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Twenty-four-hour insulin secretion rates, circulating concentrations of fuel substrates and gut incretin hormones in healthy offspring of Type II (non-insulin-dependent) diabetic parents: evidence of several aberrations

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Abstract

Aims/hypothesis. Insulin resistance is a common feature in relatives of patients with Type II (non-insulin-dependent) diabetes mellitus and abnormalities in beta-cell function can also exist. Insight into non-fasting carbohydrate metabolism in these potentially prediabetic subjects relies almost exclusively on studies in which glucose is infused or ingested or both. We aimed to characterize insulin secretion and aspects of hormonal and metabolic patterns in relatives using a physiological approach.
Methods. We examined profiles of insulin, C peptide, proinsulin, gut incretin hormones and fuel substrates in 26 glucose tolerant but insulin resistant (clamp) relatives and 17 control subjects during a 24-hour period including three meals.
Results. During the day plasma glucose was slightly raised in relatives ($p < 0.05$). Overall insulin secretion calculated on the basis of C peptide kinetics were increased in relatives ($p < 0.0005$) whereas incremental insulin secretion after all three meals were similar. Peak incremental insulin secretion tended, however, to be reduced in relatives ($p < 0.10$). Despite considerably increased insulin concentrations in relatives (70 %, $p < 0.001$), serum NEFA did not differ. Postprandial proinsulin concentrations ($p < 0.05$), but not proinsulin:insulin ratios, were increased in relatives. After meals concentrations of glucose-dependent-insulinotropic polypeptide ($p < 0.05$) were increased in relatives. Glucagon-like peptide-1 concentrations were similar.
Conclusion/interpretation. Several hormonal and metabolic aberrations are present in healthy relatives of Type II diabetic patients during conditions that simulate daily living. Increased concentrations of glucose-dependent-insulinotropic polypeptide could indicate a beta-cell receptor defect for glucose-dependent-insulinotropic polypeptide in the prediabetic stage of Type II diabetes. Incremental insulin secretion after mixed meals appear normal in relatives, although a trend towards diminished peak values possibly signifies early beta-cell dysfunction. [Diabetologia (1999) 42: 1314–1323]

Keywords Prediabetes, physiological approach, 24-h profile, glucose, insulin, insulin secretion, proinsulin, non-esterified fatty acids, gut incretin hormones, intermediary metabolites.

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Abbreviations: E%, Energy %; GIP, glucose-dependent-insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; ISGU, insulin-stimulated glucose uptake; LBW, lean body weight; 3-OHB, 3-beta-hydroxy butyrate; W/H ratio, waist-to-hip ratio.

Type II (non-insulin-dependent) diabetes mellitus is characterized by insulin resistance, increased endogenous glucose production and beta-cell dysfunction [1]. The latter has been shown in many ways, e.g. in response to mixed meals, Type II diabetic patients were found to have a decreased and delayed insulin secretion [2]. Due to the confounding effects of the metabolic derangement in diabetes [3], it is, however, difficult to determine to what extent metabolic and secretory abnormalities are of primary pathophysiological significance or reflects the metabolic derange-

Table 1. Clinical data of the two groups (mean, range)

	Relatives ($n = 26$)	Controls ($n = 17$)
Sex (men/women)	14/12	9/8
Age (years)	34.0 (21–47)	33.3 (22–46)
BMI (kg/m^2)	25.4 (20.3–30.0)	25.0 (20.9–29.9)
Waist : Hip ratio	0.89 (0.70–1.05)	0.88 (0.80–0.96)
LBW (% of BW)	75.9 (63–86)	77.8 (66–89)
ISGU (mg · kg LBW^{-1} · min^{-1})	7.76 ± 0.50	11.14 ± 0.53[c]
Fasting serum lipids (mmol/l)		
Total cholesterol	4.8 (3.2–7.0)	4.5 (3.5–6.5)
HDL-cholesterol	1.27 (0.50–2.10)	1.38 (1.00–2.10)
Triglycerides	1.35 (0.53–3.01)	1.29 (0.45–3.60)
HbA$_{1C}$ (%)	5.4 (4.3–6.4)	5.1 (4.2–5.9)[a]
Fasting plasma glucose (mmol/l)	5.3 (4.5–6.1)	5.0 (4.6–5.6)[b]
Fasting serum insulin (pmol/l)	60.5 (21.6–100.8)	34.9 (15.6–89.4)
Fasting serum C peptide (nmol/l)	0.68 (0.31–2.69)	0.46 (0.30–0.96)[a]

BW: Body Weight; [a]: $0.05 \leq p \leq 0.10$; [b]: $p < 0.05$; [c]: $p < 0.001$

ment itself. To circumvent this hindrance healthy people who are at a considerable increased risk of developing Type II diabetes, e. g. first-degree relatives of Type II diabetic patients, have often been studied to assess early metabolic abnormalities preceding development of overt Type II diabetes. First-degree relatives of Type II diabetic patients have an estimated risk of approximately 40 % for development of Type II diabetes later in life depending upon the family history of Type II diabetes [4].

Insulin resistance is a common finding in first-degree relatives of Type II diabetic patients [5–12]. The mechanisms behind the decreased insulin-stimulated glucose uptake (ISGU) – primarily affecting the skeletal muscle – are, however, only partly clarified in these potentially prediabetic people. Abnormalities in activities of important enzymes of muscle cells, such as related to glucose transport or phosphorylation or both [8], glycogen synthase [6], insulin receptor tyrosine kinase [13] and morphologic changes in skeletal muscle fibre composition [11] have been described. In addition, some studies in which the oral glucose tolerance test (OGTT) [9, 10, 14] or intravenous glucose infusion or both were used [7, 12, 14–16] have indicated an abnormal insulin secretion in relatives of Type II diabetic patients suggesting that both defects in insulin secretion and insulin action in Type II diabetes are inherited [12].

Another feature of overt Type II diabetes is a diminished incretin effect [17]. The incretin effect, denoting an augmented pancreatic insulin secretion after glucose ingestion compared with the same amount of glucose given intravenously, is to our current knowledge mediated through release of two insulinotropic hormones, glucose-dependent-insulinotropic polypeptide (GIP) [18] and glucagon-like peptide-1 (GLP-1) [19]. Recent studies have shown preserved activity of GLP-1 but not GIP in Type II diabetic patients [20]. Therefore, the reduced incretin effect in these patients has been suggested to relate to a beta-cell receptor defect for GIP [21]; a defect which could be acquired or perhaps inherited.

The OGTT provides us with a standardized tool to evaluate glucose tolerance but fails to give information on daily-life conditions, i. e. the much more complex metabolic scenario associated with mixed meals. Our study was consequently undertaken to explore the 24-hour insulin secretion and to what extent metabolic aberrations reside in healthy but potentially prediabetic subjects during daily-life conditions. This was done by calculating insulin secretion rates and measuring circulating insulin, proinsulin, C-peptide, glucagon, non-esterified fatty acids (NEFA), gut incretin hormones and fuel metabolites in a group of insulin resistant first-degree relatives of Type II diabetic parents compared with a matched control group.

Subjects and methods

Subjects. First-degree relatives of patients with Type II diabetes ($n = 26$) and control subjects ($n = 17$) without any family history of diabetes participated in the study. The two groups were matched for age, sex and body mass index (BMI). The relatives were recruited through their Type II diabetic parent(s) who attended the outpatient clinic, Medical Department M, Aarhus Community Hospital. The diagnosis of Type II diabetes mellitus was made according to WHO criteria. Diabetes duration ranged from 7–28 years. Of the patients 9 were treated with diet only, 16 were furthermore treated with oral anti-diabetic agents and 4 required insulin treatment. The patients treated with insulin had earlier been treated with oral anti-diabetic agents or diet or both for more than 8 years before receiving insulin treatment. Within the offspring group, 6 subjects had 1 parent with Type II diabetes, 13 had 1 parent and 2 or more known second-degree relatives with Type II diabetes and 7 had 2 first-degree relatives (both parents) with Type II diabetes. The 26 relatives were from 22 unrelated families; if more than 2 offspring were available in a family, a maximum of 2 were randomly selected to participate. The control group were recruited by advertising from healthy volunteers without any family history of diabetes. None in the control group were related. All participants were healthy, of Caucasian origin and were taking no medication. Insulin sensitivity in relatives and control subjects was not known before the study. No family history of any other endocrine disorder was present. Additional exclusion criteria were age older than 50 years and BMI greater than 30 kg/m^2. A questionnaire [22] dealing with daily physical activity patterns during work and leisure, respectively, did not show discrepancies between the two groups. The clinical data of the two groups are shown in Table 1. Participants were asked to consume a weight-maintaining diet containing at least 250 g of carbohydrate for 3 days before all examinations and none were engaged in heavy physical exercise during this peri-

od. None had any infectious diseases during the study or within the last 4 weeks before the study. All women were examined in the follicular phase of the menstrual cycle. The protocol was approved by the ethics committee of the county of Aarhus.

Design. All subjects were examined on three occasions (I, II and III). Examination I and II were separated by approximately 2–4 weeks but III followed immediately after II. All studies commenced at 0800 hours in the clinical research unit after a 10-h overnight fast.

I: Oral glucose tolerance test and body composition. The subjects underwent a physical examination including determination of BMI, waist:hip (W/H) ratio and lean body weight (LBW) using bioelectric impedance (Animeter, HTS-Engineering APS, Odense, Denmark). An oral glucose tolerance test (75-g glucose) was done at time 0830 hours. Blood for determination of plasma glucose was drawn at time 0, 30, 60, 90 and 120 min. Blood for measurements of HbA_{1C}, serum triglycerides, total cholesterol and HDL-cholesterol was collected at time 0.

II: Profiles over 24 h during standardized conditions. Participants arrived at the clinical research unit at 0730 hours in the fasting state. An intravenous cannula was placed in an antecubital vein for blood sampling. Throughout the subsequent 24 h subjects stayed in the clinical research unit. They were served a standardized diet prepared by a dietician. The diet was a typical Danish diet and was served as breakfast at 0800 hours, lunch at 1200 hours and dinner at 1800 hours. Participants were allowed 30 min to eat each meal. Total energy intake was approximately 10000 KJ for the men and 8000 KJ for the women distributed with 30 % on the breakfast meal (composition of breakfast in energy (E)%: carbohydrates: 50 %, fat: 37 % and protein: 13 %), 35 % on the lunch meal (composition in E%: carbohydrates: 38 %, fat: 49 % and protein: 13 %) and 35 % on the dinner meal (E%: carbohydrate: 50 %, fat: 33 % and protein: 17 %). Participants were allowed free access to tap water throughout the study period but apart from this they were not allowed to eat or drink anything not included in the diet. To maintain a normal day and night rhythm, subjects were asked to go to bed at 2230 hours (lights were off from 2300 hours to 0700 hours). No naps were allowed during daytime. Baseline blood samples were obtained at 0800 hours, before breakfast. During the 24-h profile blood samples were collected every 30 min for 2 to 3 h after each meal, every hour during daytime (0800–2400 hours) and every second hour during the night (2400–0800 hours). Blood samples were analysed for insulin, C peptide, glucose, NEFA, the intermediary metabolites lactate, 3-hydroxy-butyrate (3-OHB), glycerol and alanine, and the gut incretin hormones GIP and GLP-1. Baseline blood samples and blood samples taken after breakfast (0830, 0900, 0930, 1000, 1100 and 1200 hours) were furthermore assayed with respect to total and intact proinsulin concentrations.

III: Hyperinsulinaemic euglycaemic clamp. Insulin sensitivity was measured on the morning after the 24-h profile. At 0800 hours another catheter was inserted in a heated dorsal hand vein for sampling of arterialized blood (oxygen saturation > 90 %) and at 0830 hours (time zero) a 150-min hyperinsulinaemic (insulin infusion rate $1.0 \mathrm{mU} \cdot \mathrm{kg}^{-1} \cdot \mathrm{min}^{-1}$) euglycaemic (plasma glucose ~ 5 mmol/l) clamp was carried out. The final 30 min were regarded as "steady state". During this period indirect calorimetry (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland) was done. Blood for determination of serum insulin was drawn at times 0, 90, 120, 135 and 150 min and plasma glucose was determined every 5 to 10 min during the clamp. Mean glucose infusion rate between times 120 and 150 min was defined as "steady state" ISGU.

Analytical methods and calculations. Plasma glucose was measured in duplicate immediately after sampling (Beckman Instruments, Palo Alto, Calif., USA). The HbA_{1C} value was determined by HPLC [reference range (95 % confidence limits) = 4.4–6.4 %]. Serum insulin was determined by ELISA using a two-site immunoassay [23], which does not detect proinsulin, split(32–33), and des 31,32 proinsulin, whereas split 65,66 proinsulin and des 64,65 proinsulin cross-react 30 % and 63 %, respectively (Dako Diagnostics Ltd., Cambridgeshire, UK). The intra-assay CV was 2.0 % ($n = 75$) at a serum level of 200 pmol/l. Serum C peptide was measured using a commercial kit (Dako Diagnostics Ltd.). Total proinsulin and intact proinsulin concentrations were measured by specific immunoassays with no cross-reaction with insulin and C peptide (Dako Diagnostics Ltd., Ely, UK). Plasma glucagon was determined by RIA as described previously using wick chromatography [24] with the modifications that polythylene-glycol was used for separation before determination and that plasma was extracted with ethanol. This assay is specific for pancreatic glucagon and does not measure intestinal proglucagon-derived molecules. Serum NEFA was measured by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany) and blood lactate, glycerol, 3-OHB and alanine were assayed using the Cobas Bio centrifugal analyser (Roche Products, Welwyn Garden City, UK) with a fluorometric attachment [25]. We measured GIP and GLP-1 in plasma after extraction of plasma with 70 % ethanol (vol/vol, final concentration). For the GIP radioimmunoassay [26] we used the C-terminally directed antiserum R 65, which cross-reacts fully with human GIP but not with the so-called 'GIP 8000', whose chemical nature and relation to GIP secretion is uncertain. Human GIP and 125-I human GIP (70 MBq/nmol) were used as standard and tracer. The plasma concentration of GLP-1 were measured [27] against standards of synthetic GLP-1 7–36amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore does not react with GLP-1-containing peptides from the pancreas. The results of the assay accurately reflect the rate of GLP-1 secretion because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9amide, into which GLP-1 is rapidly converted. For both assays sensitivity was below 1 pmol/l, the intra-assay coefficient of variation below 6 % at 20 pmol/l and recovery of standard, added to plasma before extraction, about 100 % when corrected for the losses inherent in the plasma extraction procedure. Respiratory exchange ratios were assessed using indirect calorimetry. Protein oxidation rates were estimated from urinary excretion of urea. Net lipid and glucose oxidation rates were computed from the above measurements and non-oxidative glucose disposal was calculated by subtracting rates of glucose oxidation from total ISGU during the hyperinsulinaemic "steady state" period. Average concentrations of insulin and substrates during the 24-h period were calculated by adding the individual measurements weighted with respect to time.

Determination of insulin secretion rates. Standard variables for C peptide distribution and clearance were used [28] to derive insulin secretion rates from the C peptide concentration profiles as described previously [29, 30]. The C peptide concentration before breakfast was assumed to represent basal insulin secretion and incremental insulin secretion rates expressed as percentage basal secretion was calculated by dividing the insulin secretion rates measured in each subject by the basal secre-

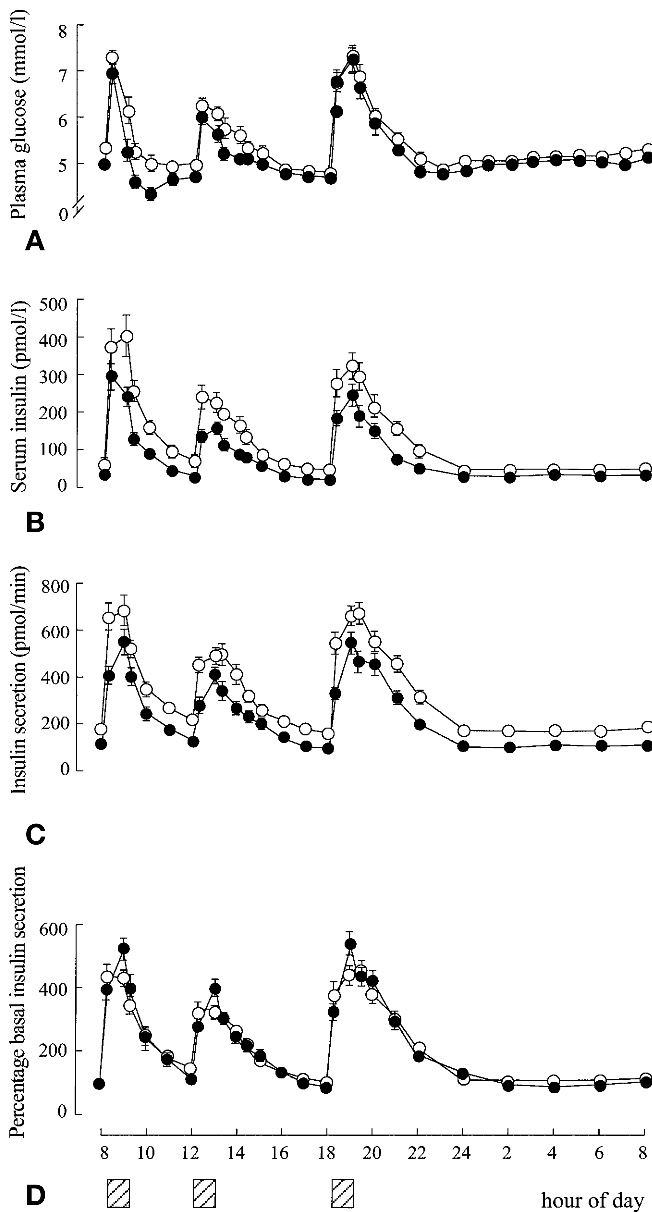

Fig. 1A–D. Profiles over 24 h of (**A**) plasma glucose and (**B**) serum insulin and (**C**) insulin secretion rates calculated on basis of C peptide kinetics expressed in pmol/min and (**D**) in percentage basal insulin secretion (insulin secretion rate/basal secretion) in 26 first-degree relatives of Type II diabetic patients (○) and 17 matched control subjects (●) (means ± SEM). ▨ indicates meals

tion rate derived in the same subject (insulin secretion rates/basal secretion).

Statistical analyses. Data in the text and figures are given as means ± SEM unless otherwise stated. Student's two-tailed *t* tests for unpaired data were used for comparison of data between the two groups. When data were not normally distributed (serum insulin and proinsulin but not calculated insulin data), Mann-Whitney's Rank sum test for unpaired data was used. Correlations were tested using Pearson product moment correlation analysis. Total area under the curve (AUC) was calculated using the trapezoidal rule and incremental area under the curve (incremental AUC) was calculated by subtracting the baseline area from AUC. Areas under the curve were calculated in the postprandial phase after each meal [breakfast (0800–1200 hours), lunch (1200–1800 hours) and dinner (1800–2400 hours), respectively] for the 24-h period (0800–0800 hours, 24-h AUC) as well as during the day (0800–2400 hours) and night-time (2400–800 hours). A two tailed value p less than 0.05 was considered to indicate statistical significance.

Results

Oral glucose tolerance test and insulin sensitivity. All subjects had a normal OGTT according to criteria as defined by The Expert Committee on the Diagnosis and Classification of Diabetes [31], but fasting plasma glucose (5.3 ± 0.1 vs 5.0 ± 0.1 mmol/l, $p < 0.05$) as well as the 120 min value (5.6 ± 0.2 vs 4.7 ± 0.2, $p < 0.01$) and AUC during OGTT (811 ± 24 vs 728 ± 29 mmol l^{-1} min, $p < 0.05$) were all significantly higher in the relatives compared with control subjects. Differences in HbA_{1C} barely reached statistical significance between the two groups whereas fasting serum lipids were similar in relatives and control subjects (Table 1). Insulin-stimulated glucose uptake was 30 % lower in the relatives compared with the control subjects (7.76 ± 0.50 vs 11.14 ± 0.53 mg · kg LBW^{-1} · min^{-1}, $p < 0.001$). This was ascribable to a diminished non-oxidative glucose utilisation in the relatives (4.01 ± 0.42 vs 7.32 ± 0.47 mg · kg LBW^{-1} · min^{-1}, $p < 0.001$), whereas rates of glucose oxidation (3.73 ± 0.14 vs 3.910.19 mg · kg LBW^{-1} · min^{-1}, p = NS) were similar in the two groups (relatives vs control subjects, respectively). Rates of lipid oxidation (0.23 ± 0.04 vs 0.16 ± 0.05 mg · kg LBW^{-1} · min^{-1}, p = NS) were similar in the relatives and the control subjects.

Profiles over 24 h : circulating concentrations of glucose, glucagon, insulin and C peptide. Fasting concentrations of plasma glucose, serum insulin and C peptide obtained from baseline blood samples are given in Table 1. Plasma glucose was raised in the relatives compared with the control subjects ($p < 0.05$) and a similar trend was observed for serum insulin ($p = 0.12$) and C peptide ($p = 0.07$). During the 24-h profile concentrations of plasma glucose, serum insulin and C peptide were increased in the relatives ($p < 0.05$, $p < 0.001$ and $p < 0.001$, respectively) (Fig. 1, Table 2). This raised glycaemia (average plasma glucose 5.2 ± 0.1 vs 5.0 ± 0.0 mmol/l, $p < 0.05$) and hyperinsulinaemia (average serum insulin 112 ± 21 vs 66 ± 6 pmol/l, $p < 0.001$) in the relatives were mainly due to the increased concentrations of plasma glucose (average concentration 5.4 ± 0.1 vs 5.1 ± 0.1 mmol/l, $p = 0.02$) and serum insulin (average concentration 146 ± 14 vs 86 ± 6 pmol/l, $p < 0.001$) during the day. In the postabsorptive state

Table 2. Area under the curve for glucose, insulin C peptide and glucagon during a standardized 24-h period and the integrated amount of insulin secreted calculated on basis of C peptide kinetics (means ± SEM)

	Breakfast AUC (0800–1200 hours) R vs C	Lunch AUC (1200–1800 hours) R vs C	Dinner AUC (1800–2400 hours) R vs C	Night-time AUC (2400–0800 hours) R vs C	24-h AUC (800–800 hours) R vs C
Plasma glucose (mmol · l^{-1} · min)	1305 ± 26 vs 1196 ± 18[a]	2004 ± 61 vs 1825 ± 19[a]	1885 ± 26 vs 1825 ± 25	2364 ± 68 vs 2409 ± 34	7559 ± 91 vs 7254 ± 62[a]
Serum insulin (pmol · l^{-1} · min)	47678 ± 4696 vs 26788 ± 1836[b]	39635 ± 4092 vs 22733 ± 1718[b]	52631 ± 5543 vs 32971 ± 3016[a]	21863 ± 1986 vs 13039 ± 942[b]	161608 ± 14977 95531 ± 6514[c]
Serum C peptide (nmol · l^{-1} · min)	354 ± 21 vs 260 ± 17[b]	414 ± 24 vs 292 ± 19[c]	521 ± 29 vs 381 ± 25[b]	324 ± 24 vs 216 ± 24[b]	1612 ± 91 vs 1149 ± 71[c]
Plasma glucagon (ng · l^{-1} · min)	12088 ± 1257 vs 11505 ± 708	16025 ± 1488 vs 14577 ± 1121	15562 ± 1265 vs 15399 ± 1631	19670 ± 1561 vs 19023 ± 2005	63344 ± 5265 vs 60504 ± 4423
Integrated amount of insulin secreted (nmol)	94.3 ± 6.3 vs 65.7 ± 5.1[b]	105.6 ± 6.9 vs 71.3 ± 5.4[c]	140.7 ± 9.0 vs 99.7 ± 7.9[b]	80.2 ± 6.6 vs 49.2 ± 3.5[c]	420.8 ± 27.2 vs 285.9 ± 20.5[c]

R = Relatives vs C = Controls. [a]: $p < 0.05$, [b]: $p < 0.01$, [c]: $p < 0.001$

during the night serum insulin (average concentration 46 ± 14 vs 27 ± 2 pmol/l, $p < 0.01$) but not plasma glucose (5.0 ± 0.1 vs 5.0 ± 0.1, p = NS) remained raised in the relatives (Fig. 1, Table 2). The differences in the circulating concentrations of C peptide between relatives and control subjects mimicked the differences observed in those of insulin during the 24-h profile (Table 2). Circulating concentrations of glucagon did not differ significantly between relatives and control subjects during the day (i. e. after each meal) or in the postabsorptive phase during night-time (Table 2).

Examination of basal and meal-stimulated insulin secretion rates over 24 h. The total integrated amount of insulin secreted was significantly higher in the relatives than in control subjects, both after meals, overnight and for the full 24-h period (Fig. 1, Table 2). Incremental insulin secretion after each meal expressed as percentage basal insulin secretion (Fig. 1) failed, however, to show statistical significance between relatives and control subjects. Thus, after breakfast, 84.4 ± 2.6 versus 92.1 ± 3.4 % (relatives vs control subjects, p = NS) of the total 4-h insulin secretory response was observed in the first 2 h after the meal. After lunch and dinner those numbers were 75.5 ± 3.0 compared with 78.3 ± 3.4 % and 66.2 ± 3.4 compared with 71.7 ± 2.0 % [p (both) = NS], respectively. Of note is that the peak postprandial insulin secretion (calculated in percentage basal insulin secretion, 60–90 min postmeal), tended to be reduced in the relatives compared with the control subjects after all meals (432 ± 29 vs 520 ± 35 %, $p = 0.06$; 333 ± 21 vs 392 ± 27 %, $p = 0.08$ and 449 ± 30 vs 532 ± 38 %, $p = 0.06$ after breakfast, lunch and dinner, respectively, Fig. 1). There was an inverse correlation throughout the entire 24-h period between calculated AUC for the circulating concentrations of insulin and the integrated amount of insulin secreted compared with insulin sensitivity (ISGU) in both relatives (24-h AUC serum insulin: $r = -0.57$, $p < 0.01$; 24-h integrated amount of insulin secreted: $r = -0.64$, $p < 0.001$) and control subjects (24-h AUC serum insulin: $r = -0.52$, $p < 0.05$; 24-h integrated amount of insulin secreted: $r = -0.70$, $p < 0.01$). In contrast, neither the incremental amount of insulin secreted after each meal nor peak incremental insulin secretion during all three meals correlated to ISGU in either of the two groups [p (all) = NS, data not shown].

Profiles over 24 h: blood intermediary metabolites and serum NEFA. Blood alanine was increased in the relatives during the 24-h profile (411 ± 12 vs 371 ± 15 mmol · l^{-1} · min, $p < 0.05$), ascribable to increased concentrations of blood alanine during daytime (293 ± 9 vs 264 ± 10 mmol · l^{-1} · min, $p < 0.05$). In contrast, blood 3-OHB appeared lower in the relatives throughout the 24-h profile, although not statistically significant (Fig. 2). Serum NEFA profiles were almost identical in the relatives and in the control subjects (Fig. 2) despite pronounced hyperinsulinaemia (as mentioned above) in the relatives. Moreover, blood lactate and glycerol were similar in the two groups (Fig. 2).

Total and intact serum proinsulin. Fasting total and intact serum proinsulin concentrations were significantly higher in the relatives compared with the control subjects [total serum proinsulin: 13.5 ± 3.6 vs 6.1 ± 0.8 pmol/l; intact serum proinsulin: 3.3 ± 0.9 vs 1.7 ± 0.1 pmol/l, p (both) < 0.05]. After breakfast, AUC for total serum proinsulin was significantly increased in the relatives (8100 ± 1883 vs 4145 ± 552 pmol · l^{-1} · min, $p < 0.05$) but differences in AUC for intact serum proinsulin did not reach statistical significance between the relatives and the control subjects (1614 ± 401 vs 961 ± 98 pmol · l^{-1} · min, $p = 0.12$) (Fig. 3). Neither total nor intact serum proinsulin concentrations differed, however, between the two

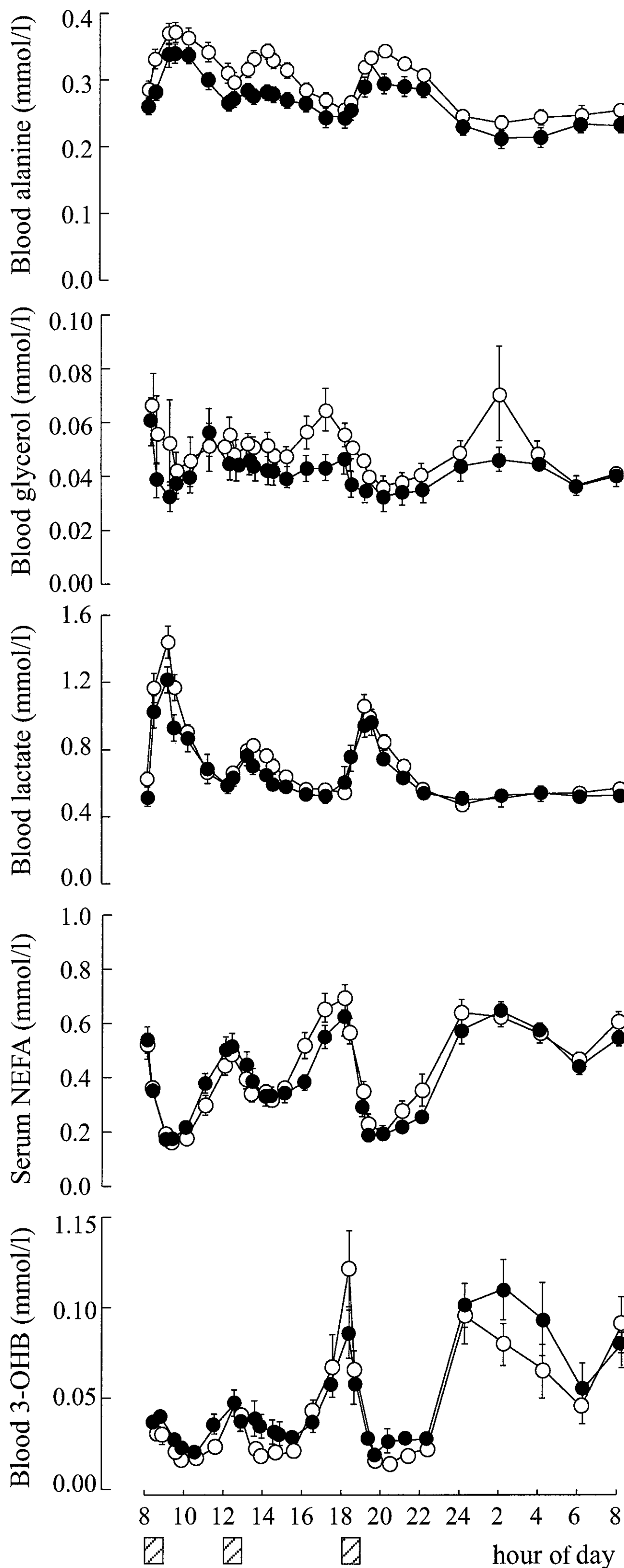

Fig. 2. Profiles over 24 h of blood alanine, glycerol, lactate, 3-hydroxy-butyrate and serum non-esterified fatty acids in first-degree relatives of Type II diabetic patients (○) and control subjects (●) (means ± SEM) ▨ indicates meals.

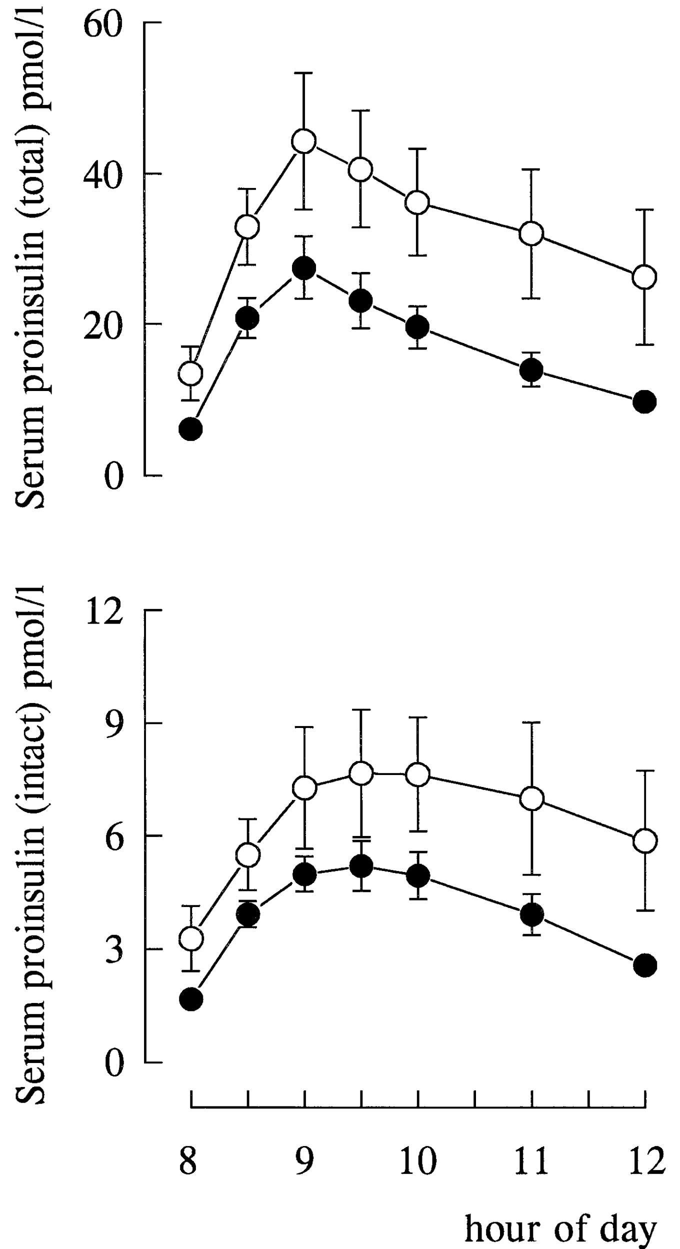

Fig. 3. Serum total and intact proinsulin in relatives of Type II diabetic patients (○) and control subjects (●) after breakfast. Data are means ± SEM

groups, when adjustments for the baseline values were made by calculating incremental AUCs (total serum proinsulin: $p = 0.14$; intact serum proinsulin: $p = 0.68$). Proinsulin (total and intact) to insulin ratios calculated at baseline [total serum proinsulin: 0.23 ± 0.03 vs 0.19 ± 0.02, intact serum proinsulin: 0.06 ± 0.01 vs 0.06 ± 0.00, relatives vs control subjects, p (both) = NS] and at each individual time point after breakfast were all similar in the relatives and control subjects (data not shown).

Gut incretin hormones. Fasting concentrations of GIP (10.0 ± 1.5 vs 11.7 ± 4.4 pmol/l, $p = 0.67$) and GLP-1

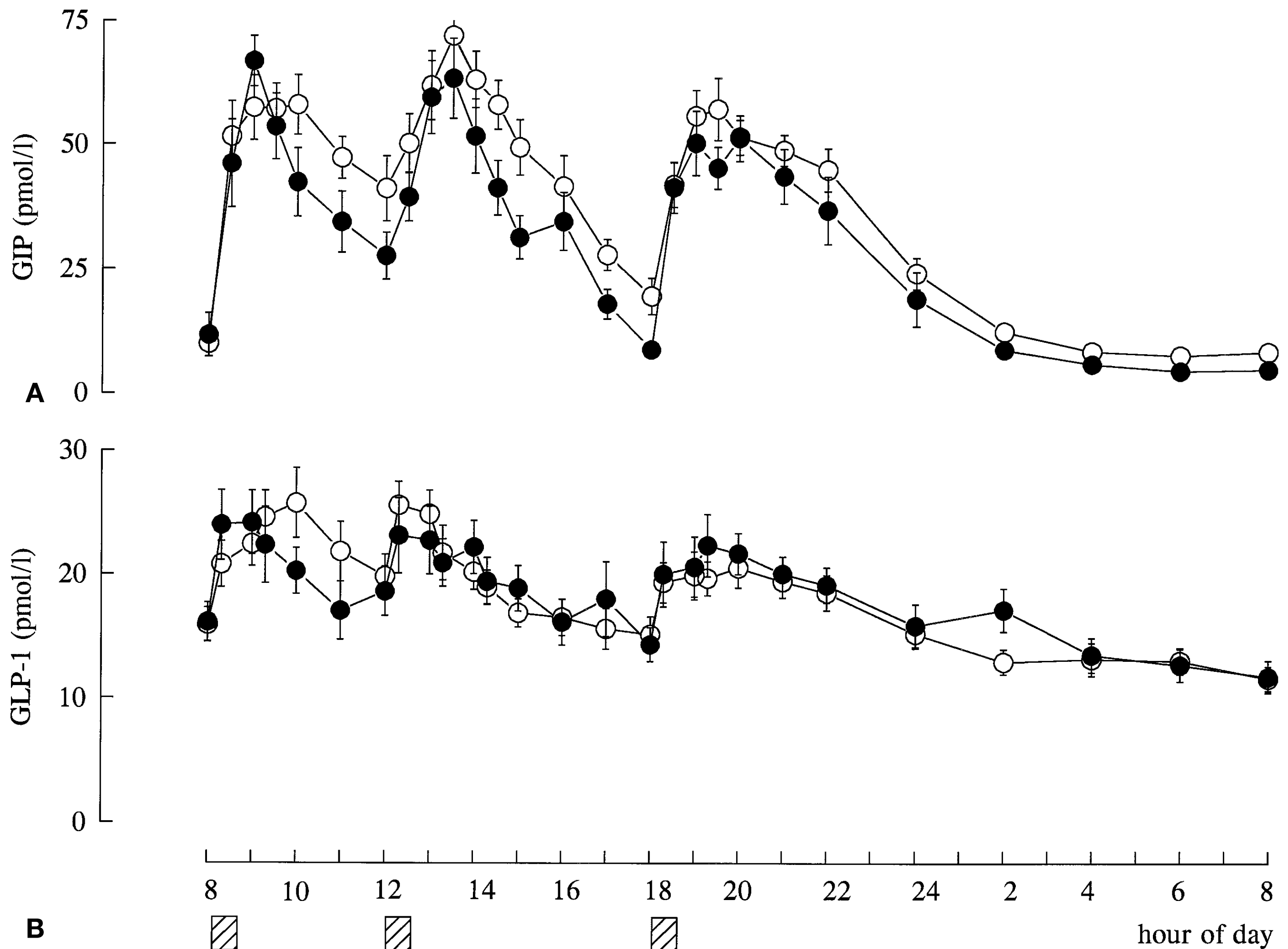

Fig. 4A, B. Circulating concentrations of the incretin hormones GIP (**A**) and GLP-1 (**B**) during a 24-h period simulating daily living in 26 first-degree relatives of Type II diabetic families (○) and 17 matched control subjects (●) (means ± SEM). ▨ indicates meals

(15.9 ± 1.4 vs 16.1 ± 1.6 pmol/l, $p = 0.88$) were similar in relatives and control subjects. During daytime (i.e. after meals, Fig. 4) AUC for plasma GIP (46002 ± 3337 vs 35006 ± 3428 pmol · l^{-1} · min, $p < 0.05$) was significantly increased in the relatives compared with control subjects, whereas AUC for plasma GLP-1 (23321 ± 3189 vs 18384 ± 1438 pmol · l^{-1} · min, $p = 0.38$) remained similar in the two groups. The AUC for plasma GIP was significantly increased in the relatives after breakfast (12999 ± 886 vs 9986 ± 913 pmol · l^{-1} · min, $p < 0.05$) and lunch (17842 ± 1698 vs 12272 ± 1311 pmol · l^{-1} · min, $p < 0.05$) though barely statistical significant after dinner (15161 ± 1088 vs 12749 ± 1589 pmol · l^{-1} · min, $p = 0.15$). In contrast, AUC for plasma GLP-1 did not differ between the two groups after any of the three meals (Fig. 4). During night-time, neither AUC for plasma GIP (18569 ± 3633 vs 12713 ± 2029 pmol · l^{-1} · min, $p = 0.23$) nor GLP-1 (11465 ± 1093 vs 13678 ± 1085 pmol · l^{-1} · min, $p = 0.12$) differed between relatives and control subjects. Due to the increased daytime concentrations of GIP in the relatives, 24-h AUC was significantly raised in relatives (64572 ± 5083 vs 47719 ± 5415 pmol · l^{-1} · min, $p < 0.05$). In contrast, and as expected, 24-h AUC for plasma GLP-1 was similar in relatives and control subjects (34768 ± 3378 vs 32062 ± 2429 pmol · l^{-1} · min, $p = 0.87$). Similar results were observed when calculating incremental AUCs (i.e. adjusting for baseline values) after each meal and during daytime and night-time for each of the two hormones (data not shown).

Discussion

The key objective of the current study was to assess insulin secretion and examine the impact of previously defined aberrations on the 24-h metabolic and hormonal response during conditions which simulate daily living with particular regard to eating pattern and dietary intake in healthy non-obese relatives of Type II diabetic patients. We found important meta-

bolic changes, an increased basal insulin secretion, albeit normal postprandial insulin secretion rates, and an exaggerated postprandial concentration of GIP in the relatives.

Although all subjects were glucose tolerant, the relatives had increased daytime plasma glucose concentrations. This tiny (4–6 %) but statistically significant raised glycaemia after mixed meals compared with matched control subjects possibly reflects an early sign of subclinical detoriation in glucose tolerance in the relatives at an increased risk for development of Type II diabetes [4]. Because the glycaemic response after meal ingestion are influenced by numerous factors, e. g. insulin sensitivity, glucose effectiveness, gastric emptying, release of gut incretin hormones, increase in insulin and suppression of glucagon, the mechanisms behind postprandial glucose excursions might, however, be complex.

During the 24-h profile, circulating concentrations of insulin and C peptide were greatly increased in the non-obese relatives. Calculated rates of insulin secretion show an increased basal insulin secretion whereas incremental insulin secretion rates in the postprandial periods were similar in relatives and control subjects, both in magnitude and pattern. This is in contrast to overt Type II diabetic patients where both diminished and delayed postprandial insulin secretion was observed [2] but analogous to the observations of insulin secretion in obese glucose tolerant subjects [32]. It should, however, be emphasized that the peak postprandial insulin secretion (60–90 min post-meal, expressed as percentage basal insulin secretion) tended to be reduced in the relatives after all meals, which possibly suggests early beta-cell dysfunction.

The increased glucose concentrations in the presence of hyperinsulinaemia indicate impaired insulin action in terms of carbohydrate metabolism, though glucose effectiveness is also of importance. Whether glucose effectiveness in first-degree relatives is decreased [33], normal [34] or even increased [7] is still disputed. In our study insulin resistance in the relatives was confirmed using the hyperinsulinaemic clamp and the findings during the 24-h profile indicate that this important metabolic feature is also apparent during conditions of daily living. Previous studies have sought to define the mechanisms underlying the insulin resistance in non-diabetic relatives. Evidence for the role of genetic factors comes among others from studies which have reported familial clustering of insulin resistance [35, 36] and the persistence of abnormal insulin mediated glucose metabolism in cultured fibroblast cells from Type II diabetic patients [37]. Hyperinsulinaemia itself possibly contributes to the insulin resistant state [38]. In our study, circulating insulin concentrations were on average increased by 70 % during the day and 68 % over 24 h in the relatives. The postprandial increase in plasma glucose in the relatives could also contribute to the insulin resistance through the operation of the hexosamine pathway [3]. The increase in circulating blood glucose concentrations in the relatives was, however, comparatively small, reaching only 4 % over 24 h and 6 % through the daytime period.

Fasting concentrations of total as well as intact serum proinsulin were increased in the relatives compared with control subjects. Proinsulin: insulin ratios were, however, almost identical in the two groups. These results are in accordance with the majority of studies focusing on proinsulin processing in normal glucose tolerant relatives of Type II diabetic patients [10, 39]. It is, however, important to note that a recent report found increased proinsulin:insulin ratios in Mexican-Americans with a family history of Type II diabetes [40], possibly indicating the occurrence of subtle abnormalities in proinsulin processing in certain groups of relatives. This issue is important as an increased proinsulin:insulin ratio is widely recognised as a key feature of Type II diabetes [41], possibly reflecting a specific beta-cell defect [42].

Non-esterified fatty acid metabolism is highly sensitive to insulin, so that small changes in circulating insulin concentrations within the physiological range exert major effects on those of circulating NEFA [43]. A further important observation from our study therefore is that NEFA concentrations were similar in relatives and control subjects, despite considerably higher circulating insulin concentrations in the relatives. In other words, we have provided evidence for insulin resistance for NEFA metabolism in the relatives in addition to that for carbohydrate metabolism. Our findings support a recent report [44] in which insulin resistance for lipid metabolism was identified in similar non-diabetic relatives. This is important as it is well recognised that insulin resistance for glucose and lipid metabolism is a key feature of established Type II diabetes. What is now emerging is that the same abnormalities of insulin action are present in at risk but non-diabetic relatives, indicating that multiple metabolic abnormalities are present well before the development of diabetes.

Another finding in the current study relates to the raised concentrations of circulating GIP during daytime in the relatives. Of note, diminished or delayed "early phase" insulin secretion after OGTT have been reported in three Danish studies dealing with glucose tolerant relatives of Type II diabetic patients [7, 9, 14]. In theory, this finding could be attributed to a secretory defect or a reduced effect of incretin hormones. Circulating concentrations of GLP-1 have been found normal in healthy relatives after an OGTT [45] and our study shows similar findings during daily-life conditions. Conflicting results of both normal and increased [18] circulating GIP con-

centrations in Type II diabetic patients have been reported. Moreover, there is a lack of effect of GIP in Type II diabetes [20]. This is in accordance with the hypothesis, that the diminished incretin effect in Type II diabetes is ascribable to defective expression or function of the GIP receptor on the beta cell [21]. It is, however, not known if such a defect is inherited or acquired secondarily to the metabolic derangement. Data on the plasma GIP response to glucose/food ingestion in potentially prediabetic subjects is only very limited. After a standard oral glucose load circulating concentrations of GIP were similar in glucose tolerant first-degree relatives of Type II diabetic patients and control subjects [45]. Simulating daily living i. e. with mixed meals containing variable insulin secretagoues, we found, however, significantly increased concentrations of GIP during the day in healthy relatives and incremental postprandial insulin secretion rates similar to a matched control group. Data from our study do not allow conclusions about causality but it is tempting to hypothesize that the increased postprandial GIP concentrations in the relatives represent a compensatory mechanism. This could reflect an underlying defect of beta-cell GIP receptor expression or function that exists even in the early prediabetic state.

Plasma glucagon, especially postprandial glucagon, is increased in Type II diabetes and probably contributes to increased postprandial plasma glucose concentrations [46]. Increased circulating glucagon concentrations have previously been shown in relatives of Type II diabetic patients including those with impaired glucose tolerance [47]. In the current study no difference was observed in plasma glucagon between glucose tolerant relatives and control subjects neither in the fasting state nor after meals suggesting that an alpha-cell dysfunction is apparently not present in normal glucose tolerant relatives of Type II diabetic patients.

One final conundrum relates to the raised alanine concentrations in the relatives. Whether this relates to changed carbohydrate or protein metabolism or both could not be determined from this study.

We have shown that normal glucose tolerant but insulin resistant relatives of Type II diabetic patients have multiple metabolic abnormalities when examined under conditions that simulate daily living. Average serum insulin was increased by approximately 70 %, attributed to an increased basal insulin secretion whereas incremental insulin secretion in the postprandial periods were similar to matched non-diabetic subjects, both of magnitude and pattern. Of note, however, peak incremental insulin secretion after all meals tended to be decreased in the relatives. Moreover, postprandial concentrations of GIP were increased in the relatives, possibly reflecting a beta-cell GIP receptor defect which predates the development of Type II diabetes.

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