Fasting and Postprandial Concentrations of Somatostatin-28 and Somatostatin-14 in Type II Diabetes in Men

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Recent evidence suggests that somatostatin-28 (SRIF-28), cleaved from prosomatostatin by cells of the upper intestine, acts as a nutrient-stimulated inhibitor of insulin secretion in healthy men. A role for SRIF-28 in the pathophysiology of diabetes has not been previously explored, although several groups have measured circulating somatostatinlike immunoreactivity (SLI) in diabetic subjects. To investigate the possible mediation of abnormal insulin secretion in diabetes by SRIF-28, plasma levels were measured in 10 non-insulin-dependent diabetic men and 9 age- and weight-matched control subjects. Concentrations of SRIF-14 and SLI were also obtained. Subjects were admitted for study after an overnight fast, blood was collected before and at 30-min intervals for 4 h after a fat meal, and plasma samples were analyzed for SRIF-28 and SRIF-14 by specific methods. Basal glucose levels in the diabetic men were significantly higher than in control subjects (10.2 ± 1 vs. 5.8 ± 0.2 mM), but insulin levels were similar (79 ± 14.2 vs. 93.3 ± 14.2 pM). The diabetic men had significantly lower basal SRIF-28 levels than the control subjects (11.4 ± 0.6 vs. 14.6 ± 1.0 pM, *P* = 0.017). After fat intake, SRIF-28 levels throughout the 4 h of study were indistinguishable in the two groups (270 vs. 292% of basal). Basal SRIF-14 and SLI levels were not significantly different in the two groups, and SRIF-14 and SLI concentrations rose similarly after the meal. There were no correlations between basal SRIF-28 and glucose or insulin levels. The lower concentrations of the β-cell inhibitor SRIF-28 in diabetic men may represent an adaptation to relative insulin deficiency. *Diabetes* 39:1198–202, 1990

Somatostatin-14 (SRIF-14) and somatostatin-28 (SRIF-28), an NH_2-terminal extension of SRIF-14, are the two bioactive peptides processed intracellularly from prosomatostatin (pro-SRIF) (1). Cleavage of pro-SRIF, resulting in either SRIF-14 or SRIF-28, occurs in distinct groups of cells. SRIF-14 is synthesized by neurons of the central and peripheral nervous systems and δ-cells of the pancreas and stomach, whereas SRIF-28 is processed from pro-SRIF in endocrine cells of the intestinal mucosa and some central nervous system neurons (2–5). This anatomical separation of the products of pro-SRIF suggests independent physiological roles. Current concepts state that SRIF-14 acts as a paracrine suppressor of glucagon and gastrin release (6,7), and SRIF-28 acts as a hormone that inhibits pancreatic exocrine and endocrine function (8,9); both substances may also act as neurotransmitters. These pro-SRIF–related peptides are normal constituents of mammalian plasma, with SRIF-28 being the most prevalent (10,11). Circulating SRIF-14 is short lived because it is rapidly metabolized by tissue aminopeptidases to SRIF-13, a metabolite that is two to three times more abundant in plasma than SRIF-14 (10). In most studies, plasma levels of pro-SRIF, SRIF-28, SRIF-14, and SRIF-13 are measured collectively as somatostatinlike immunoreactivity (SLI) by antiserums recognizing a common epitope near the COOH-terminal of pro-SRIF. However, given the separate roles of SRIF-28 and SRIF-14 and probable differences in control of their secretion, plasma SLI is a nonspecific measure and cannot provide an accurate assessment of the contribution of these peptides in various physiopathological states.

Because SRIF-14 inhibits insulin and glucagon release, there has been great interest in its possible contribution to the pathogenesis of islet cell dysfunction in diabetes mellitus (12). However, virtually no attention has been paid to SRIF-28, which is a more likely candidate than SRIF-14 for physiological regulation of insulin secretion (9). In diabetic animals, increased secretion of SLI has been observed (13,14). Patients with type I (insulin-dependent) diabetes have been

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reported to have elevations in circulating SLI, which were corrected by normalization of blood glucose (15). Patients with type II (non-insulin-dependent) diabetes have been reported to have either higher or similar basal SLI concentrations, and blunted or similar nutrient-stimulated SLI levels compared with control subjects (16–19). The discordance among animal and human studies leaves unanswered the question of a role for the pro-SRIF–related peptides in diabetes.

Based on our report that SRIF-28 acts as a physiological inhibitor of β-cells (9), we postulated that increased circulating levels might contribute to the abnormal β-cell function in diabetes. Therefore, with the availability of methods to separately analyze SRIF-14 and SRIF-28 (10), we evaluated the possibility that circulating concentrations of these two peptides are altered in patients with type II diabetes during the basal postprandial state.

RESEARCH DESIGN AND METHODS

Nineteen men were recruited from among the diabetic and healthy volunteers registered with the Diabetes and Endocrine Research Center at the University of Washington, Seattle. The mean age, body mass index, fasting blood glucose and fasting insulin values for the subjects are shown in Table 1. Seven of the diabetic volunteers were managed by dietary restriction alone, whereas three received sulfonylureas; these men stopped their oral agents the week before study. Three of the diabetic men had symptoms of peripheral neuropathy, but none had symptoms of autonomic neuropathy. Four of the control subjects were taking medicine for hypertension (2 with angiotensin-converting enzyme inhibitors and 2 with β-adrenergic blockers), and all denied use of other drugs. No control subject had symptoms of gastrointestinal disease. All of the volunteers maintained a stable body weight for at least 3 mo before study.

Subjects were admitted to beds at the Clinical Research Center at the University of Washington after an overnight fast. Details of the study were explained, and subjects signed an informed consent form approved by the institutional Human Subjects Committee. An antecubital vein was cannulated for repeated blood sampling and maintained patent with a slow infusion of 150 mM saline. Four basal blood samples were drawn every 10 min, and the subjects then consumed 162 ml of cream (63% H_2O, 31% fat, 3% carbohydrate, 2% protein) containing 50 g fat, which has been previously shown to cause a two- to threefold rise in plasma SRIF-28 levels (J.W.E., unpublished observations). Thereafter, blood samples were drawn at 30-min intervals for 240 min.

Blood was collected in heparinized tubes, centrifuged immediately, and the plasma removed. Aliquots of plasma for insulin and glucose were stored at −20°C. For SRIF-28 measurement, 1 N HCl was added to 5 ml of plasma to adjust the pH to 3 to prevent protease-mediated destruction. For assay of SRIF-14, blood was aspirated directly into syringes containing a chilled solution of 500 mM NaH_2PO_4 (pH 4.1) in a ratio of 1 ml blood/1.67 ml NaCl to achieve a final pH of 5.2, which minimizes cleavage of the NH_2-terminal Ala-Ser residues (10). Erythrocytes were removed by centrifugation, the volume of plasma was measured, and the plasma pH was immediately adjusted to 3 with 5 N HCl.

TABLE 1
Comparative values for diabetic and control subjects

	n	Age (yr)	Body mass index (kg/m²)	Fasting glucose (mM)	Fasting insulin (pM)
Diabetic	10	60 ± 2	25 ± 1	10.2 ± 1.0	79 ± 14
Control	9	59 ± 2	26 ± 1	5.7 ± 0.2	93 ± 14

SRIF-28 was measured as previously described (10). Briefly, acidified plasma samples were passed through octadecylsilyl silica cartridges (SepPak C-18, Waters, Milford, MA), and proteins and enzymes were removed by serial washing with absolute methanol and deionized water. Adsorbed peptides were eluted with 80% methanol, 1% trifluoracetic acid solution, air dried, and reconstituted in 130 mM borate buffer, pH 8.5. Aliquots were set aside for SLI measurement, and the rest of the sample was applied to a column of agarose coupled with partially purified Igs selectively binding the Asn-5 to Pro-6 sequence in the NH_2 region of SRIF-28. The column was washed with 130 mM borate buffer in which SRIF-14, SRIF-13, and pro-SRIF (fraction I) were eluted, lyophilized, and dissolved in 130 mM borate buffer. SRIF-28, bound to the immunoabsorbent, was eluted with 0.2 N HCl and 0.2% bovine serum albumin (BSA; Miles, Kankakee, IL) at pH 3.5, lyophilized, and reconstituted in 130 mM borate buffer.

Reconstituted samples were assayed for SRIF-28 concentrations by radioimmunoassay (RIA) with an antiserum (AS-10), recognizing the Phe-7, Trp-8, and Lys-9 residues of SRIF-14 shared by SRIF-28. Assays were carried out in 130 mM borate buffer, pH 8.5, with the antiserum diluted 1:100,000 with ^{125}I-labeled Tyr-11–SRIF-14 as tracer. Free and antibody-bound labeled peptides were separated by addition of 1 ml 1% activated charcoal (Norit A, Eastman-Kodak, Rochester, NY). Values were obtained by comparison with the displacement of an SRIF-28 standard (Peninsula, Belmont, CA) and expressed as picograms per milliliter. The measurements were corrected for recoveries of 50% when passed through the SepPak and immunoabsorbent. Fraction I eluted from the immunoabsorbent (pro-SRIF, SRIF-14, and SRIF-13), and aliquots for SLI were also assayed with AS-10. Synthetic SRIF-14 (Peninsula) was used as a standard, and the results were expressed as picogram equivalents of SRIF-14 per milliliter. Recoveries were corrected for 80% from the SepPak. The intra-assay coefficient of variation (C.V.) for the AS-10 assay is 6% with a minimal sensitivity of detection of 2 pg/tube.

SRIF-14 was measured by RIA with an antiserum that interacts with the NH_2-terminal Ala and Gly residues, and thus does not recognize SRIF-13 (10). Acidified plasma samples were passed through SepPak cartridges, and the peptides were eluted and air dried as described above. The eluates were dissolved in 50 mM barbital buffer, pH 8, containing 0.25% BSA and thiomerosal (1:10,000 final concn). Antiserum AS-77, was used in a final concentration of 1:4000, with SRIF-14 used as a standard and ^{125}I-[Tyr-11]SRIF-14 as tracer. Antibody-bound tracer was precipitated with 1 ml 30% polyethylene glycol (Carbowax 8000, Great Western, Seattle, WA) after the addition of 1 mg partially purified bovine γ-globulin (fraction II, Sigma, St. Louis, MO). Intra-assay

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C.V. for this measurement was 8%, with a minimal sensitivity of detection of 2 pg/tube.

Insulin levels were measured with a previously published method (20). Plasma glucose was determined with a YSI 23A glucose analyzer (Yellow Springs, OH). To eliminate interassay variance, the samples from all subjects were analyzed together in each assay.

Values for insulin, glucose, and pro-SRIF–derived peptides are presented as means ± SE. Mean postprandial levels of SRIF-28, SRIF-14, SLI, and fraction 1 peptides for the diabetic and control groups were compared with fasting levels by the paired t test with the Bonferroni adjustment for multiple comparisons ($P = 0.05/n$, where 0.05 is the selected level of significance, and n is the number of comparisons; values of <0.0063 were taken as significant). The total postprandial responses of the pro-SRIF–related peptides were calculated as the sum of the differences between the baseline concentrations and those attained at 30-min intervals during the 4 h after fat intake. These calculated postprandial responses and the mean basal levels of pro-SRIF–related peptides were compared between the diabetic and control groups with the two-tailed t test. Correlations between the various measures of pro-SRIF–related peptides and insulin and glucose were sought with linear regression.

RESULTS

The plasma concentrations of SLI in the diabetic and control groups before and after oral fat are shown in Fig. 1. SLI levels rose significantly to peak levels at 90–120 min and remained elevated for 4 h. There was no difference between the basal levels of SLI in the diabetic and control men (13.4 ± 1.2 and 15.2 ± 1.8 pM equivalents of SRIF-14/ml, respectively) or in the response to fat expressed either in absolute increment (44.5 ± 7.9 vs. 43.3 ± 9.1 pM equivalents of SRIF-14/ml) or as basal (332 vs. 318%).

Figure 2 displays the means of four fasting SRIF-28 levels in the two groups of men. The mean basal level for the diabetic group was 25% lower than that for the control subjects (11.4 ± 0.6 vs. 14.6 ± 1 pM, $P = 0.017$). There were no significant correlations between basal SRIF-28 and insulin or glucose levels. Figure 3 shows SRIF-28 concentrations in the plasma of the diabetic and control subjects before and after ingestion of fat. Both groups had similar elevations after their meal, with peak values at 90 min and elevated SRIF-28 concentrations 4 h after ingestion. There was no difference in the total response of SRIF-28 to fat between the two groups (32.1 ± 6.7 vs. 34.0 ± 10.5 pM; 270 vs. 292% of basal).

The concentrations of pro-SRIF–related peptides in fraction I (pro-SRIF, SRIF-13, and SRIF-14) and those of SRIF-

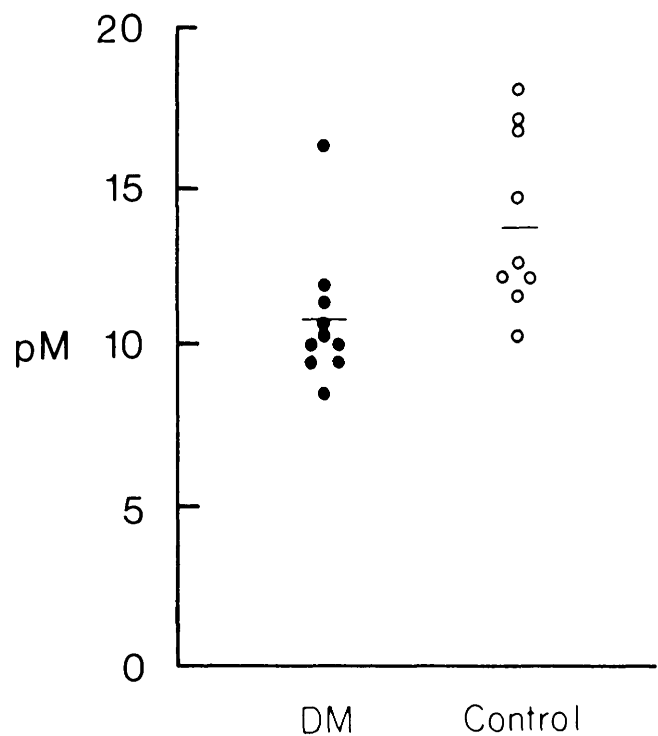

FIG. 2. Basal somatostatin-28 (S-28) levels in 10 diabetic men (●) and 9 nondiabetic control subjects (○). ***Horizontal bars*, group mean (diabetes vs. control, 36 ± 2 vs. 46 ± 3, respectively, $P < 0.017$).**

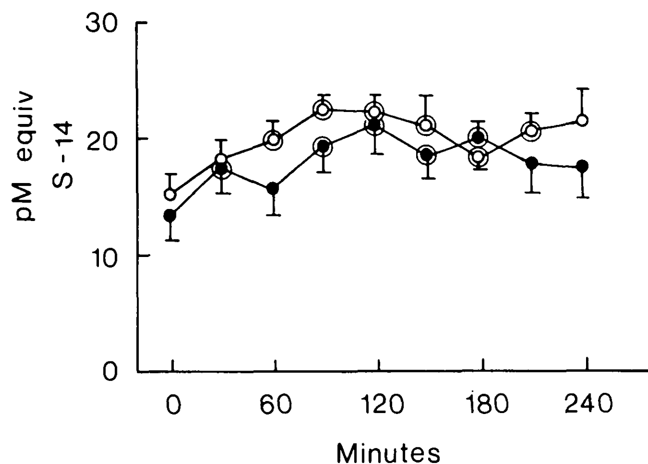

FIG. 1. Basal and postprandial levels of plasma somatostatinlike immunoreactivity in 10 diabetic men (●) and 9 nondiabetic control subjects (○). S-14, somatostatin-14. All subjects ingested 50 g fat at time 0. ***Circled points* are different from basal. *Bars*, SE.**

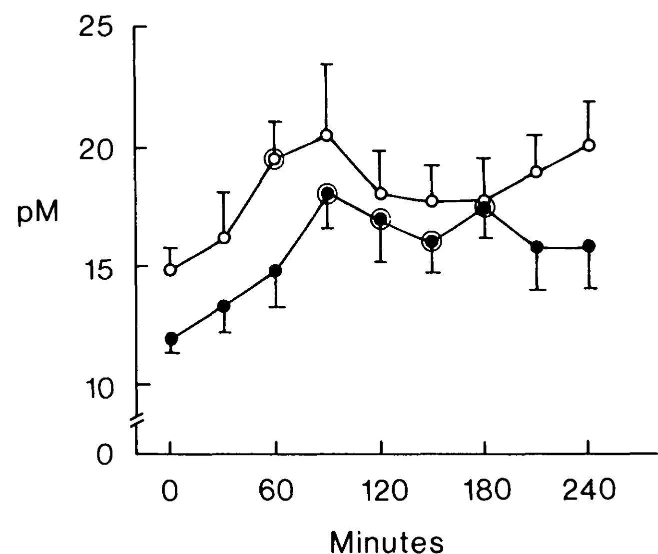

FIG. 3. Basal and postprandial levels of plasma somatostatin-28 in 10 diabetic men (●) and 9 nondiabetic control subjects (○). All subjects ingested 50 g of fat at time 0. ***Circled points* are different from basal. *Bars*, SE.**

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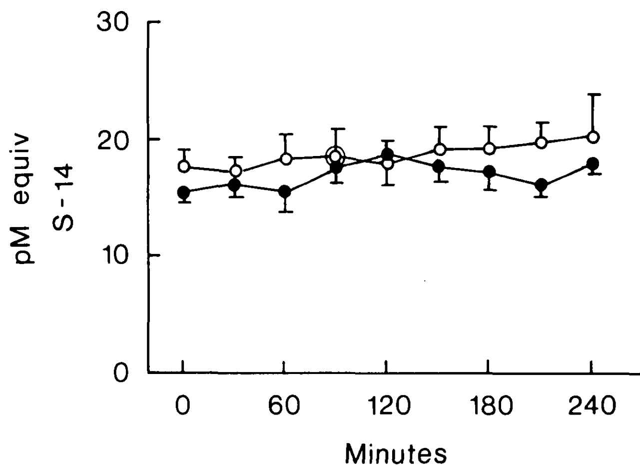

FIG. 4. Basal and postprandial levels of peptides eluted from immunoabsorbent as fraction I and comprised of prosomatostatin and somatostatin-13 and somatostatin-14 (S-14) in 10 diabetic men (●) and 9 nondiabetic control subjects (○). *Circled point* is different from basal. *Bars*, SE.

14 measured by specific RIA are shown in Figs. 4 and 5. The basal levels of fraction I were not different in the diabetic and control groups (15.9 ± 1.2 and 17.7 ± 1.8 pM equivalents SRIF-14/ml), and the values did not change after the fat meal. In contrast, levels of SRIF-14 rose significantly from basal levels of 0.73 ± 0.06 pM in both groups to peaks of 1.16 ± 0.12 pM at 120 min. SRIF-14 comprised ~5% of the peptides measured in fraction I, the rest presumably consisting of SRIF-13 and pro-SRIF.

DISCUSSION

In this study, we attempted to ascertain whether abnormalities occur in the regulation of pro-SRIF–derived peptides in patients with type II diabetes by measuring the physiologically important components of SLI. In most studies of circulating SLI in individuals with type II diabetes, there were no differences in basal levels compared with control subjects (17–19). The levels of SLI that we obtained are in agreement with most of the previous findings. However, meaningful interpretation of SLI is difficult, because it consists of several peptides secreted from cells in disparate tissues that are likely to be differentially regulated. We detected significantly lower basal concentrations of SRIF-28 in men with mild diabetes despite their having basal SLI levels comparable with levels in control subjects. This difference in basal SRIF-28 levels is likely buried in the SLI, because the AS-10 antiserum used for this measurement is only 33% as avid for SRIF-28 as for SRIF-14 and/or SRIF-13. This finding suggests that the regulation of SRIF-28–producing cells in the gastrointestinal tracts of people with type II diabetes is altered in the fasting state.

There were no differences in the nutrient-stimulated increments or peak levels of SLI and SRIF-28 in our diabetic and control groups. Conlon et al. (18) also reported comparable increases in the SLI of healthy and diabetic men after a mixed breakfast. However, others have noted deficient SLI release in response to mixed meals (16,19) or oral glucose (17). This discrepancy may be explained by methodological differences. Vinik et al. (19) and Miyazaki et al. (16) reported blunted SLI responses to a mixed meal when assayed in unextracted plasma. Because plasma globulins may interfere within SRIF RIAs (21) and spuriously contribute to the measurement of SLI, these data are open to question. Grill et al. (17) measured SLI levels after oral glucose and found very small but significant increases in healthy subjects and no change in type II diabetic subjects. Because in other investigators' studies glucose causes little or no change in SLI or SRIF-28 levels (22), it is difficult to interpret these small changes. We specifically selected 50 g of fat to induce marked changes in SLI and SRIF-28 levels (J.W.E., unpublished observations). Under these circumstances, the similar increments in SRIF-28 in our diabetic and control groups indicate that, in type II diabetes, SRIF-28 secretion with a large fat load is unimpaired. Whether responses to mixed nutrients or lesser fat intake might have been different was not examined.

There was reasonable correspondence between the aggregate measures SLI and fraction I from the immunoabsorbent (pro-SRIF, SRIF-13, and SRIF-14) and independently measured levels of SRIF-28 and SRIF-14. The variable affinities of SRIF-14 and SRIF-28 for the AS-10 antiserum prevent stoichiometric balance of SLI with the summed pro-SRIF derivatives measured independently. However, levels of SRIF-28, the predominent form of circulating SLI (10), correlated well with levels of total SLI (r = 0.73, $P < 0.05$). In contradistinction, it was not surprising that changes in the concentration of SRIF-14, which comprises only a small proportion of the non-SRIF-28 SLI (10) because of its rapid conversion to SRIF-13, were not reflected in fraction I.

The significant increase in SRIF-14 after oral fat, although small relative to concentrations of the other pro-SRIF–related peptides, was an unexpected finding. We previously reported that SRIF-14 levels do not change after ingestion of a mixed meal containing 25 g fat by healthy men (10). The postprandial increment of SRIF-14 in this study is possibly due to the larger fat load and may correspond to the nutrient-induced rise of SRIF-14 reported by others (23). We postulate that most of the postprandial rise in SRIF-14 is of gastric

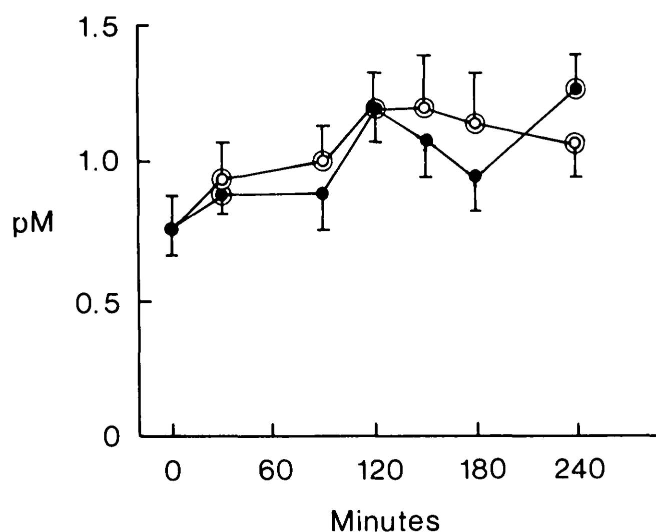

FIG. 5. Basal and postprandial levels of somatostatin-14 in 10 diabetic men (●) and 9 nondiabetic control subjects (○). All subjects ingested 50 g fat at time 0. *Circled points* are different from basal. *Bars*, SE.

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origin. However, because SRIF-14 is secreted from cells of several different tissues, it is plausible that some SRIF-14 measured in peripheral plasma originates from pancreatic or neural cells. Because SRIF-14 is probably a paracrine regulator with highest concentrations delivered to adjacent cells, its presence in the circulation probably occurs as spillover and may not accurately reflect the secretion of SRIF-14. Hence, abnormal secretion of SRIF-14 in diabetic subjects might not be detected in peripheral venous plasma. However, because there were no differences in SRIF-14 levels between our two groups of subjects, we are unable to conclude that gastric and/or pancreatic δ-cells are abnormally regulated in type II diabetes mellitus.

Considerable evidence for increased circulating SLI has accumulated in animal models of diabetes. Spontaneously diabetic BB Wistar rats have increased gastric release of SLI (24), and streptozocin-induced diabetic rats have elevated plasma SLI with pancreatic δ-cell hypertrophy and hyperplasia (14,25). Furthermore, several studies have shown that treatment of hyperglycemia with insulin in these animals can correct the abnormally increased synthesis of SLI (24,26). In contrast, the SLI and SRIF-28 levels in our diabetic subjects were independent of insulin or glucose concentrations. Animal models of type I diabetes are probably not analogous to the mildly diabetic men we studied. It is plausible that differences exist in the synthesis and secretion of pro-SRIF–derived peptides in type I and type II diabetes in humans.

In summary, we measured the bioactive peptides derived from the COOH-terminal of pro-SRIF in the plasma of men with type II diabetes and age and weight-matched control subjects. We found that basal SRIF-28 levels are lowered in the type II diabetic men but that the nutrient-induced secretory response of the gastrointestinal epithelial cells, which produce this peptide, is unaffected. The lower basal levels of SRIF-28 do not seem to be directly mediated by ambient glucose or insulin. Because the physiology of SRIF-28 has not been clearly delineated, the significance of these findings is unclear. We recently showed that SRIF-28 is a physiological inhibitor of glucose-, arginine-, or secretin-stimulated insulin secretion (9). Schusdziarra et al. (27) previously suggested that a physiological hyposomatostatinemia exists in insulin-resistant individuals. It is possible that the lower circulating levels of SRIF-28 in men with β-cell insufficiency represents such an adaptive mechanism. Further studies to examine this possibility are warranted.

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