

# Reduced Hepatic Insulin Extraction in Response to Gastric Inhibitory Polypeptide Compensates for Reduced Insulin Secretion in Normal-Weight and Normal Glucose Tolerant First-Degree Relatives of Type 2 Diabetic Patients

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**Our objective was to study whether young first-degree relatives of patients with type 2 diabetes (FDRs) have altered insulin secretion and insulin clearance in response to gastric inhibitory polypeptide (GIP) in combination with glucose and arginine. A hyperglycemic clamp (11.1 mmol/l for 115 min), followed by addition of GIP (2 pmol · kg<sup>-1</sup> · min<sup>-1</sup>, 60–115 min) and an arginine bolus and infusion (10 mg · kg<sup>-1</sup> · min<sup>-1</sup>, 90–115 min), was conducted on 14 healthy volunteers and 13 FDRs. Both groups had normal glucose tolerance. FDRs were more insulin resistant (HOMA<sub>IR</sub>) under basal conditions ( $P = 0.003$ ). FDRs demonstrated significant global impairment in insulin secretion capacity, which was not specific for one of the secretagogues. Insulin clearance was significantly reduced in the group of FDRs under basal conditions and in response to GIP, but there was no general defect in insulin clearance in response to glucose and arginine. The HOMA<sub>IR</sub> correlated negatively ( $P < 0.01$ ) with insulin clearance under basal conditions ( $r = -0.96$ ) and under GIP infusion ( $r = -0.56$ ). We propose that impairment in insulin secretion capacity and decreased insulin sensitivity is compensated for several mechanisms, one of which includes a GIP-dependent reduction of the insulin clearance that will increase peripheral insulin levels to maintain normoglycemia. *Diabetes* 53:2359–2365, 2004**

**T**ype 2 diabetes is a heterogeneous disorder with varying degrees of impaired insulin sensitivity and insulin secretion resulting from the interaction of genetic and environmental factors (1). Identification of the mechanisms of  $\beta$ -cell adaptation in relation to changes in muscle or liver insulin sensitivity is

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AUC, area under the curve; FDRs, first-degree relative of patient with type 2 diabetes; GIP, gastric inhibitory polypeptide; GLP, glucagon-like peptide; HOMA, homeostasis model assessment; IR, insulin resistance; ISR, insulin secretion rate; MCR, metabolic clearance rate.

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important to understanding the pathophysiology of type 2 diabetes.

There is increasing evidence that genetic defects in insulin secretion represent a major factor predisposing patients to the development of type 2 diabetes (2–4). Previous studies in subjects with a high risk of type 2 diabetes have demonstrated different abnormalities of insulin secretion patterns: reduced first-phase insulin secretion (3,4), reduced insulinotropic effects of incretins (5,6), and decreased overall capacity of insulin secretion (6,7). In addition, several studies of diabetes in humans have shown decreased insulin clearance and degradation associated with insulin resistance (8–14).

The fine mechanisms of regulation of insulin clearance in humans remain unclear. In nondiabetic insulin-resistant individuals, the hyperinsulinemia resulted from an increase in glucose-stimulated insulin secretion as well as from a decrease in insulin clearance (15). Moreover, increased insulin sensitivity results in low insulin secretion and increased insulin clearance (16). On the other hand, a chronically impaired ability to clear insulin from the circulation, as is seen in certain liver diseases, causes hyperinsulinemia with attendant peripheral insulin resistance as a result of impaired insulin action (17). A recent genetic study demonstrated that polymorphisms in the insulin-degrading enzyme gene are associated with type 2 diabetes (18). Thus, altered insulin clearance may not only represent an adaptive mechanism but also one of the primary defects in the pathogenesis of type 2 diabetes.

The regulation of insulin clearance has profound pathophysiological consequences for fuel metabolism. In this context, it is important to understand not only how insulin secretion is altered in insulin resistance, but also how insulin clearance is altered. One of the potential mechanisms appears to be the effect of incretin hormones on insulin secretion and insulin clearance (19,20).

The objective of our study was to characterize subtle alterations of insulin metabolism in young, normal-weight, glucose-tolerant first-degree relatives of patients with type 2 diabetes (FDRs).

## RESEARCH DESIGN AND METHODS

The study protocol was approved by the ethics committee of the Ruhr-University, Bochum, Germany. Before the study, informed written consent was obtained from all participants.

We studied 14 healthy, normal glucose tolerant volunteers and 13 healthy, normal glucose tolerant FDRs, all of Caucasian origin. They did not take any medications known to affect glucose metabolism and had been instructed to maintain their usual diet and physical activity before the study. Healthy control subjects did not have any known first- or second-degree relatives with type 2 diabetes. Impaired fasting glucose and manifested type 2 diabetes were excluded in all participants according to American Diabetes Association criteria (21). All subjects underwent a modified hyperglycemic clamp combined with gastric inhibitory polypeptide (GIP) and arginine infusion.

**Hyperglycemic clamp.** In the morning after a 12-h fast, a distal forearm vein was punctured with a Teflon cannula (Moskito 123, 18 g; Vygon, Aachen, Germany) and kept patent using physiological saline. At the same time, an antecubital vein of the contralateral arm was cannulated for infusions. Both ear lobes were made hyperemic using Finalgon (4 mg/g nonivamid, 25 mg/g nicoboxil). After baseline samples had been obtained, a hyperglycemic clamp was carried out (22). An intravenous bolus of 40% glucose was given over 1 min to instantaneously raise capillary blood glucose to 11.1 mmol/l [bolus glucose = body weight (kg) × 0.3 g glucose]. Subsequently, the glucose infusion was adjusted to maintain capillary blood glucose at 11.1 mmol/l for 2 h. After 60 min, GIP (human GIP amide; PolyPeptide Laboratories, Wolfenbüttel, Germany) was given as a continuous infusion (2 pmol · kg<sup>-1</sup> · min<sup>-1</sup>) during the next 60 min. At 90 min, a bolus (12% of infusion dose) of arginine hydrochloride (L-arginine-hydrochloride, 21% in water; Braun, Melsungen, Germany) and a continuous infusion (10 mg · kg body wt<sup>-1</sup> · min<sup>-1</sup>) of arginine were given during the next 25 min.

Synthetic GIP was purchased from PolyPeptide Laboratories and processed for intravenous infusions as described (23). In all subjects, GIP from the same batch (C-0229) was used.

Samples for insulin and C-peptide measurement were taken at -15, -5, 0, 2.5, 5, 7.5, 10, 25, 60, 62.5, 65, 67.5, 70, 85, 90, 92.5, 95, 97.5, 100, and 115 min. After centrifugation, serum for hormone analyses was kept frozen at -20°C.

**Laboratory determinations.** Capillary blood glucose concentrations (from a hyperemic ear lobe) were determined at bedside every 5 min with a glucose oxidase-based method (Hemocue glucose photometer; HemoCue, Ångelholm, Sweden). Serum insulin was measured using an enzyme-linked immunosorbent assay (Insulin ELISA; Mercodia, Uppsala, Sweden) with negligible cross-reaction with C-peptide of <0.01%. The sensitivity of this assay was <6 pmol/l. The cross-reaction with intact human proinsulin was <0.01%. Intra- and interassay coefficients of variation (CVs) were 4%. Serum C-peptide was measured by enzyme-linked immunosorbent assay (C-peptide ELISA; Mercodia), with a cross-reaction with insulin of <0.1% and with proinsulin of 100%. Intra- and interassay CVs were 5%. The sensitivity limit was <14.9 pmol/l.

**Calculations.** We calculated pancreatic β-cell function and insulin resistance (IR) from fasting glucose and insulin concentrations using homeostasis model assessment (HOMA) (HOMA<sub>β-cell</sub> and HOMA<sub>IR</sub>, respectively [24]).

The classical “steady state” cannot be achieved in the hyperglycemic clamp because the insulin secretion increases continuously (22). We used three different insulin secretagogues under clamped hyperglycemia, which progressively increased insulin secretion in our study. We restricted the “calculation window” to each of the individual insulin secretagogues separately. We calculated hepatic insulin extraction for each period of the clamp with respect to the peripheral insulin concentration and the insulin secretion rate (ISR) from the prestimulus. We used a two-compartment model of C-peptide kinetics for our calculation, as first published by Eaton et al. (25) and improved by Polonsky and colleagues (26,27).

1. This model allows estimation of ISRs by standard C-peptide kinetic parameters rather than individually derived parameters without significant loss of accuracy, even under non-steady-state conditions (26,27).
2. This model was evaluated by several previously published studies for use in the experiments, such as the hyperglycemic clamp (28,29) and even for hyperglycemic clamps with the use of three different insulin secretagogues (6).
3. This model allows estimation of insulin clearance under non-steady-state conditions by hyperglycemic clamp (28,29).

Hepatic insulin extraction was assessed in three ways: 1) from changes in ISR compared with changes in peripheral insulin concentrations, both expressed as percentages of their respective basal values, which were taken as 100% (28,29); 2) from a ratio of the incremental area under the ISR curve (AUC<sub>ISR</sub>) to the incremental area under the peripheral insulin concentration curve (AUC<sub>insulin</sub>) for each of the clamp periods with respect to the previous stimulus and from the ratio of the AUC<sub>ISR</sub> to AUC<sub>insulin</sub> (metabolic clearance rate [MCR]<sub>basal</sub>) for the basal state, which was previously established as an indicator of metabolic clearance rate of endogenous insulin (MCR<sub>insulin</sub>) (28,29); 3) from the molar ratio of the incremental area under the C-peptide curve (AUC<sub>C-peptide</sub>) to the incremental area under the insulin concentration

TABLE 1  
Subjects characteristics

	Control subjects	FDRs	<i>P</i>
Sex (F/M)	6/8	8/5	0.35
Age (years)	34 ± 2.5	29 ± 2.8	0.37
BMI (kg/m <sup>2</sup> )	23.5 ± 1.1	23.9 ± 1.2	0.68
Capillary blood glucose (mmol/l)	4.5 ± 0.1	4.9 ± 0.1	0.30
Basal insulin (pmol/l)	22 ± 2	40 ± 4	0.001
Basal C-peptide (pmol/l)	346 ± 47	493 ± 62	0.038
Basal ISR (pmol/min)	99 ± 23	132 ± 17	0.004
HOMA <sub>IR</sub> (fold normal)	0.99 ± 0.1	2.03 ± 0.5	0.003
HOMA <sub>IS</sub> (% of normal)	94 ± 13	169 ± 45	0.067

Data are means ± SE.

curve (AUC<sub>insulin</sub>) for each of the clamp periods with respect to the previous stimulus and from the ratio of the AUC<sub>insulin</sub> to AUC<sub>C-peptide</sub> for the basal state (30,31). These areas under the curve were calculated by the trapezoidal rule for each of four periods (AUC<sub>basal</sub>, AUC<sub>glucose[0-60 min]</sub>, AUC<sub>GIP[62.5-90 min]</sub>, and AUC<sub>arginine[92.5-115 min]</sub>).

**Statistical analysis.** Data are presented as means ± SE. The Student's two-tailed *t* test for unpaired variables was used for comparison of normally distributed data. When the data were not normally distributed, the Mann-Whitney rank-sum test for unpaired data was used for statistical comparisons. A *P* value <0.05 was considered statistically significant. The relationship between the MCR and HOMA<sub>IR</sub> was tested by correlation analyses (Spearman rank-order correlation). All calculations were performed with the SPSS for Windows program (version 10; SPSS, Chicago, IL).

**RESULTS**

In comparison to healthy control subjects, FDRs had higher fasting peripheral insulin concentrations, increased fasting C-peptide concentrations, increased insulin secretion rate, and higher HOMA<sub>IR</sub> (Table 1).

Steady-state glucose concentrations were achieved during the 40- to 60-min period and did not differ between the two groups (11.2 ± 0.2 vs. 11.0 ± 0.1 mmol/l, *P* = 0.10). During the infusion of GIP and arginine, similar blood glucose levels were achieved in healthy control subjects and FDRs. The mean CV of blood glucose during the 40- to 120-min period was 1.8% in healthy control subjects and 2.3% in FDRs.

The glucose infusion rates necessary to maintain hyperglycemia did not differ between the control group and FDRs (*P* = 0.87 for the 40- to 60-min period, *P* = 0.22 for the 60- to 90-min period, and *P* = 0.62 for the 90- to 120-min period, data not shown).

During the hyperglycemic clamp, insulin concentrations were significantly higher in the group of FDRs at 25, 60, 62.5, 65, 67.5, and 70 min (Fig. 1A). In contrast, C-peptide concentrations were not significantly different between both groups during the hyperglycemic clamp (Fig. 1B). ISR was significantly different between both groups only at the 7.5-min time point, after the glucose bolus (289.7 ± 62 pmol/min in the control group vs. 335.7 ± 71 pmol/min in FDRs, *P* = 0.017) (Fig. 1C).

Change in insulin concentration and change in ISR, both expressed as percentages of their basal values, are shown in Fig. 2. The increase in insulin concentration closely followed the increase in ISR in the first hour of the hyperglycemic clamp. Then, at 70 min in the FDRs and at 85 min in control subjects, the increase in insulin concentrations began to exceed the increase in ISR. Subsequently, with continuing stimulation of insulin secretion with

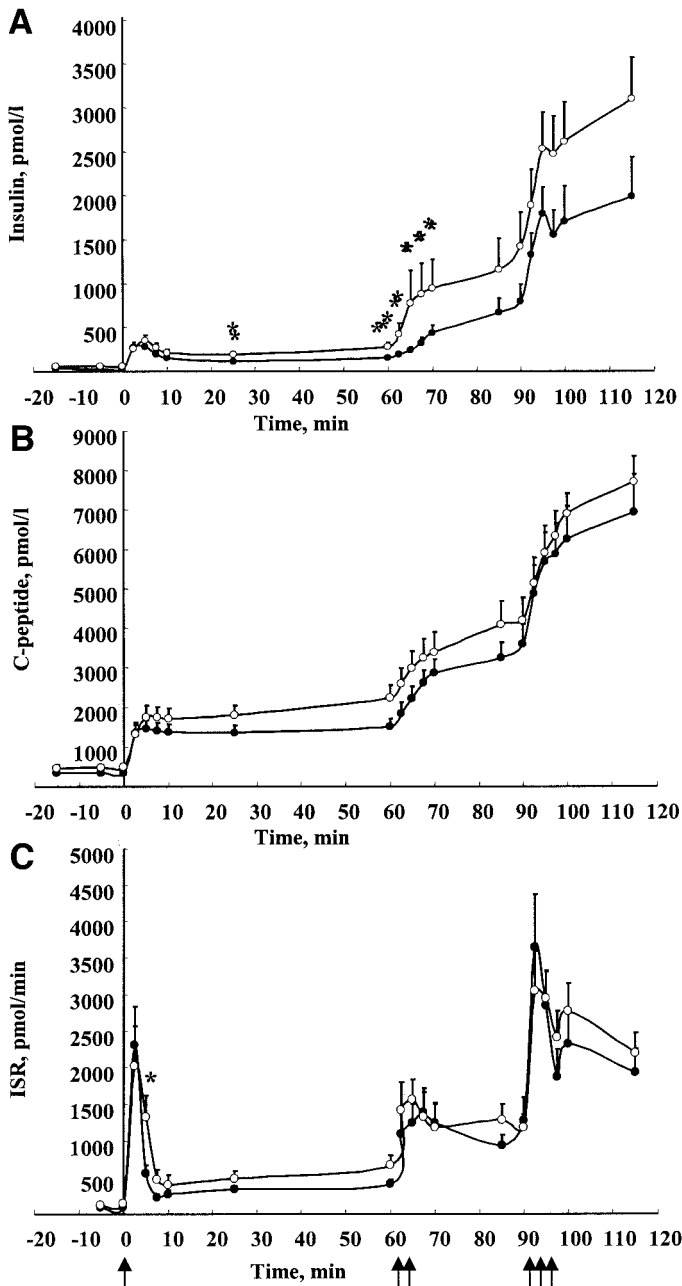


FIG. 1. Mean insulin (A), C-peptide (B), and ISR (C) values during hyperglycemic clamps. ○, FDRs; ●, control subjects. Arrows indicate the initiation of glucose, GIP, and arginine infusions. \* $P < 0.05$ .

three secretagogues, this difference became more pronounced. Thus, whereas the ISR increased to  $2,272.4 \pm 228.4\%$  of basal in the control group and to  $1,634.4 \pm 116.2\%$  of basal in FDRs ( $P = 0.04$ ) at 115 min of the hyperglycemic clamp, the peripheral insulin concentration increased to a significantly greater extent, reaching  $7,333.3 \pm 1,562.5\%$  of basal in the control group and  $7,345.8 \pm 1,455.7\%$  of basal in FDRs ( $P = 0.09$ ) (Fig. 2). The observation that the increase in the serum insulin concentration was relatively greater than the increase in the ISR can be explained only by a decrease in the clearance of endogenously secreted insulin during the clamp.

Fasting molar ratios of  $AUC_{ISR}$  to  $AUC_{insulin}$  ( $MCR_{basal}$ ) were significantly higher in the control group ( $4.0 \pm 0.3$  vs.  $2.3 \pm 0.3$  l/min,  $P = 0.001$ ) (Fig. 3A). During the first hour

of the hyperglycemic clamp, the MCRs were not significantly different in both groups ( $P = 0.99$ ). In response to GIP infusion, the MCR decreased progressively to  $2.7 \pm 0.5$  l/min in the control group and to  $1.4 \pm 0.2$  l/min in FDRs ( $P = 0.029$ ). In response to the arginine bolus and infusion, the MCRs were not significantly different between both groups ( $1.9 \pm 0.4$  l/min in control subjects vs.  $1.9 \pm 0.5$  l/min in FDRs,  $P = 0.71$ ) (Fig. 3A).

Fasting molar ratios of  $AUC_{C-peptide}$  to  $AUC_{insulin}$  were higher in the control group but not significantly different compared with FDRs ( $13.2 \pm 1.8$  vs.  $9.4 \pm 1.0$ ,  $P = 0.155$ ) (Fig. 3B). After the glucose load, there was no significant difference in the molar ratio between both groups ( $P = 0.793$ ). In response to GIP infusion, the molar ratios decreased progressively in both groups and were lower in FDRs than in control subjects ( $2.9 \pm 0.5$  vs.  $5.4 \pm 0.9$ ,  $P = 0.019$ ). Further addition of arginine bolus and infusion did not change the molar ratios, which were similar in both groups ( $P = 0.193$ ) (Fig. 3B).

$HOMA_{IR}$  correlated negatively with insulin clearance under basal conditions ( $MCR_{basal}$ ) ( $r = -0.96$ ;  $P < 0.01$ ) and under GIP infusion ( $r = -0.56$ ;  $P = 0.003$ ).

## DISCUSSION

The present study was undertaken to explore the ability of the  $\beta$ -cell to maintain the insulin secretion and mechanisms to regulate the peripheral insulin concentration in normal-weight, glucose-tolerant, young FDRs and healthy subjects in response to three different secretagogues under conditions of the hyperglycemic clamp. We designed a hyperglycemic clamp that evaluates multiple parameters during a single session: first and second phases of glucose-induced insulin secretion, insulin secretion during an incretin stimulus (hyperglycemia plus 1-h infusion of GIP), and first and second phases of arginine-induced insulin secretion (hyperglycemia plus GIP infusion plus 30 min of arginine bolus and infusion).

The IR index ( $HOMA_{IR}$ ) and basal peripheral insulin and C-peptide concentrations were significantly higher in the group of normal-weight FDRs. Previous studies have demonstrated a link between IR, including compensatory hyperinsulinemia, and both individuals with impaired glucose tolerance and FDRs. Caucasians (32–34), Pima Indians (9), African Americans (35), and Mexican Americans (14) have been found to show impaired insulin sensitivity, similar to our study. These populations show relatively high insulin responses to oral or intravenous glucose loads because of decreased insulin sensitivity. On the other hand, other groups from Europe and the U.S. described no differences in insulin sensitivity (3,4,36). It is important to point out that the matching process in our study excluded effects of obesity, body weight, fat distribution, age, and sex, which are associated with IR (37).

Thus, our data show that for a given degree of mild IR, an additional defect in insulin secretion was not detectable by direct measurement of peripheral insulin concentrations. This is not consistent with previous studies that reported a reduction in the first phase of glucose-stimulated insulin secretion in FDRs under hyperglycemic clamp conditions (3,4,36), which may relate to different degrees of  $\beta$ -cell defects by individuals in these studies and to the young age of our cohort.

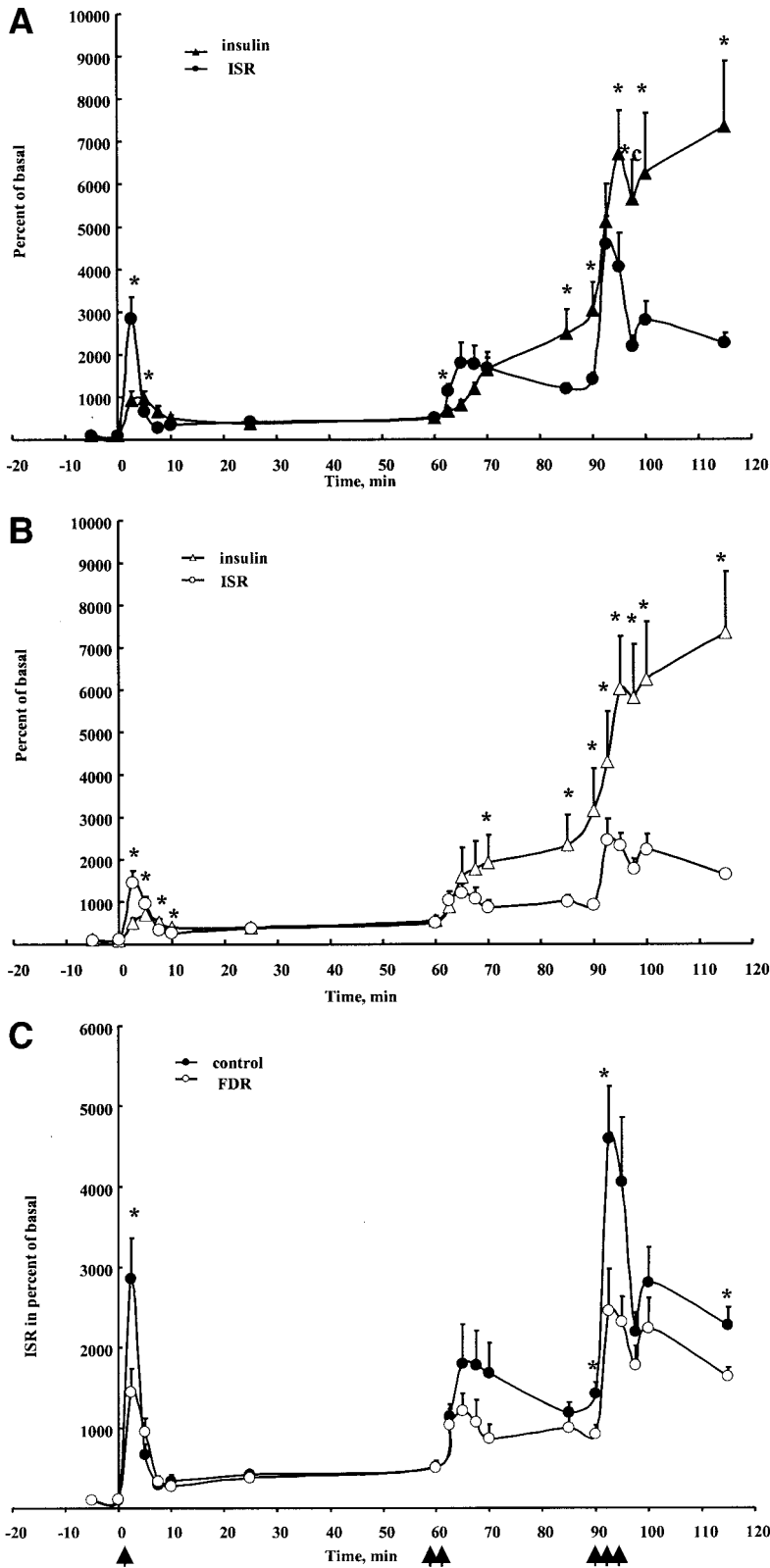


FIG. 2. *A* and *B*: Peripheral insulin concentration and ISR as percentages of their respective mean basal values, in response to three different secretagogues in the hyperglycemic clamps (means  $\pm$  SE). *A*: Control group. *B*: FDRs. *C*: ISR in percent of basal: control subjects versus FDRs. Arrows indicate the initiation of glucose, GIP, and arginine infusions. \* $P < 0.05$ .

The effects of three secretagogues were additive and caused an 80-fold increase of insulin concentrations by 115 min of the clamp in both groups. Based on the results presented in Fig. 1, no general impairment of insulin and C-peptide response was demonstrated in our group of FDRs under the conditions studied. Our data are at variance with the previous observation that the GIP effect on insulin

secretion was lower by 50% in FDRs (5). Differences in subjects (younger age, lower insulin sensitivity) and experimental design (higher glucose concentrations during the clamps) can account for this apparent discrepancy. Another study of normal glucose tolerant FDRs using hyperglycemia, exogenous glucagon-like peptide (GLP)-1, and arginine showed a decreased first-phase and de-

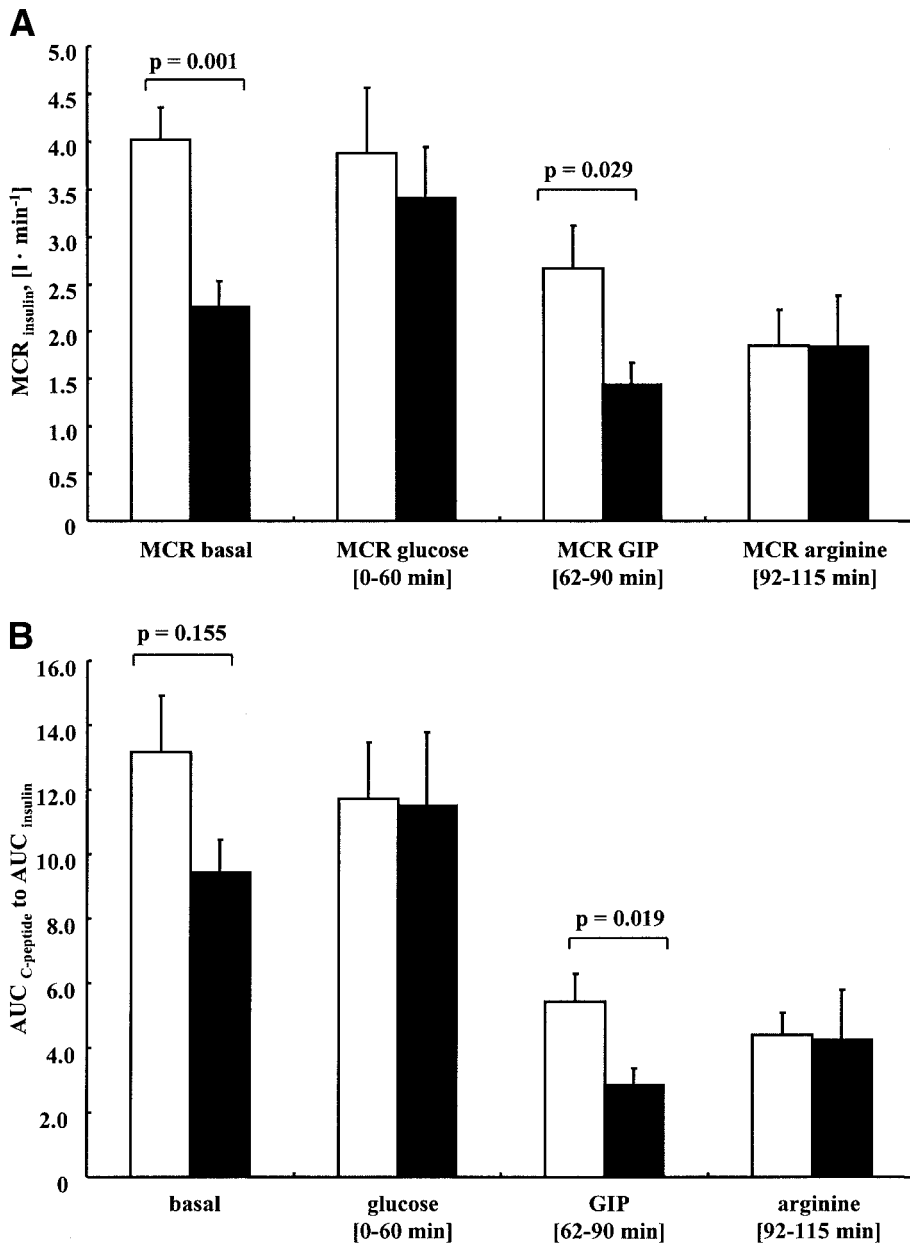


FIG. 3. Metabolic clearance rate of endogenous insulin ( $MCR_{insulin}$ ) (A) and ratio of  $AUC_{C-peptide}$  to  $AUC_{insulin}$  as an indirect index of the change in insulin extraction (B) at each period of the hyperglycemic clamp. ■, present FDRs; □, control subjects.

creased acute secretory phase in response to GLP-1 as the most impaired phase of insulin secretion in this group (6). In contrast to this study, we did not observe a specific secretory impairment in response to intravenous glucose bolus and GIP infusion.

In addition to a simple quantification of insulin secretion as such, we made an effort to elaborate the reserve of  $\beta$ -cell secretion. Insulin secretion in the FDRs expressed as the percentage of the basal ISR clearly revealed a relative and global impairment of insulin secretion in response to glucose, GIP, and arginine (Fig. 2C). It is important to point out that this relative and global impairment of insulin secretion is not detectable by direct measurement of peripheral insulin concentrations in hyperglycemic clamps (Fig. 1A). Thus, our data clearly show that for accurate characterization of insulin secretion, three variables need to be analyzed: insulin sensitivity, insulin secretion, and insulin clearance.

We observed hyperinsulinemia and a reduced insulin

clearance under fasting conditions in our participants of Caucasian origin. Moreover, a significant negative correlation between the IR index ( $HOMA_{IR}$ ) and insulin clearance was detected. Fasting hyperinsulinemia and abnormalities in insulin clearance commonly coexist in ethnic groups with severe IR (12,14). Thus, ethnicity may be a major determinant of the mechanism of peripheral hyperinsulinemia and insulin insensitivity in these populations. Compared with previously published data of these populations, our FDRs were not hyperinsulinemic and have only a borderline impairment in insulin sensitivity. We can speculate that decreased insulin clearance in FDRs observed in our study possibly represents a further adaptive response in addition to increased insulin secretion to compensate for decreased insulin sensitivity. On the other hand, a recent genetic study demonstrated that polymorphisms in the insulin-degrading enzyme gene are associated with type 2 diabetes (18). Thus, impaired hepatic insulin extrac-

tion may be a primary pathophysiological abnormality predisposing to the development of type 2 diabetes.

An important and novel finding of our study was the reduction of insulin clearance under GIP infusion in the group of FDRs (Fig. 3). This decrease in insulin clearance allowed maintaining normal, even increased, peripheral insulin concentrations despite a reduced insulin secretion capacity. This specific effect in GIP action applies to conditions of increased  $\beta$ -cell demand, as occurs in postprandial situations. However, FDRs did not show a general impairment in insulin extraction, since the clearance of insulin was not different compared with control subjects in the presence of glucose, GIP, and arginine (92–115 min of the clamp).

The liver is the primary site of insulin clearance. Approximately 50% of portal insulin is removed during first-pass transit, but this percentage varies by both physiological and pathophysiological factors (38). Stimulation of endogenous insulin secretion is associated with a reduction in insulin clearance at peripheral insulin concentrations in the high physiological range of 366–488 pmol/l (60–70 mU/l) (28). The correspondent increasing of peripheral insulin concentrations in our study was observed at 70 min in control subjects and at 62.5 min in FDRs (Fig. 1), but the reduction in insulin clearance seen here occurred in control subjects at 85 min and at 70 min in FDRs (Fig. 2). Thus, the reduction of insulin clearance in our study does not only depend on stimulation of insulin secretion. In general, nutrient intake and glucose ingestion increases hepatic insulin extraction, presumably because of signals from the gut (39,40). Also, incretins have been shown to lower hepatic insulin extraction (41). On the other hand, physiological levels of GLP-1 have no independent effect on insulin clearance in high-dose graded glucose infusion tests (42). One other recent observation demonstrated that the reduction in hepatic insulin clearance after oral glucose loading is not mediated by GIP (43) and is at variance with our data.

Some limitations are present in our study.  $HOMA_{IR}$  is not the gold standard in the evaluation of IR; nevertheless, recent studies have demonstrated that  $HOMA_{IR}$  is a better way to estimate IR in comparison with other surrogate indexes (44,45). Another aspect is the method of calculation and its accuracy to determine changes in insulin clearance in our study. A direct assessment of portal concentrations of various hormones is not feasible in human subjects. On the other hand, measurements of peripheral concentration do not always reflect the prehepatic concentration of the hormone. In our study, we measured insulin clearance indirectly in three ways based on previously reported techniques of calculation of insulin clearance: 1) as relationship between changes in ISR and changes in peripheral insulin concentrations during the hyperglycemic clamp, both in respect to basal levels (28,29); 2) as a ratio of  $AUC_{ISR}$  to  $AUC_{insulin}$  for the basal and stimulated state of the hyperglycemic clamp (28,29); and 3) as the molar ratio of  $AUC_{C-peptide}$  to  $AUC_{insulin}$  for the basal and stimulated states of the hyperglycemic clamp (30,31). We observed the reduction of insulin clearance in the basal state and under GIP infusion in FDRs by calculation of insulin clearance using methods 1 and 2. In addition, we observed the reduction of insulin clearance

under GIP infusion using method 3 and saw a borderline significant change in the basal insulin clearance in FDRs.

In conclusion, this study demonstrates the in vivo abnormality in insulin handling in marginally insulin-resistant FDRs during a hyperglycemic clamp with three different secretagogues. FDRs demonstrated relative and global impairment of insulin secretion in response to glucose, GIP, and arginine as a sign of limitation of  $\beta$ -cell secretion capacity. The reduction of insulin clearance under GIP infusion in first-degree relatives in our study was significantly greater than in control subjects and negatively correlated with the degree of insulin resistance. This suggests that decreased insulin clearance in response to GIP may represent a further pathophysiological mechanism involved in the development of type 2 diabetes.

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