was impaired in OFF, indicating insulin resistance in adipose tissue.

Vascular alterations

Studying arterial blood vessel function ultrasonographically, we did not find any differences in ABI between groups, indicating the absence of clinically relevant PAD. Analyzing, however, carotid morphology in three different sections by ultrasound, IMT was increased in the internal, but not common, carotid artery in OFF, and did not relate to insulin sensitivity. In this context it may be of note that measurement of wall thickness in the CCA was described to have less predictive power for the presence of clinically manifest atherosclerosis than a measurement made in the ICA [22]. Thus, it has been suggested that thickening of the CCA intima might be more representative of total body atherosclerotic burden, whereas thickening of the ICA-IMT might represent focal intracerebral atherosclerotic plaques with a higher risk of incident disease, possibly related to endothelial dysfunction and altered haemodynamic flow in the ICA [42,43]. This assumption would also be supported by our findings, since both groups did not differ in CCA-IMT and were in excellent health without any sign of whole body atherosclerotic disease such as CVD or PAD. In addition, T2DM family history, increased age and plasma hyperinsulinaemia were found to be predictors of ICA-IMT, as confirmed in our study by regression analysis. On the other hand, ICA-IMT was positively correlated with age, fat mass, HbA1c and fasting insulin concentrations, all of which are also increased in insulin resistant states.

Previous studies in diabetic offspring showed that the IMT of the CCA was positively correlated not only with HOMA-IR, a surrogate of insulin resistance [44], but also with GIR [45] which is in contrast to our findings. However, some of the subjects in those other studies also displayed impaired fasting glucose and impaired glucose tolerance, both of which were exclusion criteria in this study. In a number of other reports that also described an association between insulin insensitivity and higher IMT, no OGTT was performed so that people with glucose intolerance could also have been included [46–48]. Since in our study only humans with normal fasting glucose and glucose tolerance were included and we did not find any relationship between wall thickness and insulin sensitivity, it might be that IMT is not related to insulin resistance in subjects with normal glucose tolerance.

Limitations of the study

We cannot exclude that the protocol, as designed, has a regression to the mean effect because of the advice to abstain from severe physical activity for up to three days before the experiments and the absence of some days to recover following the OGTT. It would be interesting to repeat the experiments after the relatives had lost the excess BMI and corrected the waist hip ratio differences.

Conclusions

Taken together, clinically healthy, middle-aged first degree relatives of diabetic patients (T2DM-OFF) with normal OGTT and normal blood pressure show (i) whole body insulin resistance in skeletal muscle, adipose tissue and, less pronounced, in the liver (ii) impaired dynamic β-cell function unable to adapt for insulin resistance and (iii) higher IMT of the internal carotid artery, possibly indicating accelerated intracerebral atherosclerosis.

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References

1 Anderwald C, Bernroider E, Kršśák M, Stingl H, Brehm A, Bischof MG *et al*. Effects of insulin treatment in type 2 diabetic patients on intracellular lipid content in liver and skeletal muscles. *Diabetes* 2002;**51**:3025–32.
2 Warram JH, Martin BC, Krolewski AS, Soeldner JS, Kahn CR. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann Intern Med* 1990;**113**:909–15.
3 Goldfine AB, Bouche C, Parker RA, Kim C, Kerivan A, Soeldner JS *et al*. Insulin resistance is a poor predictor of type 2 diabetes in individuals with no family history of disease. *Proc Natl Acad Sci USA* 2003;**100**:2724–9.
4 Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C *et al*. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 1999;**48**:1600–6.
5 Monti LD, Barlassina C, Citterio L, Galluccio E, Berzuini C, Setola E *et al*. Endothelial nitric oxide synthase polymorphisms are associated with type 2 diabetes and the insulin resistance syndrome. *Diabetes* 2003;**52**:1270–5.
6 Selvin E, Wattanakit K, Steffes MW, Coresh J, Sharrett AR. HbA1c and peripheral arterial disease in diabetes: the atherosclerosis risk in communities study. *Diabetes Care* 2006;**29**:877–82.
7 Piatti P, Di Mario C, Monti LD, Fragasso G, Sgura F, Caumo A *et al*. Association of insulin resistance, hyperleptinemia, and impaired nitric oxide release with in-stent restenosis in patients undergoing coronary stenting. *Circulation* 2003;**108**:2074–81.
8 Piatti PM, Monti LD, Galli L, Fragasso G, Valsecchi G, Conti M *et al*. Relationship between endothelin-1 concentration and metabolic alterations typical of the insulin resistance syndrome. *Metabolism* 2000;**49**:748–52.
9 Davis PH, Dawson JD, Mahoney LT, Lauer RM. Increased carotid intimal-medial thickness and coronary calcification are related in young and middle-aged adults. The Muscatine study. *Circulation* 1999;**100**:838–42.
10 Hills SA, Balkau B, Coppack SW, Dekker JM, Mari A, Natali A *et al*. The EGIR-RISC STUDY (The European group for the study of insulin resistance: relationship between insulin sensitivity and cardiovascular disease risk): I. Methodology and Objectives. *Diabetologia* 2004;**47**:566–70.
11 Rose GA, Blackburn H, Gillum RF, Prineas RJ. *Cardiovascular Survey Methods* 1982. Geneva: World Health Organization.
12 Leng GC, Fowkes FG. The Edinburgh Claudication Questionnaire: an improved version of the WHO/Rose Questionnaire for use in epidemiological surveys. *J Clin Epidemiol* 1992;**45**:1101–9.
13 Stadler M, Anderwald C, Karer T, Tura A, Kästenbauer T, Auinger M *et al*. Increased plasma amylin in type 1 diabetic patients after kidney and pancreas transplantation: a sign of impaired beta-cell function? *Diabetes Care* 2006;**29**:1031–8.
14 Anderwald C, Brabant G, Bernroider E, Horn R, Brehm A, Waldhäusl W *et al*. Insulin-dependent modulation of plasma ghrelin and leptin concentrations is less pronounced in type 2 diabetic patients. *Diabetes* 2003;**52**:1792–8.
15 Anderwald C, Anderwald-Stadler M, Promintzer M, Prager G, Mandl M, Nowotny P *et al*. The Clamp-Like Index: a novel and highly sensitive insulin sensitivity index to calculate hyperinsulinemic clamp glucose infusion rates from oral glucose tolerance tests in nondiabetic subjects. *Diabetes Care* 2007;**30**:2374–80.
16 Anderwald-Stadler M, Krebs M, Promintzer M, Mandl M, Bischof MG, Nowotny P *et al*. Plasma obestatin is lower at fasting and not suppressed by insulin in insulin-resistant humans. *Am J Physiol Endocrinol Metab* 2007;**293**:E1393–8.
17 Brage S, Brage N, Franks PW, Ekelund U, Wong MY, Andersen LB *et al*. Branched equation modeling of simultaneous accelerometry and heart rate monitoring improves estimate of directly measured physical activity energy expenditure. *J Appl Physiol* 2004;**96**:343–51.
18 Hother-Nielsen O, Henriksen JE, Holst JJ, Beck-Nielsen H. Effects of insulin on glucose turnover rates in vivo: isotope dilution versus constant specific activity technique. *Metabolism* 1996;**45**:82–91.
19 Promintzer M, Krebs M, Todoric J, Luger A, Bischof MG, Nowotny P *et al*. Insulin resistance is unrelated to circulating retinol binding protein and protein C inhibitor. *J Clin Endocrinol Metab* 2007;**92**:4306–12.
20 Krssak M, Brehm A, Bernroider E, Anderwald C, Nowotny P, Dalla MC *et al*. Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. *Diabetes* 2004;**53**:3048–56.
21 Mazzone AM, Urbani MP, Picano E, Paterni M, Borgatti E, De Fabritiis A *et al*. In vivo ultrasonic parametric imaging of carotid atherosclerotic plaque by videodensitometric technique. *Angiology* 1995;**46**:663–72.
22 O'Leary DH, Polak JF, Kronmal RA, Savage PJ, Borhani NO, Kittner SJ *et al*. Thickening of the carotid wall. A marker for atherosclerosis in the elderly? Cardiovascular Health Study Collaborative Research Group. *Stroke* 1996;**27**:224–31.
23 Hwang JH, Perseghin G, Rothman DL, Cline GW, Magnusson I, Petersen KF *et al*. Impaired net hepatic glycogen synthesis in insulin-dependent diabetic subjects during mixed meal ingestion. A 13C nuclear magnetic resonance spectroscopy study. *J Clin Invest* 1995;**95**:783–7.
24 Anderwald C, Brunmair B, Stadlbauer K, Krebs M, Furnsinn C, Roden M. Effects of free fatty acids on carbohydrate metabolism

and insulin signalling in perfused rat liver. *Eur J Clin Invest* 2007;**37**:774–82.
25 Inzucchi SE, Maggs DG, Spollett GR, Page SL, Rife FS, Walton V *et al*. Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N Engl J Med* 1998;**338**:867–72.
26 DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;**237**:E214–23.
27 Ahren B, Pacini G. Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. *Am J Physiol* 1997;**273**:E701–7.
28 Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best Pract Res Clin Endocrinol Metab* 2003;**17**:305–22.
29 Mari A, Ahren B, Pacini G. Assessment of insulin secretion in relation to insulin resistance. *Curr Opin Clin Nutr Metab Care* 2005;**8**:529–33.
30 Stadler M, Auinger M, Anderwald C, Kastenbauer T, Kramar R, Feinbock C *et al*. Long-term mortality and incidence of renal dialysis and transplantation in type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2006;**91**:3814–20.
31 Bonadonna RC, Stumvoll M, Fritsche A, Muggeo M, Haring H, Bonora E *et al*. Altered homeostatic adaptation of first- and second-phase beta-cell secretion in the offspring of patients with type 2 diabetes: studies with a minimal model to assess beta-cell function. *Diabetes* 2003;**52**:470–80.
32 Thamer C, Stumvoll M, Niess A, Tschritter O, Haap M, Becker R *et al*. Reduced skeletal muscle oxygen uptake and reduced beta-cell function: two early abnormalities in normal glucose-tolerant offspring of patients with type 2 diabetes. *Diabetes Care* 2003;**26**:2126–32.
33 Waldhäusl WK, Roden M. The effects of free fatty acids on glucose transport and phosphorylation in human skeletal muscle. *Curr Opin Endocrinol Diabetes* 2000;**7**:211–6.
34 Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K *et al*. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. *N Engl J Med* 1996;**335**:1357–62.
35 Demacker PN. The metabolic syndrome: definition, pathogenesis and therapy. *Eur J Clin Invest* 2007;**37**:85–9.
36 Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 2004;**350**:664–71.
37 Pratipanawatr W, Pratipanawatr T, Cusi K, Berria R, Adams JM, Jenkinson CP *et al*. Skeletal muscle insulin resistance in normoglycemic subjects with a strong family history of type 2 diabetes is associated with decreased insulin-stimulated insulin receptor substrate-1 tyrosine phosphorylation. *Diabetes* 2001;**50**:2572–8.
38 DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988;**37**:667–87.
39 McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 2003;**139**:802–9.
40 Sumner AE, Vega GL, Genovese DJ, Finley KB, Bergman RN, Boston RC. Normal triglyceride levels despite insulin resistance in African Americans: role of lipoprotein lipase. *Metabolism* 2005;**54**:902–9.
41 Maheux P, Azhar S, Kern PA, Chen YD, Reaven GM. Relationship between insulin-mediated glucose disposal and regulation of plasma and adipose tissue lipoprotein lipase. *Diabetologia* 1997;**40**:850–8.
42 Psaty BM, Furberg CD, Kuller LH, Bild DE, Rautaharju PM, Polak JF *et al*. Traditional risk factors and subclinical disease measures as predictors of first myocardial infarction in older adults: the Cardiovascular Health Study. *Arch Intern Med* 1999;**159**:1339–47.
43 Fox CS, Polak JF, Chazaro I, Cupples A, Wolf PA, D'Agostino RA *et al*. Genetic and environmental contributions to atherosclerosis phenotypes in men and women: heritability of carotid intima-media thickness in the Framingham Heart Study. *Stroke* 2003;**34**:397–401.
44 Pannacciulli N, De Pergola G, Ciccone M, Rizzon P, Giorgino F, Giorgino R. Effect of family history of type 2 diabetes on the intima-media thickness of the common carotid artery in normal-weight, overweight, and obese glucose-tolerant young adults. *Diabetes Care* 2003;**26**:1230–4.
45 Cardellini M, Marini MA, Frontoni S, Hribal ML, Andreozzi F, Perticone F *et al*. Carotid artery intima-media thickness is associated with insulin-mediated glucose disposal in nondiabetic normotensive offspring of type 2 diabetic patients. *Am J Physiol Endocrinol Metab* 2007;**292**:E347–52.
46 Agewall S, Fagerberg B, Attvall S, Wendelhag I, Urbanavicius V, Wikstrand J. Carotid artery wall intima-media thickness is associated with insulin-mediated glucose disposal in men at high and low coronary risk. *Stroke* 1995;**26**:956–60.
47 Wohlin M, Sundstrom J, Arnlov J, Andren B, Zethelius B, Lind L. Impaired insulin sensitivity is an independent predictor of common carotid intima-media thickness in a population sample of elderly men. *Atherosclerosis* 2003;**170**:181–5.
48 Bokemark L, Wikstrand J, Attvall S, Hulthe J, Wedel H, Fagerberg B. Insulin resistance and intima-media thickness in the carotid and femoral arteries of clinically healthy 58-year-old men. The Atherosclerosis and Insulin Resistance Study (AIR). *J Intern Med* 2001;**249**:59–67.