# GASTRIC INHIBITORY POLYPEPTIDE DIAGNOSTIC TEST FOR DETECTING SUSCEPTIBILITY TO TYPE-2 DIABETES, IMPAIRED GLUCOSE TOLERANCE, OR IMPAIRED FASTING GLUCOSE

## BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to methods and kits for determining whether an individual is susceptible to developing Type-2 diabetes, impaired glucose tolerance (IGT), or impaired fasting glucose (IFG). These methods and kits are used to measure the response of an individual to administration of gastric inhibitory polypeptide (GIP) or a GIP variant, thereby determining whether the individual is at risk for developing Type-2 diabetes, IGT, or IFG.

Approximately 10.3 million people have been diagnosed with diabetes in the United States and it is estimated that many more have the disease but remain undiagnosed. Type-2 diabetes is believed to account for approximately 90-95% of all diabetes cases. There are currently no reliable diagnostic tests for determining if an individual is susceptible to developing Type-2 diabetes.

IGT is also common in the U.S. population, with an estimated 13.4 million Americans having this condition. IGT describes a condition where an individual has a reduced ability to tolerate glucose administration but the level of impairment is below that of definitive diabetes. Persons with IGT are at an increased risk of developing Type-2 diabetes as well as other diseases. IFG is a condition similar to IGT. IFG is also a prevalent in the U.S. and elsewhere. There are currently no reliable diagnostic tests for determining if an individual is susceptible to developing IGT or IFG.

Despite the lack of reliable diagnostic tests, it has been known for some time that GIP plays a role in the pathology of Type-2 diabetes based on data showing that the insulinotropic effects of GIP are significantly reduced in type-2 diabetes patients (7). The precise role of GIP in the pathology of Type-2 diabetes, however, remains obscure and prior to the present invention a link between GIP and an

individual's susceptibility to developing Type-2 diabetes, IGT, or IFG had not been discovered.

## SUMMARY OF THE INVENTION

The present invention is directed to, for example, methods and kits for determining whether an individual is susceptible to developing impaired glucose tolerance (IGT), impaired fasting glucose (IFG), or Type-2 diabetes. The methods include administering a GIP or GIP variant to an individual, measuring the response of the individual and determining whether the individual is susceptible to developing IGT, Type-2 diabetes, or IFG.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Fig. 1.** Plasma concentrations of glucose (capillary measurement; A) and immunoreactive GIP (B) in 21 first-degree relatives of type-2 diabetic patients (filled diamonds), 10 type-2 diabetic patients (open circles), and 10 healthy control subjects (filled circles) participating in hyperglycemic clamp experiments with intravenous infusions of GIP (2 $pmol \cdot kg^{-1} \cdot min^{-1}$). Mean ± SEM. P values: repeated-measures ANOVA (A: between subject/patient groups; B: with time; AB: interaction of group and time). *: significant difference ($p < 0.05$) to Type-2 diabetic patients; †: significant difference ($p < 0.05$) to normal subjects (Student's t-test)

**Fig. 2.** Plasma concentrations of insulin (A) and C-peptide (B) and insulin secretion rates (C) in 21 first-degree relatives of Type-2 diabetic patients (filled diamonds), 10 Type-2 diabetic patients (open circles), and 10 healthy control subjects (filled circles) participating in hyperglycemic clamp experiments with intravenous infusions of GIP (2 $pmol \cdot kg^{-1} \cdot min^{-1}$). Mean ± SEM. P values: repeated-measures ANOVA (A: between subject/patient groups; B: with time; AB: interaction of group and time). *: significant difference ($p < 0.05$) to Type-2 diabetic patients; †: significant difference ($p < 0.05$) to normal subjects (Student's t-test). In the right panels, individual plasma concentrations of insulin (D) and C-peptide (E) and insulin

secretion rates (F) in 21 first-degree relatives are shown in relation to the upper and lower 95 % confidence interval for normal subjects (dashed lines).

**Fig. 3.** Differences (Δ) between the mean values at time points 15 and 30 min ("hyperglycemia") and 75 and 90 min ("hyperglycemia plus exogenous GIP) for insulin (A) and C-peptide (B) concentrations and for insulin secretion rates (C) in 21 first-degree relatives of Type-2 diabetic patients (filled diamonds), 10 Type-2 diabetic patients (open circles), and 10 healthy control subjects (filled circles). P values: ANOVA (overall comparison) and Student's t-test (comparison of individual groups).

**Fig. 4.** Plasma concentrations of glucagon in 21 first-degree relatives of Type-2 diabetic patients (filled diamonds), 10 Type-2 diabetic patients (open circles), and 10 healthy control subjects (filled circles) participating in hyperglycemic clamp experiments with intravenous infusions of GIP (2 $pmol \cdot kg^{-1} \cdot min^{-1}$). Mean ± SEM. P values: repeated-measures ANOVA (A: between subject/patient groups; B: with time; AB: interaction of group and time). *: significant difference ($p < 0.05$) to Type-2 diabetic patients; †: significant difference ($p < 0.05$) to normal subjects (Student's t-test).

**Fig. 5.** Plasma concentrations of proinsulin in 21 first-degree relatives of Type-2 diabetic patients (filled diamonds), 10 Type-2 diabetic patients (open circles), and 10 healthy control subjects (filled circles) participating in hyperglycemic clamp experiments with intravenous infusions of GIP (2 $pmol \cdot kg^{-1} \cdot min^{-1}$). Mean ± SEM. P values: repeated-measures ANOVA (A: between subject/patient groups; B: with time; AB: interaction of group and time). *: significant difference ($p < 0.05$) to Type-2 diabetic patients; †: significant difference ($p < 0.05$) to normal subjects (Student's t-test).

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

"Impaired glucose tolerance," abbreviated herein as "IGT," refers to an intermediate stage between normal (an individual without impaired glucose

metabolism) and definitive or "frank" diabetes. As an illustrative, but non-limiting criterion, using an oral glucose tolerance test, impaired glucose tolerance is defined as a two-hour value between 140 mg per dL (7.8 mmol per L) and 199 mg per dL (11.0 mmol per L).

"Impaired fasting glucose" refers to a condition similar to IGT. As an illustrative, but non-limiting criterion, "impaired fasting glucose" is defined as a fasting plasma glucose concentration between 110 mg per dL (6.1 mmol per L) and 125 mg per dL (6.9 mmol per L).

"Type-2 diabetes" (also referred to as non-insulin-dependent diabetes mellitus or adult-onset diabetes) is a classification used to describe individuals who exhibit insulin resistance and who usually exhibit relative, rather than absolute, insulin deficiency. The precise cause of Type-2 diabetes has not been identified. Illustrative, but non-limiting criteria for determining whether an individual has Type-2 diabetes, include one or more of the following: (1) a confirmed fasting plasma glucose value of greater than or equal to 126 milligrams/deciliter (mg/dL), (2) in the presence of symptoms of diabetes, a confirmed non-fasting plasma glucose value of greater than or equal to 200 mg/dL (3) with an oral glucose tolerance test (by administering 75 grams of anhydrous glucose dissolved in water, in accordance with World Health Organisation standards, and then measuring the plasma glucose concentration 2 hours later), a confirmed glucose value of greater than or equal to 200 mg/dL.

The above illustrative diagnostic criteria for IGT, IFG, and Type-2 diabetes are those proposed in 1997 by the "Expert Committee on the Diagnosis and Classification of Diabetes Mellitus." See Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, Diabetes Care 20:1183 (1997). It is understood, however, that the criteria for diagnosing IGT, IFG, and Type-2 diabetes are set by institutional bodies and may be changed from time to time and may vary from organisation to organisation. Notwithstanding these variations, the terms IGT, IFG, and Type-2 diabetes, as used herein, are intended to be interpreted broadly and to be inclusive of the varying classification criteria used in the art.

"Susceptible to developing IGT, IFG, or Type-2 diabetes" means at risk for developing one or more of these conditions. It is understood that even if a given

individual is determined, via the inventive methods or kits, to be susceptible to developing these diseases, the individual may in fact never develop either condition. It is further understood that the present invention can be used to assess individuals who already exhibit one or more risk factors of developing these diseases. For example, in a preferred embodiment, the inventive methods are applied to an individual who is a first-degree relative of a Type-2 diabetes patient. In this case, the individual is already in a high risk category, from an epidemiological standpoint. However, prior to the present invention, there was no way to predict with more certainty whether a particular individual within a high risk category would develop IGT, IFG, or Type-2 diabetes. The present invention provides such a predictive diagnostic tool.

"Gastric inhibitory polypeptide," (also known as glucose-dependent insulinotropic hormone), abbreviated herein as "GIP," is an insulinotropic hormone synthesised and secreted from upper gut cells, particularly cells in the duodenum and proximal jejunum. GIP is referred to as an incretin because it is responsible, in part, for what is called "the incretin effect," namely the enhancement of insulin secretion following oral but not intravenous glucose administration. In a preferred embodiment, the GIP used is synthetic human GIP, having the following amino acid sequence of human GIP: Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln (SEQ ID NO: 1). However, the present invention includes the use of recombinant human GIP as well as GIP derived from other species, whether recombinant or synthetic. Also included in the present invention are biologically active variants of GIP. "Biologically active," in this context, means having GIP biological activity, but it is understood that the activity of the variant can be either less potent or more potent than native GIP. GIP biological activity can be determining by *in vitro* and *in vivo* animal models and human studies as is well known to the skilled artisan. Included as GIP variants are any molecules, whether they be peptides, peptide mimetics, or other molecules that bind to or activate the GIP receptor and its second messenger cascade. The GIP receptor has been characterised in the art. See, for example, Gremlich et al., Diabetes 44: 1202 (1995). Methods of determining whether a chemical or peptide binds to or activates a GIP receptor are known to the skilled

artisan and are preferably carried out with the aid of combinatorial chemical libraries and high throughput screening techniques. Also included in the present invention are polynucleotides that express GIP or GIP variants as defined herein.

Also included in the present invention are GIP peptides containing one or more amino acid substitutions, additions or deletions. In one embodiment the number of substitutions, deletions, or additions is 30 amino acids or less, 25 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less or any integer in between these amounts. In one aspect of the invention, the substitutions include one or more conservative substitutions. A "conservative" substitution denotes the replacement of an amino acid residue by another, biologically active similar residue. Examples of conservative substitution include the substitution of one hydrophobic residue, such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The following table lists illustrative, but non-limiting, conservative amino acid substitutions.

| ORIGINAL RESIDUE | EXEMPLARY SUBSTITUTIONS |
|---|---|
| ALA | SER, THR |
| ARG | LYS |
| ASN | HIS, SER |
| ASP | GLU, ASN |
| CYS | SER |
| GLN | ASN, HIS |
| GLU | ASP, GLU |
| GLY | ALA, SER |
| HIS | ASN, GLN |
| ILE | LEU, VAL, THR |
| LEU | ILE, VAL |
| LYS | ARG, GLN, GLU, THR |

| | |
|---|---|
| MET | LEU, ILE, VAL |
| PHE | LEU, TYR |
| SER | THR, ALA, ASN |
| THR | SER, ALA |
| TRP | ARG, SER |
| TYR | PHE |
| VAL | ILE, LEU, ALA |
| PRO | ALA |

An embodiment of a GIP variant is a polypeptide comprising amino acids 19-30 of SEQ ID NO: 1 (see Morrow et al., Canada J. Physiol Pharmacol. 74:65 (1996)), where 1-5 or more amino acid substitutions, deletions, or additions can be made within this 19-30 amino acid sequence. It is further understood that GIP variants include the above described peptides which have been chemically derivatized or altered, for example, peptides with non-natural amino acid residues (e.g., taurine residue, beta and gamma amino acid residues and D-amino acid residues), C-terminal functional group modifications such as amides, esters, and C-terminal ketone modifications and N-terminal functional group modifications such as acylated amines, Schiff bases, or cyclization, such as found for example in the amino acid pyroglutamic acid.

Also included in the present invention are peptide sequences having greater than 50 percent sequence identity, and preferably greater than 90 percent sequence identity to (1) SEQ ID NO: 1, (2) to truncated sequences thereof; and (3) to amino acids 19-30 of SEQ ID NO: 1. As used herein, sequence identity refers to a comparison made between two molecules using standard algorithms well known in the art. The preferred algorithm for calculating sequence identity for the present invention is the Smith-Waterman algorithm, where SEQ ID NO: 1 is used as the reference sequence to define the percentage identity of polynucleotide homologs over its length. The choice of parameter values for matches, mismatches, and inserts or deletions is arbitrary, although some parameter values have been found to yield more biologically realistic results than others. One preferred set of parameter values for the Smith-

Waterman algorithm is set forth in the "maximum similarity segment" approach, which uses values of 1 for a matched residue and -1/3 for a mismatched residue (a residue being a either a single nucleotide or single amino acid) (Waterman, Bulletin of Mathematical Biology 46:473-500 (1984)). Insertions and deletions (indels), x, are weighted as

$$X_k = 1 + k/3,$$

where k is the number of residues in a given insert or deletion (Id.).

For instance, a sequence that is identical to the 42 amino acid residue sequence of SEQ ID NO: 1, except for 18 amino acid substitutions and an insertion of 3 amino acids, would have a percent identity given by:

[(1 x 42 matches) - (1/3 x 18 mismatches)

- (1 + 3/3 indels)] /42 = 81% identity

"Exhibits at least one indicia of susceptibility for developing Type-2 diabetes, IFG, or IGT" refers to epidemiological risk factors for developing one or both of these diseases. Such risk factors include, but are not limited to, older age, particularly, 45 years or older, obesity, family history of diabetes, particularly, individuals who are first-degree relatives of one or more Type-2 diabetes patients; genotype, prior history of gestational diabetes, physical inactivity, and race/ethnicity (African Americans, Hispanic/Latino Americans, American Indians, and some Asian Americans and Pacific Islanders are at particularly high risk for developing these diseases). Moreover, IGT and IFG are themselves risk factors for developing Type-2 diabetes.

"Polypeptide" as used herein refers to two or more amino acids joined by one or more peptide bonds.

"Pharmaceutically acceptable carrier or excipient" includes, for example, saline, buffered saline, dextrose, water, glycerol, ethanol, lactose, phosphate, mannitol, arginine, trehalose and combinations thereof and further includes agents which enhance the half-life in vivo of GIP, or a biologically active variant thereof, in order to enhance or prolong the biological activity of the peptide or variant. For example, a molecule or chemical moiety may be covalently linked to GIP, or a

biologically active variant thereof; or the enhancing agent can be administered simultaneously with GIP, or a biologically active variant thereof.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to methods and kits using GIP or GIP variant to determine whether an individual is susceptible to developing Type-2 diabetes, IGT, or IFG.

The present invention includes A method for determining whether an individual is susceptible to developing impaired glucose tolerance (IGT), impaired fasting glucose (IFG), or Type-2 diabetes, comprising: (i) administering to at least one individual at least one polypeptide selected from the group consisting of a gastric inhibitory polypeptide (GIP) and a biologically active GIP variant, or any combination thereof; wherein the polypeptide is optionally combined with a pharmaceutically acceptable carrier or excipient; (ii) assessing the response of the individual to the administration; (iii) comparing the response to a constant; and (iv) determining from the comparison whether the individual is susceptible to developing IGT, IFG, or Type-2 diabetes.

It is understood that the above method can be applied to test any individual, even if the individual is not considered to fall within a risk category for developing Type-2 diabetes, IGT, or IFG. The above method can also be applied more selectively to those who are at risk for developing these diseases, namely individuals exhibiting at least one indicia of susceptibility for developing Type-2 diabetes, IGT, or IFG. Thus, the present invention includes applying the above method to, for example, a blood relative of at least one individual who has Type-2 diabetes, IGT, or IFG; an individual who is 45 years of age or older; an individual who is obese; or an individual who already has IGT or IFG (in the context of diagnosing risk for developing Type-2 diabetes).

The above method can be used with or without the further step of administering a nutrient. In a simple and commercially advantageous form, the above method simply involves the administration of GIP or GIP variant in a single dose and

then blood is drawn and a response to the GIP or GIP variant is assessed. There is no need to administer a nutrient in this embodiment.

In a further embodiment, where nutrient is administered, in a simple and commercially advantageous form, the nutrient can be in the form of a meal, akin to a glucose tolerance test or can be administered intravenously or otherwise. If administered, the nutrient is preferably administered prior to the assessing step. When nutrient is administered, the preferred nutrient is glucose. However, other nutrients can be used including carbohydrates, amino acids, lipids, monoglycerides, diglycerides, triglycerides, fatty acids, or any combination thereof. Carbohydrate nutrients useful in the present method include hexoses or pentoses, specific examples of which are glucose, as mentioned above, dextrose, fructose, galactose, xylitol, mannitol, and sorbitol or any combination thereof. Nutrients also include nutrient derivatives, such as pyruvate and lactate, which are carbohydrate derivatives. Nutrients also include intermediates of nutrient metabolic pathways. For example, pyruvic acid is an intermediate of carbohydrate metabolism.

In the above method, the preferred route of administration of the GIP or GIP variant and nutrient is by intravenous infusion, at the same time or at different times. However, it is understood that the GIP or GIP variant and nutrient can be administered orally, enterally, parenterally, or otherwise as would understood to the skilled artisan.

In the above method, the preferred method of assessing the response of an individual to GIP or GIP variant alone or with nutrient is to assess plasma levels of insulin, C-peptide, glucose, or levels of insulin secretion, insulin secretion rate, or any combination thereof. As used herein, "insulin response" denotes the insulin level in an individual to whom a GIP or GIP variant has been administered. As used herein, "C-peptide response" denotes the C-peptide level in an individual to whom a GIP or GIP variant has been administered. As used herein, "glucose response" denotes the glucose level in an individual to whom a GIP or GIP variant has been administered. This assessment is made optionally, before, after, or during the administration step. In one embodiment, one or more of these levels are assessed before administration and then after administration in order to assess the difference in the levels, which indicates the

response to the administration. Methods of assessing these levels, either in the plasma or elsewhere, are known to the skilled artisan. See, for example, Insulin: Andersen, et al., "Enzyme immunoassay of intact insulin in serum and plasma," Clinical Chemistry 39: 578-582 (1993) (insulin levels); Heding, L. G., "Specific and direct radioimmunoassay for human C-peptide in serum," Diabetologia 11: 547-548 (1975) (C-peptide); Kjems, et al., "Validation of methods for measurement of insulin secretion in humans in vivo," Diabetes 49: 580-588 (2000) (insulin secretion rate). In a preferred commercially advantageous embodiment, insulin levels are assessed. This is commercially advantageous because radioimmuno and ELISA tests for assessing peripheral blood insulin levels are widely available and inexpensive.

The plasma levels are assessed after the administration of the GIP or GIP variant, preferably within 5-60 minutes after and more preferably within about 15 minutes after administration. In a preferred embodiment, a catheter is placed in the individual and the GIP or GIP variant is administered through the catheter, and then blood is drawn through the same catheter, the catheter being suitable flushed, optionally, in between.

In the above method, the response to the administration is compared to a "constant." The constant is a value which is used to determine whether the response to the administration described above is significantly different from the. response of a normal individual, i.e., an individual who has a normal, non-reduced, GIP response. In a simple form, the constant can be the arithmetic mean of the response of a population of individuals that do not have reduced levels of c-peptide or insulin or increased levels of glucose after administration of GIP or GIP variant and glucose. The constant can of course be more complex in a mathematical sense as would be understood to the skilled artisan. The constant can also be more complex in the sense that it can based on data from particular sub-populations of individuals, where the sub-populations are selected to provide a more accurate control for the test individual or individuals. In a preferred embodiment, the constant is predetermined, meaning that the constant has been calculated prior to the administration of the above described method.

In the above method, the "determining step" involves a comparison of the response of the tested individual or individuals to the constant and a determination, based on the comparison, of whether the tested individual or individuals is susceptible to developing IGT, IFG, or Type-2 diabetes. In a simple form, the mathematical difference between the response of the tested individual or individuals and the constant is calculated and this amount is compared to a second constant. The second constant represents a number or range of numbers, wherein, if the above mathematical difference corresponds to the number or falls within the rage of numbers of the second constant, the tested individual or individuals is determined to be susceptible to developing IGT, IFG, or Type-2 diabetes. In a preferred embodiment, the second constant is predetermined, meaning that the second constant has been calculated prior to the administration of the above described method.

In a preferred embodiment, a determining means is used to carry out the determining step. Examples of determining means include a computer which is programmed to make the above described calculations or a table, chart, or other similar device which can be used to quickly select the appropriate first and second constants or to make the above described calculations. Computer calculations can also be made on an internet site or similar venue, which contains suitable database for making the above calculations. In a preferred embodiment, a database is used which is stratified by age, gender, ethnic group, weight, genotype, and other factors, where for each population a constant, including a suitable ranges, can be selected depending on the characteristics of the individual being tested.

The present invention further includes a kit for determining whether an individual is susceptible to developing IGT, IFG, or Type-2 diabetes, comprising at least one polypeptide selected from the group consisting of a gastric inhibitory polypeptide (GIP) and a biologically active GIP variant, or any combination thereof: wherein the polypeptide is optionally combined with a pharmaceutically acceptable carrier or excipient, and at least one means for determining whether the individual is susceptible to developing IGT, IFG, or Type-2 diabetes.

The term "kit" refers to a one or more containers or one or more packaging materials used to house the contents of the kit. Preferably the container or

packaging material provides a sterile, contaminant free environment and preferably the kit contains one or more instructions for directing one on how to use the contents of the kit to carry out the above described methods.

In a further embodiment, the kit includes at least one syringe with GIP or GIP variant, or a combination thereof: (1) in powder form, for example lyophilized, to be reconstituted with, for example, saline, or (2) in a liquid form. The kit can either include or not include a nutrient. In an embodiment where the kit includes a nutrient, the kit includes at least one syringe containing one or more nutrients, preferably glucose, (1) in powder form, for example lyophilized, to be reconstituted with, for example, saline, or (2) in a suitable stable liquid form.

In a further embodiment, an I.V. line is installed in the hand or other location of an individual and, if nutrient is to be administered, a glucose or other nutrient syringe is discharged into the I.V. line and then a GIP or GIP variant syringe is discharged into the line. After a suitable time interval, blood is drawn from the I.V. line to obtain C-peptide, insulin, or glucose levels. The levels of C-peptide, insulin, or glucose are evaluated as described above to determine whether the individual is susceptible to developing IGT, IFG, or Type-2 diabetes.

The kit further includes at least one means for determining whether the individual is susceptible to developing IGT, IFG, or Type-2 diabetes. The means as described above can include a chart or table, or other similar device which can be used to quickly select the appropriate above-described first and second constants or make the above described calculations. The means can also include software or a database for making the calculation or other similar electronic means. The kit can also include a password or other similar code which enables a user to log on to an internet site or other suitable venue as described above to make the calculations described above.

## EXAMPLES

It is understood that the following examples are illustrative and non-limiting.

Study Protocol

The study protocol was approved by the ethics committee of the medical faculty of the Ruhr-University, Bochum, on April, 1998 (registration number 1114) prior to the study. Written informed consent was obtained from all participants.

Subjects

Ten healthy control subjects, ten Type-2 diabetic patients, and 21 first-degree relatives of Type-2 diabetic patients were studied. Subject/patient characteristics are presented in Table 1. The groups were matched for sex, obesity, and age. Non-diabetic participants were subjected to an oral glucose tolerance test (75 g; Boebringer O.G.T., Roche Diagnostics, Mannheim, Germany) with the determination of capillary glucose in the fasting state and 120 min after the ingestion of glucose. 1 subject with diabetes was excluded from the group of relatives. In healthy control subjects, any first- or second-degree relatives with Type-2 diabetes were excluded by history taking.

From all participants, blood was drawn in the fasting state for measurements of standard hematological and clinical chemistry parameters. Spot urine was sampled for the determination of albumin, protein and creatinine by standard methods. Anemia (hemoglobin $<$ 12 g/dl), an elevation in liver enzymes (ALAT, ASAT, AP, $\gamma$-GT) to higher activities than double the respective normal value, or elevated creatinine concentrations ($>$1.5 mg/dl) were excluded. One female first-degree relative had an elevated $\gamma$-GT activity (90 U/l, normal $<$28 U/l), which most likely was caused by cholelithiasis. Body height and weight were determined and waist- and hip-circumference were measured in order to calculate body mass index and the waist-to-hip ratio, respectively (Table 1). Blood pressure was determined according to the Riva-Rocci method.

Five Type-2-diabetic patients had been treated with diet alone, and 5 patients received oral antidiabetic treatment (glibenclamide, 3,5 mg/d, in 1 case, acarbose 150 mg/d in 3 cases, metformin, 1700 mg/d in 1 case). None of the patients had been treated with insulin. In these patients, the usual antidiabetic medication was withdrawn the day before the study.

Study Design

All participants were studied on two or three occasions:

(a) At a screening visit, an oral glucose tolerance test was performed in all subjects with unknown oral glucose tolerance in the fasting state, and laboratory parameters were screened. If subjects met the inclusion criteria, they were recruited for the second test.

(b) A hyperglycemic clamp test aiming at a steady capillary plasma glucose concentration of 7.8 mmol/l (140 mg/dl) was started by injecting 40 % glucose as a bolus and maintained by infusing glucose (20 % in water, weight/vol) as appropriate, based on glucose determinations performed every 5 min. From 30 to 90 min, gastric inhibitory polypeptide (glucose-dependent insulinotropic peptide; GIP) was administered intravenously at an infusion rate of 2.0 pmol $kg^{-1}$ $min^{-1}$.

(c) Six subjects (5 healthy controls and one first-degree relative) participated in a third experiment (hyperglycemic clamp experiment with the administration of placebo instead of GIP)) in order to judge the insulin secretory response to prolonged hyperglycemia alone.

Peptides

Synthetic GIP was purchased from PolyPeptide Laboratories GmbH, Wolfenbüttel, Germany. The lot number (pharmaceutical grade) was: C-0229, net peptide content was 80.3 %. The peptide was dissolved in 0.9 % NaCl/1 % human serum albumin (HSA Behring, salt poor, Marburg, Germany), filtered through 0.2 $\mu$m nitrocellulose filters (Sartorius, Göttingen, Germany) and stored frozen at -28°C as previously described. HPLC profiles (provided by the manufacturer) showed that the preparation was > 99 % pure (single peak coeluting with appropriate standards). Samples were analysed for bacterial growth (standard culture techniques) and for pyrogens (Laboratory Dr. Balfanz, Münster, Germany). No bacterial contamination was detected. Endotoxin concentrations in samples from the GIP stock solution were 1:61 EU/ml.

Experimental Procedures

The tests were performed in the morning after an overnight fast in a supine position throughout the experiments with the upper body lifted by approximately

30°. Two forearm veins were punctured with a teflon cannula (Moskito 123, 18 gauge, Vygon, Aachen, Germany), and kept patent using 0.9 % NaCl (for blood sampling and for glucose and GIP administrations, respectively). Both ear lobes were made hyperemic using Finalgon® (Nonivamid 4 mg/g, Nicoboxil 25 mg/g).

After drawing basal blood specimens at - 15 and 0 min, at 0 min a bolus of 40% glucose (in water weight/vol) was administered to elevate capillary glucose concentrations to 7.8 mmol/l. The dose was based on the fasting plasma glucose concentrations and body weight. Then, an intravenous infusion of glucose 20 % (in water; weight/vol) was started and maintained at a rate that adjusted capillary plasma glucose concentrations to approximately 7.8 mmol/l (Fig. 1 A). 30 min later, an infusion of human synthetic GIP (2.0 pmol $kg^{-1}min^{-1}$ was begun and maintained for 60 min (rate: 20 ml/h, Perfusor secura, Braun Melsungen, Germany; diluted in 0.9 % NaCl with 1 % human serum albumin). At 5 min intervals, plasma glucose was determined in 100 µl capillary samples drawn from an ear lobe. The glucose infusion rates and time points of changing it were recorded in order to allow a calculation of the amount of glucose infused.

Blood Specimens

Blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol®; 20,000 KIU/ml, 200 µl per 10 ml blood; Bayer AG, Leverkusen, Germany and kept on ice. A sample (approximately 100 µl) was stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the immediate measurement of glucose. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at -28°C.

Laboratory Determinations

Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Munich, Germany). Insulin was measured using an insulin microparticle enzyme immunoassay (MEIA), IMx Insulin, Abbott Laboratories, Wiesbaden, Germany. Intra-assay coefficients of variation were ≈ 4 %. C-peptide was measured using C-peptide-antibody-coated microtitre wells (C-peptide MTPL EIA) from DRG Instruments GmbH, Marburg, Germany. Intra-assay coefficients of variation were ≈ 6 %. Human insulin and C-peptide were used as standards.

Proinsulin was measured using a commercially available ELISA (DAKO Diagnostics Ltd., Cambrigeshire, UK). This assay also cross-reacts with split (65,66) proinsulin (100 %), and split (31,32) proinsulin (100%). Detection limit was <0.2 pmol/. Intra-assay coefficient of variation was 3.2 - 5.7 %, inter-assay coefficients of variation were 3.6 - 6.0 %.

IR-GIP was determined as previously described (26), using antiserum R 65 (final dilution 1:150 000) and synthetic human GIP for tracer preparation and as standard. The experimental detection limit was < 1 pmol/l. Antiserum R 65 binds to the midportion of the GIP molecule.

Intra-assay coefficients of variation were ≈ 8%, inter-assay coefficients of variation were < 6%.

IR-Glucagon was measured using porcine antibody 4305 in ethanol-extracted plasma, as previously described (27). The detection limit was < 1 pmol/l. Intra-assay coefficients of variation were 6.7 %, inter-assay coefficients of variation were 16%.

Calculations

Insulin-resistance and B-cell function were calculated according to the HOMA-model (28).

The calculation of the insulin secretion rates was performed by deconvolution analysis (29, 30) using the software "ISEC", version 2.0a, kindly provided by Dr. Roman Hovorka, Centre for Measurement & Information in Medicine, Department of Systems Science, City University, Northampton Square, London, UK (31).

Statistical Analysis

Results are reported as mean ± SEM. All statistical calculations were carried out using repeated-measures analysis of variance (ANOVA) using NCSS Version 5.01 (Jerry Hintze, Kaysville, Utah, USA). If a significant interaction of treatment and time was documented ($p < 0.05$), values at single time points were compared by one-way ANOVA and Student's t-test (paired analyses). A two-sided p-value <0.05 was taken to indicate significant differences.

Results

Comparing hyperglycaemic clamp experiments with and without exogenous GIP in 6 subjects, insulin, C-peptide, and insulin secretion increased to higher concentrations with GIP than with placebo (all $p < 0.0001$). The differences in integrated incremental responses between experiments with and without exogenous GIP correlated with the increments between the mean values at 15 and 30 min (hyperglycemia alone) and at 75 and 90 min (hyperglycemia plus GIP) determined during the experiments with exogenous GIP (insulin: $r^2 = 0.532$, $p = 0.099$; C-peptide: $r^2 = 0.94$, $p = 0.0014$; insulin secretion: $r^2 = 0.898$ , $p = 0.004$). Therefore, it appeared possible to judge the insulinotropic action of GIP based on a single experiment (details not shown).

In comparison to healthy control subjects and first-degree relatives, Type-2 diabetic patients had higher fasting plasma glucose and $HbA_{1c}$ concentrations, but lower HDL cholesterol and creatinine concentrations (Table 1). There were no significant differences in any parameter between healthy control subjects and first degree relatives (Table 1):

Type-2 diabetic patients were hyperglycemic in the basal state (Fig. 1 A). Steady state glucose concentrations did not differ between the groups (Fig. 1 A). During the infusion of GIP, similar steady-state plasma levels were determined in healthy control subjects, first-degree relatives, and Type-2 diabetic patients, respectively (Fig. 1 B).

Basal plasma insulin concentrations were significantly lower in normoglycemic relatives than in hyperglycemic Type-2 diabetic patients (Fig. 2 A). Raising plasma glucose concentrations to 7.8 mmol/l (30 min) (Fig. 2 A) increased plasma insulin to similar values in healthy control subjects, first-degree relatives, and Type-2 diabetic patients, respectively (Fig. 2 A; $p = 0.29$). In response to exogenous GIP, plasma insulin further increased to $39.5 \pm 7.0$, by $26.8 \pm 2.6$, and $21.2 \pm 4.3$ mU/l in healthy control subjects, first-degree relatives, and Type-2 diabetic patients, respectively (Fig. 2 A; $p = 0.031$). The insulinotropic response to GIP, as judged by the $\Delta$ (after minus before GIP), which is a measure of the secretory response to GIP corrected by the response to hyperglycemia alone, was significantly lower in Type-2

diabetic patients in comparison to healthy control subjects, no matter whether based on insulin (Fig. 3 A), C-peptide (Fig. 3 B), or the insulin secretion rates (Fig. 3 C). Δ-values were significantly lower in first-degree-relatives than in Type-2 diabetic patients. Between control subjects and the first-degree-relatives, however, there were significant differences for the insulin secretion rates ($p = 0.022$), but not for Δ insulin ($p = 0.19$) or Δ C-peptide ($p = 0.061$). However, judging individual responses in relation to 95% confidence intervals based on the results in healthy subjects, 7 out of 21 relatives had insulin values totally below the lower normal limits (Fig. 3 D). 11 relatives had C-peptide concentrations totally below the 95 % confidence interval for normal subjects (Fig. 3 E), and 12 out of 21 relatives were characterised by insulin secretion rates below the lower 95 % confidence interval (Fig. 3 F).

When expressing B cell secretory responses as a percentage value of the mean concentrations observed in the control subjects, a reduced activity was present in first-degree relatives in the fasting state (insulin: 75± 8 %, C-peptide: 60± 8 %, insulin secretion: 63 ± 8 %), under hyperglyemic conditions (15/30 min) (insulin: 79 ± 7 %, C-peptide: 55 ± 8 %, insulin secretion 53 ± 7 %), as well as in response to exogenous GIP (75/90 min) (insulin: 77 ± 7 %, C-peptide: 62 ± 6 %, insulin secretion: 65 ± 6 %). After GIP, these numbers were 61 ± 13 % (insulin), 61 ± 11 % (C-peptide) and 50 ± 10 % (insulin secretion; details not shown).

Basal proinsulin concentrations were significantly higher in the Type-2 diabetic patients compared to normal subjects (Fig. 4; $p = 0.049$) and to first-degree-relatives of Type-2-diabetic patients ($p = 0.012$). The difference between normal subjects and the first-degree-relatives was not significant ($p = 0.16$). Expressing proinsulin as its relative proportion of insulin-like immunoreactivity, significantly higher values were found in Type-2 diabetic patients (26 ± 12 % than in the first-degree-relatives (12 ± 5 %, $p = 0.0067$) or healthy subjects (16 ± 8 %).

Glucagon concentrations in the fasting state did not show any significant differences between the groups (Fig. 5; $p = 0.26$). Hyperglycaemia induced a reduction in glucagon concentrations in control subjects and the first-degree-relatives, whereas in Type-2 diabetic patients the values did not change significantly. With

exogenous GIP, glucagon concentrations continued to decline in control subjects and first-degree-relatives, but did not change in Type-2 diabetic patients (Fig. 5).

HOMA analysis (Table 2) revealed no significant differences in B cell secretory function between the groups ($p = 0.27$). Type-2 diabetic patients were more insulin resistant than control subjects ($p = 0.034$) and first-degree-relatives ($p = 0.022$). Between control subjects and first-degree-relatives no significant differences existed in this respect ($p = 0.25$).

Discussion

GIP has lost part of its insulinotropic effect in at least a subgroup of first-degree relatives of Type-2 diabetic patients (Figs. 2 and 3). This is similar to a well-recognised phenotypic abnormality in Type-2 diabetic patients (7, 24, 25). According to the present study, this reduced insulinotropic effect of GIP precedes any clinically relevant disturbance of glucose tolerance, because the first-degree relatives all had a normal or (in one subject) an impaired oral glucose tolerance.

The distribution of insulin secretory responses to the exogenous administration of GIP suggests that approximately 50 % of the first-degree relatives show a normal response, while at least half of them respond very much like Type-2 diabetic patients, i.e. with a markedly reduced insulin secretory response towards GIP (Fig. 3, right panels). This proportion is similar to the percentage of first-degree relatives of Type-2 diabetic patients that ultimately will develop diabetes mellitus themselves (22). The present invention therefore comprehends that a reduced insulinotropic response after GIP is an early marker of a predisposition to develop Type-2 diabetes. The present invention further comprehends that the reduced insulinotropic response after GIP also precedes other metabolic disturbances characteristic for Type-2 diabetes like insulin resistance (32, 33), hyperproinsulinemia (34, 35), and diminished B-cell secretory capacity (15, 18), as none of these factors was present in the first-degree-relatives in the present study. Along these lines, the present invention contemplates that a reduced insulinotropic effectiveness of GIP is an early marker that characterises an abnormality of B cell function which might predispose to Type-2 diabetes.

The cause of the reduced insulinotropic effectiveness of GIP in Type-2 diabetic patients and in the tested first-degree relatives is not known. It could be a specific defect, for example concerning the level of expression of GIP receptors on pancreatic B cells in Type-2 diabetic patients (14). One possibility is mutations in the GIP receptor leading to an impaired interaction with its ligand, GIP, or a reduced expression of the GIP receptor due to reduced mRNA transcription, translation, or posttranslational modifications affecting its biological activity. Polymorphisms in the GIP receptor coding (36) or promoter region in humans, however, have not been found associated with Type-2 diabetes. It is not very likely that other components of the GIP signal transduction pathway are defective, because even in Type-2 diabetic patients, GLP-1 still is very effective in augmenting insulin secretory responses (3, 7, 37, 38). GIP and GLP-1 share most of the components of intracellular signal transduction apart from their receptor molecules, which are different and do not crossreact with the other ligand, respectively (39-45). This also would point to a GIP-specific rather than a general impairment of B cell function in Type-2 diabetic patients and, with all likelihood, also in their first-degree relatives.

The present invention further comprehends that the impairment in GIP function found in the present study is one of several aspects of reduced B cell function in more general terms, including a reduced responsiveness to glucose, arginine and possibly other secretagogues (34, 46, 47). Such a reduced B cell function has also been found in first-degree relatives of Type-2 diabetic patients with different stimuli (18, 20, 48,49). The reduction in measures of B cell secretory parameters relative to normal subjects also in the fasting state and under hyperglycemic conditions found in the present examination could be interpreted in favour of this hypothesis. Another mechanism contemplated by the present invention is that GIP and glucose might act in a synergistic way in stimulating B-cells in the fasting state as well as under hyperglycemic conditions. Holz et al recently showed 100 pmol/l of the other incretin hormone, GLP-1, to be necessary to make B cells responsive to glucose (50). They named this phenomenon induction of "glucose competence". However, fasting concentrations of 30-100 pmol/l are more typical for GIP (51) (7) than for GLP-1, where concentrations of approximately 2-10 pmol/l are typically measured in fasting

humans (3, 52-54). But antagonising GLP-1 effects using exendin [9-36 amide] in the basal state increased glucagon, pointing to an effect on islets at these low, fasting concentrations (55). Considering the almost equivalent dose-response relationships for both incretin hormones in the perfused pancreas (56), it may be hypothesised that basal GIP is necessary for the induction of "glucose competence" as well. Therefore, the reduced effect of hyperglycemia (under clamp conditions, Fig. 2) on insulin secretion in first-degree relatives may be viewed as the consequence of reduced GIP activity in these subjects.

Insulin resistance is another phenotypic peculiarity of Type-2 diabetic patients, and it has also been recognised in first-degree relatives (16-21, 49). Since it is generally accepted that both secretion defects and a reduced insulin sensitivity have to be present in order to explain all facets of Type-2 diabetes, it was of interest whether our first-degree relatives display features of both insulin resistance and impaired insulin secretion. Using HOMA analysis may have limitations (60), but as far as it can be said, insulin resistance (as determined by the HOMA model) was not a characteristic feature of the same subjects that displayed a reduced insulinotropic effectiveness of exogenous GIP. Therefore, we have. characterised a reduction in B cell secretory function and not the combined occurrence of B cell dysfunction and insulin resistance.

In conclusion, we have demonstrated a reduced insulinotropic effectiveness of GIP in normal glucose-tolerant first-degree relatives of Type-2 diabetic patients in comparison to healthy control subjects. This is a new phenotypic abnormality in such subjects, which may be genetically determined.

**References**

1. Creutzfeldt, W. 1979. The incretin concept today. Diabetologia 16: 75-85.

2. Creutzfeldt, W. and Nauck, M. 1992. Gut hormones and diabetes mellitus. Diabetes/Metab Rev 8: 149-177.

3. Nauck, M,A., Bartels, E., Ørskov, C., Ebert, R. and Creutzfeldt, W. 1993. Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-l-(7-36) amide infused at near-

physiological insulinotropic hormone and glucose concentrations. J Clin Endocrinol Metab 76: 912-917.

4. Kreymann, B., Williams, G., Ghatei, M.A. and Bloom, S.R. 1987. Glucagon-like peptide-l [7-36]: a physiological incretin in man. Lancet 2: 1300-1304.

5. Nauck, M.A., Homberger, E., Siegel, E.G., Allen, R.C., Eaton, R.P., et al. 1986. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 63: 492-498.

6. Tillil, H., Shapiro, E.T., Miller, A., Karrison, T., Frank, B.H., et al. 1988. Dose-dependent effects of oral and intravenous glucose on insulin secretion and clearance in normal humans. Am J Physiol (Endocrinol Metab) 254: E349-357.

7. Nauck, M.A., Heimesaat, M.M., Ørskov, C., Holst, J.J., Ebert, R., et al. 1993. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with Type-2 diabetes mellitus. J Clin Invest 91: 301-307.

8. Nauck, M.A., Holst, J.J., Willms, B. and Schmiegel, W. 1997 a. Glucagon-like peptide 1 (GLP-1) as a new therapeutic approach for Type-2-diabetes. Exp Clin Endocrinol Diabetes 105: 187-195.

9. Holst, J.J. 1999. Glucagon-like peptide 1 (GLP-1): an intestinal hormone, signalling nutritional abundance, with an unusual therapeutic potential. TEM 10: 229-235.

10. Göke, R., Trautmann, M.E., Haus, E., Richter, G., Fehmann, H.C., et al. 1989. Signal transmission after GLP-1 (7-36) amide binding in RINm5F cells. Am J Physiol 257: G397-401.

11. Gallwitz, B., Witt, M., Morys-Wortmann, C., Fölsch, U.R. and Schmidt, W.E. 1996. GLP-1/GIP chimeric peptides define the structural requirements for specific ligand-receptor interaction of GLP-1. Regul Pept 63: 17-22.

12. Gromada, J., Holst, J.J. and Rorsman, P. 1998. Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. Pflügers Arch - Eur J Physiol 435: 583-594.

13. Nauck, M., Stöckmann, F., Ebert, R. and Creutzfledt, W. 1986. Reduced incretin effect in Type-2 (non-insulin-dependent) diabetes. Diabetologia 29: 46-54.

14. Holst, J.J., Gromada, J. and Nauck, M.A. 1997. The pathogenesis of niddm involvers a defective expression of the GIP receptor. Diabetologia 40: 984-986:

15. O'Rahilly, S.P., Rudenski, A.S., Burnett, M.A., Nugent, Z., Hosker, J.P., et al. 1986. Beta-cell dysfunction, rather than insulin insensitivity, is the primary defect in familial type-2 diabetes. Lancet 16: 360-364.

16. Haffner, S., Stern, M., Hazuda, H., Mitchell, B. and Patterson, J. 1988. Increased insulin concentrations in nondiabetic offspring of diabetic parents. N Engl J Med 319: 1297-1301.

17. Laws, A., Stefanick, M. and Reaven, G. 1989. Insulin resistance and hypertriglyceridemia in nondiabetic relatives of patients with noninsulin-dependent Diabetes Mellitus. J Clin Endocrinol Metab 69: 343-347.

18. Eriksson, J., Franssila-Kallunki, A., Ekstrand, A., Saloranta, C., Widen, E., et al. 1989. Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. N Engl J Med 321: 337-343.

19. Humphriss, D.B., Stewart, M.W., Berrish, T.S., Barripcanal, L.A., Trajano, L.R., et al. 1997. Multiple metabolic abnormalities in normal glucose tolerant relatives of niddm families. Diabetologia 40: 1185-1190.

20. Vauhkonen, I., Niskanen, L., Vanninen, E., Kainlainen, S., Uusitupa, M., et al. 1998. Defects in insulin secretion and insulin action in non-insulin-dependent Diabetes Mellitus are inherited. Metabolic studies on offspring of diabetic probands. J Clin Invest 101: 86-96.

21. Ishikawa, M., Pruneda, M.L., Adams-Huet, B. and Raskin, P. 1998. Obesity-independent hyperinsulinemia in nondiabetic first-degree relatives of individuals with type-2 diabetes. Diabetes 47: 78-792.

22. Köbberling, J., Tillil, H. and Lorenz, H.-J. 1985. Genetics of type-2A- and type-2B- Diabetes Mellitus. Diabetes Res Clin Pract 1(suppl 1): 311.

23. Leahy, J.L., Bonner-Weir, S. and Weir, S. and Weir, G.C. 1992. β-cell dysfunction induced by chronic hyperglycemia. Current ideas on mechanism of impaired glucose-induced insulin secretion. Diabetes Care 15: 442-455.

24. Jones, I.R., Owens, D.R., Moody, A.J., Luzio, S.D., Morris, T., et al. 1987. The effects of glucose-dependent insulinotropic polypeptide infused at physiological concentrations in normal subjects and Type-2 (non-insulin-dependent) diabetic patients on glucose tolerance and B-cell secretion. Diabetologia 30: 707-712.

25. Krarup, T., Saurbrey, N., Moody, A.J., Kühl, C. and Madsbad, S. 1988. Effect of porcine gastric inhibitory polypeptide on β-cell function in Type I and Type II diabetes mellitus. Metabolism 36: 677-682.

26. Krarup, T., Madsbad, S., Moody, A.J., Regeur, L., Faber, O.K., et al. 1983. Diminished gastric inhibitory polypeptide (GIP) response to meal in newly diagnosed Type 1 (insulin dependent) diabetics. J Clin Endocrinol Metab 56: 1306-1312.

27. Holst, J.J. 1982. Evidence that peak II GLI or enteroglucagon is identical to the C-terminal sequence (residues 33-69) of glicentin. Biochem J207: 381-388.

28. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., et al. 1985. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419.

29. Eaton, R.P., Allen, R.C., Schade, D.S., Erickson, K.M. and Standefer, J. 1980. Prehepatic insulin production in man: kinetic analysis using peripheral C-peptide behaviour. J Clin Endocrinol Metab 51:

30. Polonsky, K.S., Licinio-Paixao, J., Given, B.D., Pugh, W., Rue, P., et al. 1986. Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type 1 diabetic patients. J Clin Invest 77: 98-105.

31. Hovorka, R. and Jones, R.H. 1994. How to measure insulin secretion. Diabetes Metab Rev 10: 91-117.

32. Olefski, J.M. 1981. Insulin resistance and insulin action. An in vitro and in vivo perspective. Diabetes 30: 148-162.

33. DeFronzo, R.A., Gunnarsson, K, Björkman, 0., Olsson, M. and Wahren, J. 1985. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (Type-2) diabetes mellitus. J Clin Invest 76: 149-155.

34. Porte, D. and Kahn, S.E. 1989. Hyperproinsulinemia and amyloid in niddm: Clues to etiology of islet ß-cell dysfunction. Diabetes 38: 1333-1336.

35. Kahn, S.E. and Halban, P.A. 1997. Release of incompletely processed proinsulin is the cause of the disproporttionata proinsulinemia of niddm. Diabetes 46: 1725-1732.

36. Kubota, A., Yamada, Y., Hayami, T. and et al. 1996. Identification of two missense mutations in the GIP receptor gene: A functional study and association analysis with niddm. Diabetes 45: 1701-1705.

37. Nathan, D.M., Schreiber, E., Fogel, H., Mojsov, S. and Habener, J.F. 1992. Insulinotropic action of glucagon-like peptide 1 (7-37) in diabetic and non-diabetic subjects. Diabetes Care 15: 270-276.

38. Nauck, M.A., Kleine, N., Ørskov, C., Holst, J.J., Willms, B., et al. 1993. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type-2 (non-insulin-dependent) diabetic patients. Diabetologia 36: 741-744.

39. Göke, R. and Conlon, J.M. 1988. Receptors for glucagon-like peptide-1 (7-36) amide on rat insulinoma-derived cells. J Endocrinol 116: 357-362.

40. Thorens, B., Porret, A., Buhler, L., Deng, S.P., Morel, P., et al. 1993. Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. Diabetes 42: 1678-1682.

41. Gallwitz, B., Witt, M, Paetzold, G., Morys-Wortmann, C., Fölsch, U.R., et al. 1995. Binding characteristics of N-terminal GIP/GLP-1 hybrid peptides. Endocrinology and Metabolism 2: 39-46.

42. Gremlicli, S., Porret, A., Hani, E.H., Cherif, D., Vionnet, N., et al. 1995. Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. Diabetes 44: 1202-1208.

43. Volz, A., Göke, R., Lankat Buttgereit, B., Fehmann, H.C., Bode, H.P., et al. 1995. Molecular cloning, functional expression, and signal transduction of the GIP-receptor cloned from a human insulinoma. FEBS Lett 373: 23-29.

44. Gromada, J., Dissing, S., Bokvist, K., Renström, E., Froekjaer-Jensen, J., et al. 1995. Glucagon-like peptide I increases cytoplasmic cacium in insulin-secreting ßTC3-cells by enhancement of intracellular calcium mobilization. Diabetes 44:

45. Ding, W.-G. and Gromada, J. 1997. Protein kinase A-dependent stimulation of exocytosis in mouse pancreatic ß-cells by glucose-dependent insulinotropic polypeptide. Diabetes 46: 615-621.

46. Faber, O.K. and Binder, C. 1977. C-peptide response to glucagon. A test for the residual beta-cell function in diabetes mellitus. Diabetes 26: 605-661.

47. Ward, W.K., Bolgiano, D.C., McKnight, B., Halter, J.B. and Porte, D. 1984. Diminished B cell secretory capacity in patients with non-insulin-dependent diabetes mellitus. J Clin Invest 74: 1318-1328.

48. O'Rahilly, S., Turner, R.C. and Matthews, D.R. 1988. Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. N Engl J Med 318: 1225-1230.

49. Elbein, S., Hasstedt, S., Wegner, K. and Kahn, S. 1999. Heritbility of pancreatic B-cell function among nondiabetic members of caucasian familial type-2 diabetic kindreds. J Clin Endocrinol Metab 84: 1398-1403.

50. Holz, G.G., Kuhtreiber, W.M. and Habener, J.F. 1993. Pancreatic beta-cells are rendered glucose-competent by the unsulinotropic hormone glucagon-like peptide-1(7-37). Nature 361: 362-365.

51. Krarup, T. 1988. Immunoreactive gastric inhibitory polypeptide. Endocrin Reviews 9: 122-133.

52. Ørskov, C., Rabenh¢j, L., Wettergren, A., Kofod, H. and J.J., H. 1994. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide 1 in humans. Diabetes 43: 535-539.

53. Willms, B., Werner, J., Holst, J.J., Ørskov, C., Creutzfeldt, W., et al. 1996. Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in type-2 (nonunsulin-dependent) diabetic patients. J Clin Endocrinol Metab 81: 327-332.

54. Schirra, J., Katschinski, M., Weidmann, C., Schafer, T., Wank, U., et al. 1996. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 97: 92-103.

55. Schirra, J., Sturm, K., Leicht, P., Arnold, R, Göke, B., et al. 1998. Exendin (9-39)amide is an antagonist of glucagon-like peptide-1 (7-36)amide in humans. J Clin Invest 101: 1421-1430.

56. Jia, X., Brown, J.C., Ma, P., Pederson, R.A. and McIntosh, C.H. 1995. Effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-I-(7-36) on insulin secretion. Am J Physiol 268: E645-651.

57. Tseng, C.-C., Kieffer, T.J., Jarboe, L.A., Usdin, T.B. and Wolfe, M.M. 1996. Postprandial stimulation of insulin release by glucose-dependent insulinotropic peptide (GIP). Effect of a specific glucose-dependent insulinotropic polypeptide receptor antagonist in the rat. J Clin Invest 98: 2440-2445.

58. Elrick, H., Stimmler, L., Hlad, C.J. and Arai, Y. 1964. Plasma insulin response to oral and intravenous glucose administration. J Chin Endocrinol Metab 24: 1076-1082.

59. Nyholm, B., Walker, M., Gravholt, C.H., Shearing, PA., Sturis, J., et al. 1999. 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. Diabetologia 42: 1314-1323.

60. Hermans, M.P., Levy, J.C., Morris, R.J. and Turner, R.C. 1999. Comparison of tests of B-cell function across a range of glucose tolerance from normal to diabetes. Diabetes 48: 1779-1786.

**Table 1.** Characteristics of the subjects and patients participating in hyperglycaemic clamp experiments with GIP infusion

| Parameter [unit] | Healthy Control Subjects | First-degree relatives of Type 2 diabetic patients | Type 2 diabetic patients | Significance (p-value)[a] |
|---|---|---|---|---|
| Gender (female/male) | 4/6 | 15/6 | 3/7 | 0.059 |
| Age [years] | 49 ± 17 | 49 ± 12 | 52 ± 9 | 0.83 |
| Body-mass-index [kg/m$^2$] | 25.7 ± 3.6 | 26.0 ± 4.2* | 28.6 ± 5.1 | 0.23 |
| Waist-hip ratio [cm/cm] | 0.89 ± 0.1 | 0.84 ± 0.1 | 0.93 ± 0.07 | 0.028 |
| Subjects with first-degree-relatives (Type 2 diabetes) | 0/10* | 21/21†* | 4/10† | <0.0001 |
| Father | - | 8 | 2 | |
| Mother | - | 15 | 2 | |
| Siblings | - | 3 | 3 | |
| $HbA_{1c}$ [%][c] | 5.0 ± 0.5* | 5.1 ± 0.3* | 6.2 ± 0.7† | <0.0001 |

Continued on next page



SUBSTITUTE SHEET (RULE 26)

Table 1 (continued):

| | | | | |
|---|---|---|---|---|
| Oral glucose tolerance | | | | |
| | | | | |
| Fasting plasma glucose [mg/dl] | 94 ± 7* | 88 ± 8* | 117 ± 15† | <0.0001 |
| | | | | |
| 120 min-plasma glucose [mg/dl] | 101 ± 16 | 112 ± 15 | n.e.[b] | 0.81 |
| | | | | |
| Blood pressure | | | | |
| | | | | |
| systolic [mmHg] | 128 ± 9 | 130 ± 18 | 131± 14 | 0.93 |
| | | | | |
| diastolic [mmHg] | 81 ± 4 | 80 ± 9 | 77 ± 5 | 0.36 |
| | | | | |
| Triglycerides [mg/dl] | 113 ± 65 | 117 ± 75 | 189± 107 | 0.06 |
| | | | | |
| HDL-cholesterol [mg/dl] | 80 ± 26 | 71 ± 24* | 43± 19 | 0.013 |
| | | | | |
| LDL-cholesterol [mg/dl] | 140 ± 30 | 120 ± 35 | 146± 25 | 0.70 |
| | | | | |
| Creatinine [mg/dl] | 1.1 ± 0.1* | 1.0 ± 0.1† | 1.0 ± 0.1† | 0.017 |
| | | | | |
| Bilirubin [mg/dl] | 0.6 ± 0.2 | 0.5 ± 0.2 | 0.7± 0.3 | 0.26 |
| | | | | |
| Albumin creatinine ratio [mg/g] | 10.0 ± 9.0 | 8.1 ± 6.2 | 41.4± 78.1 | 0.078 |
| | | | | |
| Mean ± SD, | | | | |



SUBSTITUTE SHEET (RULE 26)



Continued on next page

Table 1, continued

a: ANOVA or $\chi^2$ tests

b: not examined

c: normal range: 4.0-6.2%

$^{\dagger}$: significant difference ($p < 0.05$) versus healthy controls (Student's t-test)..

$^{*}$: significant difference ($p < 0.05$) versus Type 2 diabetic patients (Student's t-test)..

**Table 2.** Insulin sensitivity and B cell function (homeostasis model assessment according to (28)) of the subjects and patients participating in hyperglycemic clamp experiments with GIP infusion.

| Parameter [unit] | Healthy Control Subjects | First-degree relatives of Type 2 diabetic patients | Type 2 diabetic patients | Significance (p-value); ANOVA) |
|---|---|---|---|---|
| B-cell function [%] | 67 ± 23 | 49 ± 23 | 60 ± 48 | 0.27 |
| Insulin resistance[a] | 1.3 ± 0.8* | 1.0 ± 0.5* | 3.6 ± 1.1† | 0.0002 |

Mean ± SD, P-values were calculated by analysis of variance (continuous variables).

†: significant difference ($p < 0.05$) versus healthy controls (Student's t-test).

*: significant difference ($p < 0.05$) versus Type 2 diabetic patients (Student's t-test).

a: expressed as fold-value of control subjects



SUBSTITUTE SHEET (RULE 26)

WE CLAIM:

1. A method for determining whether an individual is susceptible to developing impaired glucose tolerance (IGT), impaired fasting glucose (IFG), or Type-2 diabetes, comprising:

(i) administering to at least one individual at least one polypeptide selected from the group consisting of a gastric inhibitory polypeptide (GIP) and a biologically active GIP variant, or any combination thereof; wherein said polypeptide is optionally combined with a pharmaceutically acceptable carrier or excipient;

(ii) assessing the response of said individual to said administration;

(iii) comparing said response to a constant; and

(iv) determining from said comparison whether said individual is susceptible to developing IGT, IFG, or Type-2 diabetes.

2. The method according to claim 1, wherein said individual exhibits at least one indicia of susceptibility for developing Type-2 diabetes, but does not have Type-2 diabetes.

3. The method of claim 1, wherein the assessed response is an insulin response.

4. The method of claim 1, wherein the assessed response is a C-peptide response.

5. The method of claim 1, wherein the assessed response is a glucose response.

6. A kit for determining whether an individual is susceptible to developing IGT, IFG, or Type-2 diabetes, comprising at least one polypeptide selected from the group consisting of a gastric inhibitory polypeptide (GIP) and a biologically active GIP variant, or any combination thereof; wherein said polypeptide is optionally combined with a pharmaceutically acceptable carrier or excipient, and at least one means for

determining whether said individual is susceptible to developing IGT, IFG, or Type-2 diabetes.

7. The kit of claim 6, wherein said polypeptide is in a liquid solution or is lyophilized.

8. The method of claim 2, wherein said indicia is that said individual is a first-degree relative of at least one individual who has Type-2 diabetes.

9. The method of claim 2, wherein said indicia is that said individual is 45 years of age or older.

10. The method of claim 2, wherein said indicia is that said individual is obese.

11. The method of claim 2, wherein said indicia is that said individual has IGT.

12. The method of claim 2, wherein said indicia is that said individual has IFG.

13. The method according to claim 1, wherein said individual exhibits at least one indicia of susceptibility for developing IGT, but does not have IGT.

14. The method of claim 1, wherein said polypeptide is GIP.

15. The kit of claim 6, wherein said polypeptide is GIP.

16. The method of claim 1, further comprising, prior to said assessing step, administering at least one nutrient to said individual.

17. The method of claim 16, wherein said nutrient is glucose.

18. The method according to claim 1, wherein said individual exhibits at least one indicia of susceptibility for developing IFG, but does not have IFG.