Demonstration of Glycated Insulin in Human Diabetic Plasma and Decreased Biological Activity Assessed by Euglycemic-Hyperinsulinemic Clamp Technique in Humans

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The presence and biological significance of circulating glycated insulin has been evaluated by high-pressure liquid chromatography (HPLC), electrospray ionization mass spectrometry (ESI-MS), radioimmunoassay (RIA), receptor binding, and hyperinsulinemic-euglycemic clamp techniques. ESI-MS analysis of an HPLC-purified plasma pool from four male type 2 diabetic subjects (HbA1c 8.1 ± 0.2%, plasma glucose 8.7 ± 1.3 mmol/l [means ± SEI]) revealed two major insulin-like peaks with retention times of 14–16 min. After spectral averaging, the peak with retention time of 14.32 min exhibited a prominent triply charged (M + 3H)³⁺ species at 1,991.1 m/z, representing monocarbamylated insulin with an intact A-chain, of 5,970.3 Da. The second peak (retention time 15.70 min) corresponded to native insulin (M, 5,807.6 Da), with the difference between the two peptides (162.7 Da) representing a single glucitol adduct (theoretical 164 Da). Measurement of glycated insulin in plasma of type 2 diabetic subjects by specific RIA gave circulating levels of 10.1 ± 2.3 pmol/l, corresponding to ~9% total insulin. Biological activity of pure synthetic monocarbamylated insulin (insulin B-chain Phe¹-glucitol adduct) was evaluated in seven overnight-fasted healthy nonobese male volunteers using two-step euglycemic-hyperinsulinemic clamps (2 h at 16.6 µg·kg⁻¹·min⁻¹, followed by 2 h at 83.0 µg·kg⁻¹·min⁻¹; corresponding to 0.4 and 2.0 mU·kg⁻¹·min⁻¹). At the lower dose, the exogenous glucose infusion rates required to maintain euglycemia during steady state were significantly lower with glycated insulin (P < 0.01) and ~70% more glycated insulin was required to induce a similar rate of insulin-mediated glucose uptake. Maximal responses at the higher rates of infusion were similar for glycated and native insulin. Inhibitory effects on endogenous glucose production, insulin secretion, and lipolysis, as indicated by measurements of C-peptide, nonesterified free fatty acids, and glycerol, were also similar. Receptor binding to CHO-T cells transfected with human insulin receptor and in vivo metabolic clearance revealed no differences between glycated and native insulin, suggesting that impaired biological activity is due to a postreceptor effect. The present demonstration of glycated insulin in human plasma and related impairment of physiological insulin-mediated glucose uptake suggests a role for glycated insulin in glucose toxicity and impaired insulin action in type 2 diabetes. Diabetes 52:492–498, 2003

Considerable evidence now exists to indicate that the extent and duration of hyperglycemic disarray is an important determinant of the onset and severity of complications frequently observed in type 1 and type 2 diabetes, including neuropathy, nephropathy, retinopathy, and cardiovascular disease (1,2). It has been suggested that hyperglycemia is a cause as well as a consequence of β-cell dysfunction in type 2 diabetes (3–5). Convincing and mounting evidence now exists to support the idea that both hyperglycemia and hyperlipidemia of type 2 diabetes contributes to disturbances in gene expression, insulin biosynthesis, and insulin secretion (6–8).

Particular attention has been devoted in recent years to the role of glycation in complications of diabetes (9–12). It is now becoming increasingly evident that glycation may also compromise the biological activity of functional proteins, including glucose-6-phosphate dehydrogenase (13), aldolase (14), glutathione reductase (15), Cu-Zn superoxide dismutase (16,17), HDL (18), and both IgM and IgG (19,20). Studies also suggest that glycation of β-cell proteins may contribute to defective insulin secretion, and glucokinase has been implicated (21,22). It is also apparent that glycation of the single most important β-cell protein, insulin, occurs in diabetes (11). Thus, we have previously demonstrated that insulin and proinsulin are glycated in the pancreas of various animal models of diabetes (23). Islets and clonal β-cells cultured in hyperglycemic medium also contain increased amounts of glycated cellular insulin that can be readily secreted in response to a variety of physiological stimuli (23,24). Peptide chemistry studies have revealed the site of glycation to correspond to the NH2-terminal Phe¹ of the insulin B-chain (25).

Observations in animals and isolated muscle and adi-
pose tissues suggest that the glycation of insulin results in a significant compromise of biological activity (26,27), raising the possibility that glycation of insulin might contribute to the insulin resistance and glucose intolerance characteristic of type 2 diabetes (21). Although insulin resistance is influenced by multiple factors, glycation may also explain why insulin action improves rather than deteriorates when exogenous insulin is used to improve glycemic control in patients with type 2 diabetes (28). The present study was conducted to evaluate the significance of this proposal to human type 2 diabetes. Electro spray ionization mass spectrometry (ESI-MS) has been used to detect and characterize glycated insulin in plasma from patients with type 2 diabetes. Hyperinsulinemic-euglycemic clamps have been used to evaluate the antihyperglycemic potency of a pure synthetic human NH2-terminally Phe1 glycated insulin.

**RESEARCH DESIGN AND METHODS**

**Reagents.** Chemicals of analytical grade were used throughout, and all water was purified using a Milli-Q Water Purification System (Millipore, Milford, MA). The National Reference Preparation (Codex 55/04) was obtained from the World Health Organization International Laboratory for Biological Standards, National Institute for Biological Standards and Control (South Mimms, Hertfordshire, U.K.). Other chemicals and their sources were as follows: high-pressure liquid chromatography (HPLC) grade acetonitrile from Rathburn (Walkerburn, Scotland); trifluoroacetic acid (TFA; sequencing grade) from Aldrich Chemical Dorset, U.K.; human insulin (E. coli recombinant) for glycation and biological studies from Eli Lilly (Basingstoke, U.K.); F-12 tissue culture media and FCS from Invitrogen (Paisley, Scotland); sodium azide, sodium barbitone, and sodium cyanoborohydride (NaBH3CN) from Sigma (Poole, Dorset, U.K.); PBS tablets (pH 7.4) from Oxoid (Basingstoke, U.K.); Na15O3 from Amersham International (Buckinghamshire, U.K.); and d-glucose and other reagents from BDH Chemicals (Dorset, U.K.).

**Subjects.** Four male type 2 diabetic subjects (age range 70–81 years, HbA1c 8.1 ± 0.2%, plasma glucose 8.7 ± 1.3 mmol/l) were recruited for combined HPLC/mass spectrometry analysis of plasma. For clamp studies to evaluate biological activity of glycated insulin, seven healthy nonobese (BMI 22.2 ± 0.9 kg/m2) male volunteers (age range 19–21 years) were recruited. None of the subjects in this group had a family history of diabetes. For the measurement of glycated insulin concentrations using a specific radioimmunoassay (RIA), 15 type 2 diabetic subjects (7 men/8 women, age range 38–67 years, HbA1c 7.3 ± 0.4%, plasma glucose 7.9 ± 0.4 mmol/l) were recruited. All subjects gave written informed consent, and the protocols were approved by the Ethics Committee of the Queen’s University, Belfast.

**HPLC analysis of human type 2 diabetic plasma.** Human plasma from overnight-fasted subjects (containing 12 mg/ml NaBH3CN) was pooled and peptides extracted using two C-18 Sep-Pak cartridges (Millipore) in series connected to a peristaltic pump (flow rate 1.0 ml/min, P-1; Pharmacia, Uppsala, Sweden). Briefly, the cartridges were activated and washed with 0.12% (vol/vol) TFA in water. Plasma (20 ml) containing 0.1% TFA was applied to the column followed by washing with 20 ml 0.12% (vol/vol) TFA in water. Bound material was eluted using 0.1% TFA (vol/vol) in 35% acetonitrile/64.9% water. The eluent was concentrated using an AES 1000 Speed-Vac (Savant Instruments, Uppsala, Sweden). Brieﬂy, the interface to the mass spectrometer was operated with the heated capillary at a temperature of 220°C. ESI-MS profiles were examined, and the molecular masses of glycated insulin and insulin were determined using prominent multiple charged ions and the equation: M/z = IM − m/z, where M is molecular mass, m is the m/z ratio, and n is the number of charges, and Mm is the molecular mass of a proton.

**Preparation of glycated human insulin.** The synthesis and purification of glycated human insulin has been previously described in detail (25). Brieﬂy, human insulin (100 μg/ml; Eli Lilly) was incubated with 220 nmol/l l-glucose in 10 mmol/l sodium phosphate buffer (pH 7.4) and a 1,000-fold molar excess of the reducing agent NaBH3CN for 24 h at 57°C. Nonglycated control insulin was treated under similar conditions in the absence of glucose. The reaction was terminated by the addition of 0.5 mol/l acetic acid to lower the pH to 4.0. Insulin and glycated insulin samples were pooled and puriﬁed in solid-phase extraction using two preequilibrated (0.1% vol/vol TFA/water) C18 Sep-Pak cartridges in series connected to a peristaltic pump (flow rate 1.0 ml/min) to concentrate the peptide. The bound material was eluted with 10 ml acetonitrile/water (70:30) and concentrated under vacuum (Speed-Vac; Savant Instruments).

**Puriﬁcation and characterization of glycated human insulin.** Reconstituted samples (1 ml) were applied to a Vydac (C-18) HPLC (4.6 × 250 mm; The Separations Group) equilibrated with 0.12% (vol/vol) TFA in water (solvent A); solvent B consisted of 0.1% (vol/vol) TFA in 70% acetonitrile and 29.9% water. The concentration of acetonitrile in the eluting solvent was increased from 35% to 56% over 5 min, 70% over 5 min, and 100% over 5 min. Elution proﬁles were monitored at 206 nm, and peptide peaks corresponding to glycated and nonglycated insulin were collected by hand. Pooled fractions were taken to dryness using the Speed-Vac concentrator and reconstituted with 0.12% (vol/vol) TFA/water. Insulin and glycated insulin were puriﬁed to homogeneity by repeated HPLC runs on a (4.6 × 150 mm) Supelcosil LC-8 analytical column at a ﬂow rate of 1.0 ml/min. The concentration of acetonitrile in the eluting solvent was increased from 35% to 56% over 10 min, 56% over 20 min, and 70% in 5 min using linear gradients. Glycated and nonglycated insulin in pooled fractions were evaluated by ESI-MS as described above with collection of spectra using full ion scan mode (300–3,000 m/z mode). Peptides were quantiﬁed by comparison of peak areas obtained from RP-HPLC with a standard curve of known concentrations of human insulin standard. Glycated and control insulin preparations were lyophilized and stored at −20°C in aliquots for subsequent experiments. Samples of the two peptides were tested for sterility and pyrogenicity before use in clamp studies.

**Assessment of biological activity in hyperinsulinemic-euglycemic clamps.** Insulin action was assessed on three occasions in each of the volunteers using the euglycemic clamp technique as previously described (29). Brieﬂy, infusions of control insulin were compared directly with the same dose of glycated insulin with 70% higher insulin. Subjects were admitted to the Metabolic Unit at 7:45 a.m. on the morning of the study after a 12-h overnight fast. An antecubital vein was cannulated (18-gauge cannula; Venflon Viggo, Helsingborg, Sweden) and used for all infusions. A dorsal hand vein in the contralateral arm was cannulated (21-gauge; Venflon Viggo) and the hand placed in a thermoregulated plexiglass box maintained at 55°C to allow intermittent sampling of arterialized venous blood. Freeze-dried samples of glycated or control human insulin were reconstituted under sterile conditions immediately before use in physiological saline (0.9% NaCl; Antigen Pharmaceuticals, Roscera, Ireland) and ﬁltered using a Millex-GV low protein-binding ﬁlter (0.22 μm; Millipore SA, Mostraime, France) into 15 ml evacuated sterile vials (Ethmic II; CIS UK, High Wycombe, U.K.).

A primed-continuous infusion of HPLC-puriﬁed [3H]glucose (net 100 Ci/nmol) was given during a 2-h equilibration period (~120 min to time zero), after which insulin or glycated insulin were infused at 16.6 μg · kg−1 · min−1 for the ﬁrst 2-h period and at 83.0 μg · kg−1 · min−1 for the second 2-h period. This corresponded to 0.4 and 2.0 μl · kg−1 · min−1, respectively, for control insulin. A two-step clamp was used to evaluate the impact of glycation at different sites of insulin action and to provide information regarding the dose-response relationship of any observed effect. In a further set of infusions, 70% higher doses of glycated insulin were similarly administered at 28.2 and 141.1 μg · kg−1 · min−1 during the ﬁrst and second periods, respectively. Plasma glucose was maintained at the fasting concentration by an exogenous glucose infusion (20% wt/vol). Exogenous glucose was prelabeled with [3H]glucose to match the predicted basal plasma glucose speciﬁc activity, as previously described (30,31), with the modiﬁcation that the primed-continuous tracer infusion was reduced to 50% of the basal rate after 20 min and to 25% of basal after 140 min (to maintain tracer steady state) and was terminated by this rate thereafter for the remainder of the clamp period. The mean speciﬁc activity levels of tracer glucose achieved during the clamp were 200.7, 224.7, 211.6 (basal), 240.0, 255.7, 220.9 (steady state 1
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Calculateds. During each clamp, –30 to 0 min before insulin infusion is referred to as the basal period, with the final 30 min of the low-insulin infusion rate, i.e., 90–120 min, referred to as SS1 and the final 30 min of the high-insulin infusion rate, i.e., 210–240 min, referred to as SS2. The non–steady-state equations of Steele et al. (32), as modified by De Bodo et al. (33), were used to determine rates of glucose appearance and disappearance basally and to establish a dextran-coated charcoal RIA using glycated insulin (25) as standard. HPLC-purified mono-iodinated 125I-tyrosylated tracer was prepared from glycated insulin peptide using the solid-phase iodogen method (35). Assay sensitivity was 9 pmol/l, with an intra-assay coefficient of variation of 1.8%. Cross-reaction with nonglycated insulin or proinsulin was negligible, but the antibody cross-reacted 52% with glycated proinsulin (37). Insulin was measured by RIA using an antisera (GPB1) that cross-reacts fully with glycated insulin and proinsulin (23,24,38). Glucose was measured by an automated glucose oxidase method (39) using a Beckman glucose analyzer 2.

RESULTS

Evaluation of glycated insulin in type 2 diabetic plasma. HPLC analysis of a partially purified pool of plasma from subjects with type 2 diabetes revealed multiple peptide peaks (Fig. 1). ESI-MS analysis of fractions around the expected elution time of insulin revealed two major peaks with retention times of 14–16 min. After spectral averaging, the peak with retention time of 15.70 min exhibited a prominent multiply charged (M+3H)3+ species at 1,936.8 m/z (Fig. 2A), corresponding to human glycated insulin with a single glucitol adduct (M+3H)r of 5,970.3 Da). This species at 1,936.8 m/z, representing an intact M of 5,970.3 Da. This corresponds to human insulin with a single glucitol adduct (164 Da; total theoretical M, 5,971.6 Da). Synthetic glycated insulin used for infusion studies exhibited a (M+3H)3+ species at 1,990.9 m/z, corresponding to a molecular mass of 5,969.7 Da (data not shown). Measurement of glycated insulin in plasma of type 2 diabetic subjects by specific RIA gave circulating levels of 10.1 ±...
2.3 pmol/l. Insulin concentrations in this group were 108.2 ± 15.9 pmol/l, indicating that ~9% of insulin is glycated in type 2 diabetes.

Evaluation of biological activity of glycated insulin in euglycemic clamps. Mean basal arterialized venous plasma glucose and serum insulin concentrations were similar before insulin, matched glycated insulin, and high-dose glycated insulin infusions (Fig. 3A and B). Plasma glucose concentrations during the clamps were also similar through both steady-state periods during the sequential low and high insulin infusions, with mean coefficients of variation of 4.4% (4.9–5.3 mmol/l glucose). Insulin infusions led to comparable steady-state levels for matched insulin and glycated insulin (Fig. 3B). Infusion of the higher dose of glycated insulin resulted in significantly (P < 0.001 to P < 0.01) higher insulin levels compared with the other two groups during SS1 (51–67%) and SS2 (67–95%). As shown in Fig. 3C, endogenous insulin secretion was suppressed to a comparable extent in the three groups, as assessed from proinsulin C-peptide concentrations during SS1 (0.33–0.50 nmol/l) and SS2 (0.20–0.36 nmol/l).

The exogenous glucose infusion rate required to maintain euglycemia (an index of peripheral insulin action) was significantly reduced, by 46%, during matched glycated insulin infusion compared with control insulin infusion at SS1 (Fig. 4A). This reduction in glucose disposal was a consistent finding in all subjects. At SS2, however, no significant difference was present between exogenous glucose infusion rates for matched glycated insulin and control insulin infusions. High glycated insulin infusion resulted in similar exogenous glucose infusion rates compared with control insulin at SS1. However, at SS2, the exogenous glucose infusion rate was significantly greater (P < 0.05) with the 70% higher dose of glycated insulin.

Suppression of endogenous glucose production (EGP) (an index of hepatic insulin action) was similar during SS1 and SS2 for matched glycated insulin and control insulin infusions (Fig. 4B). At SS1, the high-glycated insulin infusion suppressed EGP by 45.2% from basal (13.31 ± 0.89 μmol·kg⁻¹·min⁻¹), as compared with 58.3% for matched glycated insulin infusion from basal (12.01 ± 0.50 μmol·kg⁻¹·min⁻¹) and 61.6% for control insulin from basal (12.9 ± 0.49 μmol·kg⁻¹·min⁻¹). At SS2, high-dose glycated insulin suppressed EGP by 46.7% from basal, as compared with 59% for matched glycated and 63% for monoglycated insulin. The molecular masses was determined using prominent multiple-charged ions and the equation: \( M_i = iM_i M_h \), where \( M_i \) is molecular mass, \( M_i \) is m/z ratio, \( i \) is number of charges, and \( M_h \) is mass of a proton.
control insulin. At SS2, the high-dose glycated insulin infusion did not suppress glucose production as effectively as control insulin ($P < 0.05$).

Basal levels of nonesterified fatty acids and glycerol in the three groups were in the ranges 0.5–0.6 mmol/l and 32–39 μmol/l, respectively. Control insulin, matched glycated insulin, and high-dose glycated insulin infusion suppressed serum levels to a similar extent during both SS1 and SS2 (80–90% for nonesterified fatty acids, $P < 0.01–0.001$ and 64–75% for glycerol, $P < 0.02–0.001$). Estimation of metabolic clearance rates for matched glycated and native insulin ([34] during SS1 (20 ± 2 and 20 ± 2 ml·kg⁻¹·min⁻¹, respectively) and SS2 (17 ± 1 and 16 ± 1 ml·kg⁻¹·min⁻¹) revealed no significant differences.

**Evaluation of insulin receptor binding.** The results of in vitro receptor binding studies conducted using CHO-T cells transfected with the human insulin receptor are shown in Fig. 5. Nonlinear regression analysis of competitive binding assays using labeled insulin and increasing concentrations of the peptides, from $10^{-10}$ to $10^{-6}$ mol/l, indicated a comparable affinity of glycated insulin for the insulin receptor when compared with insulin, with $IC_{50}$ (half-inhibitory concentration) values of 128 and 196 μmol/l, respectively.

**FIG. 5.** Displacement of $^{125}$I-labeled insulin by human insulin (■) and glycated human insulin (□). CHO-T cells were incubated for 16 h at 4°C with $^{125}$I-labeled insulin (30,000 cpm) and increasing concentrations of the peptide ligand. Results are expressed as a percentage of the $^{125}$I-labeled insulin bound in the absence of unlabeled insulin. Data represent the mean of triplicates from representative experiments.

**DISCUSSION**

This study has provided definitive evidence for the first time that glycated insulin circulates in the plasma of poorly controlled type 2 diabetic patients. This was achieved by chemical purification and HPLC separation of circulating proteins from a plasma pool, followed by ESI-MS of two peptide peaks corresponding to intact insulin (5,807.6 Da) and glycated insulin (5,970.3 Da). The difference in molecular mass corresponding to 162.7 Da indicates a comparable affinity of glycated insulin for the insulin receptor. Instead, experiments performed to date indicate that a substantial proportion of glycated insulin in the circulation (37) represents a single glucitol adduct, as previously described for in vitro glycation and structural characterization of insulin (25). The mass spectrometry fingerprint of plasma glycated insulin revealed molecular ion peak of (M+3H)+ at 1,991.1 m/z, corresponding to $M_r$ of 5,970.3 Da. This was very close to that observed with the synthetic glycated insulin preparation ($M_r$ 5,969.7 Da), providing clear evidence for the glycated molecule in plasma of type 2 diabetic subjects.

It is highly unlikely that insulin is normally glycated to any significant extent, as it circulates in the plasma of diabetic patients. Thus, the short half-life estimated at 5–10 min is unlikely to provide a significant opportunity for such structural modification. Instead, experiments performed to date indicate that a substantial proportion of insulin and proinsulin (10–20%) is glycated in the pancreatic β-cells during the stages of insulin synthesis and storage. Thus, glycated insulin has been measured in the pancreas of various animal models of type 2 diabetes (23,27) and in both isolated islets and clonal β-cells exposed to elevated glucose concentrations in tissue culture (23,24). These latter studies have also shown that glycated insulin is secreted from the β-cells with the nonglycated insulin in response to established secretagogues (24). Initial plasma measurements in diabetic obese hyperglycemic ob/ob mice using an affinity chromatography method revealed raised concentrations of glycated insulin in the circulation (37). These observations in animal models have been confirmed and extended with the recent advent of a sensitive RIA for glycated insulin in plasma and biological tissues (37,40). Exploitation of this new assay in the present study revealed that ~9% insulin in type 2 diabetic subjects circulates in glycated form.

The possible mechanism underlying glycation of insulin in the β-cell is worthy of consideration. It is likely to follow from glucose transporter-mediated entry into the β-cell and rapid metabolism by glucokinase to glucose-6-phos-
phate (41), which is a particularly potent glycating agent (42,43). This is in turn transported to the inner leaflet of the endoplasmic reticulum membrane by glucose-6-phosphatase (44), where proinsulin and insulin are produced at high concentrations for incorporation into vesicles for transport to the Golgi for packaging into secretory granules, where storage and further processing take place (45). Observations using clonal insulin-secreting cells in culture indicate that glycation of insulin is an efficient process that is neither increased nor decreased by variable rates of β-cell stimulation and insulin secretion (24). However, the level of glycated insulin was significantly decreased by culture of insulin-secreting cells under hyperglycemic conditions with established inhibitors of glycation (24).

The second part of this human study evaluated the possible significance of circulating glycated insulin in type 2 diabetes, using a pure and fully chemically characterized preparation of human insulin glycated at the NH₂-terminal Phe¹ of the insulin B-chain (25). Using healthy male volunteers, hyperinsulinemic-euglycemic clamps were performed with control insulin, an equivalent amount of glycated insulin, or a 70% greater amount of glycated insulin. Changes in circulating concentrations of C-peptide were similar in the three groups, suggesting that endogenous insulin secretion was suppressed equally in the three tests. However, insulin action, judged from the amount of glucose needed to be infused to maintain normoglycemia, was significantly impaired after glycated insulin compared with the control preparation. Potency was about the same when a 70% greater dose was infused, clearly indicating that glycated insulin exhibits a substantial impairment of glucose lowering action in normal humans. This observation parallels previous in vitro studies using muscle or adipose tissue and in vivo studies using simple undefined mixtures of nonstabilized glycated insulin (26,27,46,47).

Interestingly, the decrease of insulin-mediated glucose uptake was overcome at higher supraphysiological concentrations of glycated insulin during the second steady-state period, suggesting a shift to the right of the dose-response relationship. Decreased sensitivity but normal responsivity of the insulin dose-response curve has previously been suggested to indicate a receptor abnormality (48). An earlier study using human monocytes and an impure mixture of glycated insulin suggested that glycation of insulin compromised hormone action at the postreceptor level (47). Consistent with this view, the binding of glycated and native insulin to CHO-T cells transfected with human insulin receptor in the present study revealed no significant change in binding affinity for the glycated peptide. Since insulin clearance also involves binding to the insulin receptor, the observed similarity in the in vivo clearance rates of glycated and native insulin also suggests that the difference in insulin action is due to a postreceptor effect. Thus, although glycation at B1 Phe may appear away from the A-chain residues (A1 Gly, A5 Gln, A19 Tyr, and A21 Asn) and adjacent B-chain residues (B24 Phe, B25 Phe, B26 Tyr, B12 Val, and B16 Tyr) thought to be involved in binding (49), glycation may affect the charge density or insulin tertiary structure, affecting postreceptor signaling. Recent studies using analogs of glucagon-like peptide-1(7–36) amide and exendin clearly demonstrate that very simple structural modifications, such as glycation, can result in substantial agonist or antagonist properties independent of changes in receptor binding (50–53).

In contrast to the detrimental effect of glycation on insulin-mediated glucose uptake, hepatic glucose production was suppressed to a similar extent following infusions of either control or glycated insulin. This suggests that the effect of glycation on biological activity does not extend to actions on hepatic insulin action, but variability of hepatic glucose output between individuals during the first steady-state period may be a contributing factor. Similarly, glycation of insulin had no deleterious effects on the suppression of lipolysis and circulating concentrations of nonesterified free fatty acids or glycerol. Since nonesterified free fatty acid levels were low with each of the infusion regimens, it is possible that a near-maximal suppression of lipolysis was already achieved by the steady-state period. These observations, together with normal suppression of C-peptide, are likely to also reflect differences in dose-response relationships compared with sites, such as muscle, that are involved in cellular glucose uptake (48).

In conclusion, this study has provided the first definitive evidence and measurement of glycated insulin in the plasma of patients with type 2 diabetes. Using the euglycemic clamp technique, a pure and chemically characterized glycated insulin has been shown to exhibit an ~70% decrease in glucose-lowering action in normal humans compared with physiological concentrations of native insulin. These observations support a role of glycated insulin in glucose toxicity and impairment of insulin action in type 2 diabetes (4,5).

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