Effect of Aging and Diabetes on the Enteroinsular Axis

Judit Korosi,1 Christopher H.S. McIntosh,2 Raymond A. Pederson,2 Hans-Ulrich Demuth,3 Joel F. Habener,4 Ronald Gingerich,5 Josephine M. Egan,6 Dariush Elahi,4 and Graydon S. Meneilly1

Departments of 1Medicine and 2Physiology, the University of British Columbia, Vancouver, Canada.
3Hans Kneill Institute for Natural Product Research, Halle, Germany.
4Department of Medicine, Harvard University, Boston, Massachusetts.
5Linco Research Inc., St. Charles, Missouri.
6Laboratory of Clinical Physiology, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Maryland.

Background. The current studies were designed to examine the effect of aging and diabetes on the enteroinsular axis.

Methods. Healthy young control subjects (n = 10; age 23 ± 1 years; body mass index [BMI] 24 ± 1 kg/m²), healthy elderly subjects (n = 10; age 80 ± 2 years; BMI 26 ± 1 kg/m²), and elderly patients with type 2 diabetes (n = 10; age 76 ± 2 years; BMI 26 ± 2 kg/m²) underwent a 3-hour oral glucose tolerance test (glucose dose 40 gm/m²).

Results. Insulin responses were not different between young controls and elderly patients with diabetes but were significantly lower in elderly patients with diabetes and young controls than in elderly controls (young control: 178 ± 27 pM; elderly control: 355 ± 57 pM; elderly diabetes: 177 ± 30 pM; p < .05 elderly control vs young control and elderly diabetes). Total glucagon-like peptide 1 (GLP-1) responses were not significantly different between young and elderly controls and patients with diabetes (young control: 15 ± 2 pM; old control: 8 ± 2 pM; elderly diabetes: 12 ± 3 pM; p = ns). Active GLP-1 responses were also not different between young and elderly controls and patients with diabetes (young control: 5 ± 1 pM; old control: 6 ± 1 pM; elderly diabetes: 7 ± 1 pM; p = ns). However, the difference between total and active GLP levels was significantly greater in the young controls (young control: 10 ± 2 pM; old control: 2 ± 2 pM; elderly diabetes: 4 ± 2 pM; p < .05, young vs elderly). Glucose-dependent insulinotropic polypeptide responses were not different between young and elderly controls and between elderly controls and patients with diabetes but were significantly higher in elderly patients with diabetes than in young controls (young control: 97 ± 12 pM; elderly control: 121 ± 16 pM; elderly diabetes: 173 ± 27 pM; p < .05, young vs elderly diabetes). Glucagon responses were reduced in elderly controls but were similar in young controls and elderly patients with diabetes and young controls with diabetes (young control: 15 ± 1 pM; elderly control: 9 ± 1 pM; elderly diabetes: 16 ± 1 pM; p < .01 elderly control vs young control and elderly diabetes). Dipeptidyl peptidase IV (DPIV) activity that occurs in concert with these incretin response to oral glucose and the changes in these metabolic abnormalities (6). In normal elderly subjects, glucose-induced insulin release from the pancreas is impaired, and this defect is accentuated in elderly patients with diabetes (7–10). Previous studies have demonstrated that the responses of GIP and GLP-1 to oral glucose are both normal (11–13) or increased (14) in healthy elderly subjects when compared with healthy young controls. In elderly patients with diabetes, GIP responses to oral glucose are clearly enhanced (12). The response of the β cell to both GIP and GLP-1 is impaired in normal elderly subjects and to a much greater extent in elderly patients with diabetes (7,12,14,15). The mechanism for this effect is unclear, but one possibility for the reduced effectiveness of incretins in elderly persons is that these peptides are inactivated to a greater extent by DPIV.

Conclusions. We conclude that normal aging and diabetes are associated with multiple changes in the enteroinsular axis.
METHODS

**Experimental Subjects**

Subject characteristics are shown in Table 1. Healthy controls had a normal history and physical examination, normal laboratory tests, normal kidney and liver function, and a normal electrocardiogram. All healthy controls had a normal glucose tolerance test by the National Diabetes Data Group Criteria. Patients with diabetes were recruited from the Vancouver Hospital Diabetes Centre. The mean duration of diabetes was 7 ± 1 years, and Hgb A1C was 8.0 ± 0.4%. All subjects were free of clinically significant microvascular, macrovascular, or neuropathic complications from their diabetes. Patients with hypertension were not excluded. Five of the elderly subjects were treated with sulfonylurea drugs, and five were being treated with angiotensin-converting enzyme inhibitors for hypertension. All medications were stopped at least 2 weeks prior to the test. This study was approved by the committee on Human Investigation at the University of British Columbia. All subjects gave written, informed consent prior to participation.

**Experimental Protocol**

All subjects underwent an oral glucose tolerance test. Subjects consumed a diet containing at least 200 g carbohydrate for 3 days prior to each test and were studied after a 12-hour overnight fast. Blood samples were taken at −10 and 0 minutes. Subjects were then given glucose (40 g/m²), and blood samples were taken every 30 minutes to 180 minutes. Plasma glucose was measured immediately at the bedside using a YSI Glucose Analyzer (Yellowsprings Instruments, Yellowsprings, OH). Samples for DPIV were collected in heparin only. The remaining blood was placed in pre-chilled test tubes containing isoleucine thiazolidine (for measurement of GIP and GLP-1) (17), aprotinin (400 KIU/ml) and ethylenediaminetetraacetic acid (1.5 mg/ml) (for measurement of glucagon) and centrifuged at 4°C. Samples were stored at −70°C until analysis. Insulin, glucagon, and GIP were measured by radioimmunoassays as previously described (15). Total GLP-1 antibody is directed at the C-terminal end of the molecule and thus recognizes the intact forms of GLP-1 as well as the truncated forms. Active GLP-1 was measured in the assay services department at Linco Research Inc. using an ELISA technique. This assay measures both the 7-36 and the 7-37 GLP-1 moieties and excludes completely the 9-36 and 9-37 truncated forms. Plasma DPIV activity was measured by a colorimetric assay as previously described (4,18).

Data are presented as mean ± standard error of the mean. The area under the curve was calculated according to the Trapezoidal Rule. All values in the results for insulin, glucose, glucagon, GLP-1, and GIP are presented as the AUC. Differences between groups were determined using analysis of variance followed by Bonferroni’s correction for pairwise comparisons. A p value of .05 was considered significant in all analyses.

**RESULTS**

Elderly controls and patients with diabetes were similar in body mass index. Young control subjects appeared to have a lower BMI than elderly subjects, but the difference did not reach statistical significance.

Glucose, insulin, and glucagon values during the oral glucose tolerance test are shown in Figure 1. As expected, the AUC for glucose values was higher in patients with diabetes than in both young and elderly controls (p < .0001), but there was no significant difference between young and elderly controls. Insulin responses were not different between young controls and elderly patients with diabetes but were significantly lower in young controls and elderly patients with diabetes than in elderly controls (young control: 178 ± 27 pM; elderly control: 355 ± 57 pM; elderly diabetes: 177 ± 30 pM; p < .05, elderly control vs young control and elderly diabetes). Glucagon values were greater in elderly patients with diabetes and young controls when compared with elderly controls (young control: 15 ± 1 pM; elderly control: 9 ± 1 pM; elderly diabetes: 16 ± 1 pM; p < .01, elderly control vs young control and elderly diabetes).

GLP-1 values are shown in Figure 2. Total GLP-1 responses were not significantly different between young and elderly controls and patients with diabetes (young control: 15 ± 2 pM; old control: 8 ± 2 pM; elderly diabetes: 12 ± 3 pM; p = ns). Active GLP-1 responses were also not different between young and elderly controls and patients with diabetes (young control: 5 ± 1 pM; old control: 6 ± 1 pM; elderly diabetes: 7 ± 1 pM; p = ns). However, the difference between total and active GLP levels was significantly greater in the young controls (young control: 10 ± 2 pM; old control: 2 ± 2 pM; elderly diabetes: 4 ± 2 pM; p < .05, young control vs elderly control and elderly diabetes). GIP and DPIV responses are shown in Figure 3. GIP responses were not different between young and elderly controls and between elderly controls and patients with diabetes but were significantly higher in elderly patients with diabetes than in young controls (young control: 97 ± 12 pM; elderly control: 121 ± 16 pM; elderly diabetes: 173 ± 27 pM; p < .05, young control vs elderly diabetes). The AUC for DPIV activity was lower in both elderly controls and patients with diabetes when compared with young controls (young control: 0.17 ± 0.01; elderly control: 0.15 ± 0.01; elderly diabetes: 0.15 ± 0.01 ΔOD/20 minutes; p < .05, both elderly groups vs young).

**DISCUSSION**

In this study, we examined multiple components of the enteroinsular axis in healthy young and old and elderly patients with diabetes. Insulin responses to the oral glucose challenge appeared to be greater in the healthy elderly subjects than in the healthy young subjects; however, this may have been a result of the higher glucose levels in elderly persons, and we
believe that our results are consistent with previous studies that have demonstrated subtle alterations in glucose-induced insulin release in healthy elderly individuals (7,8). Similar to our previous results, we demonstrated a profound impairment in glucose-induced insulin release in relatively lean elderly patients with diabetes when compared with age- and weight-matched controls (9,10). Few studies have evaluated the changes with age in glucagon physiology. Fasting glucagon levels have been reported to be normal in elderly persons and to suppress normally in response to intravenous glucose and insulin (11,19–21). However, suppression of glucagon levels in response to oral glucose has been found to be impaired in healthy elderly subjects (11). We and others have documented that the glucagon responses to hypoglycemia are impaired with aging (22–24). In this study we found that glucagon levels were reduced in elderly controls, but glucagon responses to oral glucose were similar in elderly patients with diabetes and young controls. We demonstrate for the first time that, similar to middle-aged patients with diabetes, glucagon levels are elevated in elderly patients with diabetes relative to controls.

Few studies have evaluated the changes in the physiology of GLP-1 with age. Ranganath and colleagues reported substantial increases in GLP-1 responses to oral glucose in healthy elderly women when compared with young controls (14). In contrast, MacIntosh and colleagues (13) found that the GLP-1 responses to oral glucose were similar in healthy young and old men. In a mixed group of men and women, we could find no differences between young subjects and either normal elderly subjects or elderly patients with diabetes in the total GLP-1 responses to oral glucose. In this study, we report for the first time active GLP-1 values in elderly controls and patients with diabetes and found no difference in these values between groups. However, we found that the difference between total and active GLP-1 was lower in elderly controls and patients with diabetes, suggesting reduced inactivation of this peptide with age (see below). Despite preserved levels of active GLP-1 in these patients, glucose-induced insulin release was markedly impaired, consistent with a reduction in β-cell sensitivity to GLP-1 in elderly patients with diabetes.

Previous reports suggested that GIP responses to oral glucose are either normal (11–13) or increased (14) in healthy elderly subjects when compared with young controls. In this study and other previous studies that did not find an age-related increase in GIP responses to oral glucose (11–13), there was a trend, which did not reach statistical significance, toward increased GIP levels in healthy elderly subjects. We suspect that the lack of a significant effect in our study and the failure of previous reports to find a type 2 error and
In this study, we demonstrate that, contrary to our initial hypothesis, DPIV activity was reduced in both elderly controls and patients with diabetes. It is possible that the body has adapted to reduced glucose-induced insulin secretion by decreasing the activity of DPIV, which would result in reduced degradation of incretins. This would result in increased active levels of these peptides and enhance glucose-induced insulin secretion. The DPIV enzyme is produced in multiple tissues and has been found in plasma in many different forms (28–31). Circulating DPIV activity therefore appears to consist of a mixture of different proline dipeptidases (32). There are several potential mechanisms whereby the activity of the peptide could be reduced, including decreased biosynthesis, reduced release into plasma, or increased breakdown and clearance of the circulating form. Intuitively, we would have expected a greater reduction in DPIV activity in patients with diabetes to compensate for their marked impairment in insulin secretion. Further studies that characterize the form of DPIV specific to the catabolism of incretins will be needed to resolve this issue.

DPIV inhibitors are being investigated as therapeutic interventions in middle-aged patients with type 2 diabetes (16). Our current findings suggest that these interventions may be less effective in elderly patients due to reduced activity of DPIV. To enhance the effectiveness of incretins, it will be necessary not only to inhibit degradation but to increase secretion and enhance activity at the cellular level. Further studies are clearly needed to determine the effectiveness of DPIV inhibitors and other manipulations of the enteroinsular axis in elderly patients.

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Address correspondence to Dr. G.S. Meneilly, Rm S 169, Vancouver Hospital and Health Sciences Centre, UBC Site 2211, Wesbrook Mall, Vancouver, BC V6T 2B5. E-mail: meneilly@interchange.ubc.ca

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