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Glucagon-like Peptide 1 Content of Intestinal Tract in Adult Rats Injected with Streptozotocin Either During Neonatal Period or 7 d Before Sacrifice

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Glucagon-like peptide 1 (GLP-1) content of the intestinal tract was recently found to be lower in diabetes-prone BioBreeding (BBdp) rats than in the corresponding control animals (BBc rats), a finding compatible with the idea that an inflammatory intestinal state precedes insulitis in these diabetes-prone animals. This study aimed at measuring GLP-1 content of the intestinal tract both in another animal model of type 1 diabetes and in an animal model of type 2 diabetes. GLP-1 content of the jejunum, ileum, colon, and cecum was measured in male and female adult control rats and animals injected with streptozotocin (STZ) either during the neonatal period or 7 d before sacrifice. GLP-1 content of the intestinal tract was higher in type 1 diabetic rats than in control animals. Such was not the case in the type 2 diabetic rats. The findings recorded in the rats injected with STZ either during the neonatal period or later in life indicate that hyperglycemia and/or insulin deficiency do not cause a decrease in GLP-1 content of the intestinal tract. On the contrary, such a content may increase when the glucose intolerance and hypoinsulinemia are sufficiently pronounced, as was the case in the type 1 diabetic rats. These findings are thus compatible with the view that the decreased GLP-1 content of the intestinal tract in BBdp rats may result from intestinal inflammation.

Key Words: Glucagon-like peptide 1; intestinal tract; streptozotocin-induced diabetes.

Introduction

Glucagon-like peptide 1 (GLP-1) is secreted by L-cells located in the small and large intestine in response to the presence of nutrients in the gut lumen and communicates this information to the pancreas, where it acts as a potent insulin secretagogue *(1)*. It was recently reported that the GLP-1 content of the jejunum, ileum, and colon was abnormally low in diabetes prone BioBreeding (BBdp) rats, compared with the corresponding control animals (BBc rats), when killed at 60–66 d of age *(2)*. This prior study was motivated by the view that the development of immune-mediated diabetes in BBdp rats may involve an inflammatory state of the intestinal tract, as suggested by both increased gut permeability *(3)* and alteration in hydrolase activity in the intestinal mucosa *(4)* and as documented by histologic findings *(5)*. Such results were indeed considered compatible with the idea that a proinflammatory state of the gastrointestinal (GI) tract, associated with compromised function, may precede the occurrence of pancreatic insulitis in these diabetes-prone animals and, possibly, in humans *(2)*.

In the present study, the validity of our proposal was further assessed by measuring the GLP-1 content of the intestinal tract in another animal model of type 1 diabetes: adult rats injected with a diabetogenic dose of streptozotocin (STZ) 1 wk before sacrifice *(6)*. The present experiments were also extended to a model of type 2 diabetes: adult rats injected with STZ during the neonatal period *(7)*. The selection of these two animal models of diabetes was motivated by the fact that, at variance with the situation found in BBdp rats, they do not involve an autoimmune process.

Results

Body Weight

Mean body weight of male rats was higher than that of females, this difference achieving statistical significance ($p < 0.025$ or less) in both the control and type 2 diabetic animals (Table 1). At sacrifice, body weight of the female rats averaged 75.5 ± 5.0% ($n = 13$; $p < 0.005$) of that of the males within the same group (100.0 ± 2.7%; $n = 9$). The type 1 diabetic rats lost 46.6 ± 4.4 g ($n = 8$; $p < 0.005$) over the period of 7 d following the administration of STZ. Body

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Table 1
Metabolic and Hormonal Data

	Control		STZ (type 1)		STZ (type 2)	
	Male	Female	Male	Female	Male	Female
Body weight (g)	262 ± 10 (3)	223 ± 4 (3)	200 ± 12 (4)	184 ± 17 (4)	296 ± 1 (2)	177 ± 8 (6)
Blood glucose (mmol/L)	5.94 ± 0.44 (3)	4.89 ± 0.22 (3)	30.22 ± 2.06 (4)	19.94 ± 1.11 (4)	7.06 ± 1.17 (2)	5.56 ± 0.22 (6)
Plasma glucose (mmol/L)	6.78 ± 0.22 (3)	5.44 ± 0.28 (3)	37.78 ± 2.17 (4)	24.83 ± 1.11 (4)	7.50 ± 0.61 (2)	7.11 ± 0.50 (6)
Plasma insulin (pmol/mL)	200 ± 29 (3)	122 ± 39 (3)	58 ± 25 (4)	83 ± 35 (3)	275 ± 75 (2)	125 ± 36 (4)
Pancreas						
Wet weight (g)	0.91 ± 0.08 (3)	0.91 ± 0.03 (3)	0.77 ± 0.02 (4)	0.89 ± 0.07 (4)	1.32 ± 0.05 (2)	1.06 ± 0.07 (6)
Protein content (mg)	112 ± 3 (3)	110 ± 2 (3)	104 ± 2 (4)	100 ± 6 (4)	120 ± 3 (2)	119 ± 4 (6)
Insulin content (μg)	55.6 ± 10.1 (3)	47.0 ± 7.4 (3)	0.96 ± 0.26 (4)	0.66 ± 0.16 (4)	22.2 ± 3.0 (2)	25.1 ± 2.5 (6)
Insulin/protein content (ng/mg)	493 ± 74 (3)	431 ± 73 (3)	9 ± 2 (4)	7 ± 2 (4)	185 ± 29 (2)	209 ± 18 (6)
Insulin content/wet weight (μg/g)	60.2 ± 6.0 (3)	52.3 ± 9.4 (3)	1.2 ± 0.3 (4)	0.7 ± 0.1 (4)	16.7 ± 1.7 (2)	23.5 ± 1.0 (6)

weight of the type 2 diabetic rats averaged 87.8 ± 6.1% ($n = 8$; $p < 0.13$) of that of control animals of the same sex.

Blood and Plasma D-Glucose Concentration

As expected, plasma D-glucose concentration was higher ($p < 0.001$) than blood D-glucose concentration, the former value averaging 121.3 ± 2.7% ($n = 21$) of the paired latter one (Table 1). The type 1 diabetic rats were severely hyperglycemic ($p < 0.001$). At sacrifice, the blood and plasma D-glucose concentrations were higher in the type 2 diabetic rats than in the control animals of the same sex. For instance, plasma D-glucose concentration averaged in type 2 diabetic rats 125.6 ± 7.7% ($n = 8$) of the mean value found in control animals of the same sex (100.0 ± 2.7%; $n = 6$). As shown in Fig. 1, when examined 1 d before sacrifice, these type 2 diabetic rats displayed, during an intravenous glucose tolerance test (IVGTT), a mean glucose disappearance constant (K value) not exceeding $1.9 \pm 0.2 \times 10^{-2}/\text{min}^{-1}$ ($n = 7$).

Plasma Insulin Concentration

At sacrifice, plasma insulin concentration was lower ($p < 0.02$) in type 1 diabetic rats (68.3 ± 20.0 pmol/L; $n = 7$) than in control animals (161.7 ± 28.3 pmol/L; $n = 6$) and type 2 diabetic rats (175.0 ± 43.3 pmol/L; $n = 6$). These animals displayed, however, an abnormally low increase in plasma insulin concentration in response to the iv administration of D-glucose (Fig. 1). Thus, in these animals, the paired increment in plasma insulin concentration above basal value (time zero) averaged, 2 min after administration of D-glucose, 178.3 ± 75.0 pmol/L ($n = 7$), as distinct ($p < 0.005$) from 1141.7 ± 203.3 pmol/L ($n = 10$) in control animals *(8)*.

Pancreas Protein and Insulin Content

Protein content of the pancreas, relative to its wet weight, was not significantly different in control rats (122.4 ± 3.5 mg/g; $n = 6$), type 1 diabetic rats (124.4 ± 4.5 mg/g; $n = 8$), and type 2 diabetic rats (108.4 ± 5.7 mg/g; $n = 8$). Insulin content of the pancreas, however, whether expressed in absolute terms (micrograms) or relative to either protein content (nanograms/milligram) or pancreas wet weight (micrograms/gram), was decreased to about half its control value ($p < 0.001$) in type 2 diabetic rats and to <2% of such a control value in type 1 diabetic animals (Table 1).

Jejunum

Despite comparable length, wet weight and protein content of the jejunum were lower in female rats than in males. Thus, while the length of the jejunum averaged, in the female rats, 97.5 ± 2.3% ($n = 13$) of that found in male rats within the same group(s) (100.0 ± 1.4%; $n = 9$), wet weight and protein content of the jejunum in female rats represented no more than 74.9 ± 4.6% ($n = 13$; $p < 0.001$) and 80.5 ± 5.9% ($n = 13$; $p < 0.05$), respectively, of the mean corresponding value found in male rats of the same group(s) (i.e., 100.0 ± 3.1 and 100.0 ± 5.7%: $n = 9$ in both cases). In the control and type 1 diabetic rats, GLP-1 content (nanograms) of the jejunum was also lower ($p < 0.005$) in females than in males, averaging 52.7 ± 3.7% ($n = 7$) of the mean corresponding value found in male rats of the same group(s) (100.0 ± 13.2%; $n = 7$). Such was not the case, however, in the type 2 diabetic rats. When GLP-1 content of the jejunum was expressed relative to the paired protein content (nanograms/milligram), these sex-related differences were attenuated. Thus, when pooling all available data, such a paired GLP-1/protein ratio averaged in the female rats 4.63 ± 0.42 ng/mg ($n = 13$), compared with 6.47 ± 1.34 ng/mg ($n = 9$; $p > 0.1$) in males.

Mean wet weight, protein content, and GLP-1 content of the jejunum were all higher in type 1 diabetic rats than in control animals. In the former rats, they averaged 131.8 ± 10.2% ($n = 8$; $p < 0.05$), 138.8 ± 17.5% ($n = 8$), and 175.4 ± 14.9% ($n = 8$; $p < 0.01$), respectively, of the mean corresponding values found in control rats of the same sex (i.e.,

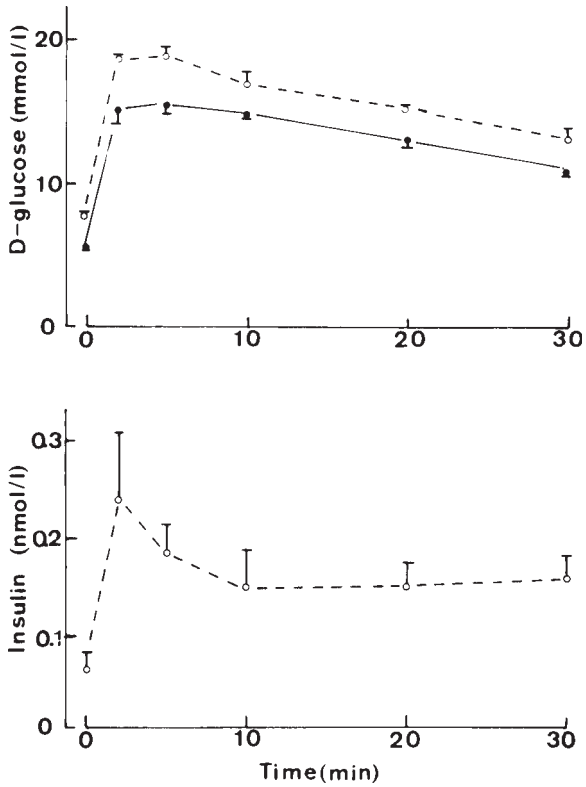

Fig. 1. Time course for changes in blood D-glucose (closed circles and solid line) and plasma D-glucose and insulin (open circles and dotted lines) concentrations during IVGTT in type 2 diabetic rats. Mean values (±SEM) refer to seven to eight individual measurements.

100.0 ± 2.8, 100.0 ± 5.7, and 100.0 ± 13.4%; $n = 6$ in all cases). Mean paired ratio between GLP-1 and protein content was higher, albeit not significantly so ($p < 0.1$), in the male type 1 rats than in the male control animals. Such was not the case in the female rats. When pooling all available data, the paired ratio between GLP-1 and protein content was somewhat higher in type 1 diabetic rats (138.9 ± 19.7%; $n = 8$) than in control animals of the same sex (100.0 ± 14.5%; $n = 6$); such a difference failed, however, to achieve statistical significance ($p > 0.1$).

A vastly different situation prevailed in the jejunum of type 2 diabetic rats. First, no significant difference was observed between these rats and control animals in terms of either the wet weight, length, or protein content of the jejunum. Indeed, the values recorded in the diabetic animals averaged 106.0 ± 4.8 ($n = 8$), 95.2 ± 1.8 ($n = 8$), and 116.9 ± 9.8% ($n = 8$), respectively, of the mean corresponding values found in control rats of the same sex (i.e., 100.0 ± 2.8, 100.0 ± 2.1, and 100.0 ± 5.7%; $n = 6$ in all cases). Second, whether in male or female animals, GLP-1 content (nanograms) of the jejunum was not significantly different ($p > 0.1$ or more) in the control and type 2 diabetic rats. In the latter rats, it averaged 73.9 ± 15.4% ($n = 8$; $p > 0.2$) of the mean corresponding value found in control animals of the same sex. When expressed relative to the paired protein content, the mean GLP-1 content of the jejunum was also lower in type 2 diabetic rats (3.3 ± 0.6 ng/mg; $n = 8$) than in control animals (5.4 ± 0.8 ng/mg; $n = 6$), in mirror image of the situation found in type 1 diabetic rats.

Ileum

The situation in the ileum was comparable in virtually all respects with that described in the jejunum. First, despite comparable length ($p > 0.2$) in male rats (49.0 ± 1.4 cm; $n = 9$) and females (46.5 ± 1.6 cm; $n = 13$), wet weight and protein content of the ileum was lower in females than in males. Thus, they averaged, in the female rats, 72.8 ± 4.5% ($n = 13$; $p < 0.001$) and 72.2 ± 5.8% ($n = 13$; $p < 0.005$), respectively, of the mean corresponding value found in male animals of the same group (i.e., 100.0 ± 4.1 and 100.0 ± 5.5%; $n = 9$ in both cases). Second, GLP-1 content of the ileum was also lower ($p < 0.02$) in female (442 ± 59 ng; $n = 13$) than in male rats (1.23 ± 0.34 μg; $n = 9$). Such a difference remained significant ($p < 0.03$) when the GLP-1 content of the ileum was expressed relative to the paired protein content, with mean values of 30.0 ± 6.9 ng/mg ($n = 9$) and 15.3 ± 1.8 ng/mg ($n = 13$) in male and female rats, respectively.

Third, despite comparable length of the ileum in control rats (46.2 ± 1.6 cm; $n = 6$) and type 1 diabetic animals (50.5 ± 2.0 cm; $n = 8$), its mean wet weight, protein content, and GLP-1 content were all higher in the latter rats. They averaged, in the type 1 diabetic rats, 133.2 ± 9.9% ($n = 8$; $p < 0.05$), 137.7 ± 16.5% ($n = 8$), and 209.9 ± 32.0% ($n = 8$; $p < 0.05$), respectively, of the mean corresponding values found in control animals of the same sex (i.e., 100.0 ± 4.9, 100.0 ± 7.2, and 100.0 ± 7.0%; $n = 6$ in all cases). Even when expressed relative to the paired protein content, the GLP-1 content of the ileum remained higher in type 1 diabetic rats (161.1 ± 23.5%; $n = 8$) than in control animals of the same sex (100.0 ± 5.8%; $n = 6$).

Finally, no significant difference between control rats and type 2 diabetic animals was observed in terms of wet weight, length, and GLP-1 content of the ileum. The values recorded in the type 2 diabetic rats averaged 115.5 ± 6.8% ($n = 8$), 98.4 ± 4.0% ($n = 8$), and 76.2 ± 18.4% ($n = 8$), respectively, of the mean corresponding values found in control rats of the same sex (i.e., 100.0 ± 4.9, 100.0 ± 3.4, and 100.0 ± 7.0%; $n = 6$ in all cases). Protein content of the ileum in type 2 diabetic rats averaged 120.6 ± 5.7% ($n = 8$) of that found in control rats of the same sex (100.0 ± 7.2%; $n = 6$). The mean paired ratio between the GLP-1 and protein contents of the ileum was lower in the type 2 diabetic rats than in the control animals. It averaged, in the former rats, 65.0 ± 17.4% ($n = 8$) of the mean corresponding value found in control rats of the same sex (100.0 ± 5.8%; $n = 6$). Such a difference failed, however, to achieve statistical significance.

Colon

The findings recorded in the colon were reminiscent in several respects of those made in either the jejunum or ileum. First, despite comparable length ($p > 0.2$) of the colon in male rats (14.5 ± 0.8 cm; $n = 9$) and female rats (13.6 ± 0.4 cm; $n = 13$), its wet weight, protein, and GLP-1 contents were all lower in females than in males. They averaged, in the female rats, 73.5 ± 4.1% ($n = 13$; $p < 0.05$), 71.1 ± 5.6% ($n = 13$; $p < 0.005$), and 47.7 ± 7.9% ($n = 13$; $p < 0.001$), respectively, of the mean corresponding values found in male rats of the same group (i.e., 100.0 ± 7.0, 100.0 ± 6.3, and 100.0 ± 11.6%; $n = 9$ in all cases). Even when expressed relative to the paired protein content, the GLP-1 content of the colon remained significantly lower ($p < 0.01$) in female (18.6 ± 1.3 ng/mg; $n = 13$) than in male rats (51.3 ± 12.7 ng/mg; $n = 9$).

Second, mean weight, length, protein content, and GLP-1 content of the colon were all higher in type 1 diabetic rats than in control animals. They averaged, in the type 1 diabetic rats, 130.0 ± 10.1% ($n = 8$), 113.9 ± 5.0% ($n = 8$), 156.1 ± 19.2% ($n = 8$; $p < 0.05$), and 270.2 ± 47.4% (GLP-1; $n = 8$; $p < 0.01$), respectively, of the mean corresponding values found in control animals of the same sex (i.e., 100.0 ± 4.9, 100.0 ± 3.5, 100.0 ± 4.7, and 100.0 ± 5.4%; $n = 6$ in all cases). The paired ratio between GLP-1 and protein content averaged, in the type 1 diabetic rats, 189.6 ± 39.1% ($n = 8$) of the mean corresponding value found in control animals of the same sex (100.0 ± 10.4%; $n = 6$). Such a difference failed, however, to achieve statistical significance.

Finally, in type 2 diabetic rats, wet weight, length, protein content, GLP-1 content, and paired ratio between GLP-1 and protein content of the colon were not significantly different from the corresponding values found in control rats of the same sex. Thus, they averaged 106.7 ± 7.9% ($n = 8$), 107.7 ± 5.3% ($n = 8$), 128.6 ± 6.5% ($n = 8$), 126.7 ± 18.3% ($n = 8$), and 98.2 ± 15.4% ($n = 8$), respectively, of such control values (i.e., 100.0 ± 4.9, 100.0 ± 3.5, 100.0 ± 4.7, 100.0 ± 5.4, and 100.0 ± 10.4%; $n = 6$ in all cases).

Cecum

Whether in control or type 1 diabetic rats, weight, protein content, and GLP-1 content of the cecum were lower ($p < 0.06$ or less) in female than in male rats. They averaged, in the female rats, 79.1 ± 3.6%, 85.4 ± 4.3%, and 50.2 ± 2.4%, respectively, of the mean corresponding values found in males within the same group (i.e., 100.0 ± 3.9, 100.0 ± 5.2, and 100.0 ± 6.4%; $n = 7$ in all cases). Even when expressed relative to paired protein content, the GLP-1 content remained lower ($p < 0.001$) in female rats (control and type 1 diabetic animals) than in male rats, with mean values of 10.7 ± 0.7 ng/mg (females) and 18.1 ± 1.3 ng/mg (males; $n = 7$ in both cases).

Whether in male or female rats, mean wet weight, protein content, and GLP-1 content of the cecum were higher ($p < 0.01$) in type 1 diabetic rats than in control animals of the same sex. Thus, they averaged, in type 1 diabetic rats, 131.5 ± 6.5% ($n = 8$), 131.8 ± 6.3% ($n = 8$), and 154.1 ± 9.1% ($n = 8$), respectively, of the mean corresponding values found in control rats of the same sex (i.e., 100.0 ± 2.7, 100.0 ± 5.8, and 100.0 ± 4.7%; $n = 6$ in all cases). However, when expressed relative to paired protein content, GLP-1 content of the cecum in the type 1 diabetic rats averaged 115.1 ± 6.3% ($n = 8$) of the mean corresponding value found in control animals of the same sex (100.0 ± 6.8%; $n = 6$), such a difference failing to achieve statistical significance.

Weight, protein content, and GLP-1 content of the cecum in the six female type 2 diabetic rats were not significantly different from the corresponding value found in the three female control animals (Table 2).

Intestinal Tract

The data summarized in the top of Fig. 2 illustrate that, except in the two male type 2 diabetic rats, the GLP-1 content of the four segments of the intestinal tract displayed a comparable hierarchy in all other groups, i.e., jejunum < ileum > colon > cecum. Likewise, when expressed relative to paired protein content, that of GLP-1 always followed a comparable hierarchy in all groups of rats: jejunum < ileum ≤ colon > cecum (Fig. 2, bottom).

Total GLP-1 content of the intestinal tract averaged, in the type 1 diabetic rats, 210.4 ± 22.2% ($n = 8$; $p < 0.01$) of that found in the control animals of the same sex (100.0 ± 4.6%; $n = 6$). Comparable findings applied to GLP-1 content of the intestinal tract excluding the cecum (Fig. 3). In the latter case, such a content was lower ($p < 0.01$) in the two male rats with type 2 diabetes than in the three male control animals. However, when pooling all available data, GLP-1 content of the intestinal tract (excluding the cecum) averaged, in the type 2 diabetic rats, 86.2 ± 16.4% ($n = 8$) of that found in control animals of the same sex (100.0 ± 5.8%; $n = 6$).

Discussion

The GLP-1 content of the intestinal tract, when expressed relative to protein content, displayed the following hierarchy: jejunum < cecum < ileum ≤ colon. In each of these four intestinal segments, wet weight, as well as protein and GLP-1 content, were higher in male rats than in female rats. For instance, when pooling all available data, the paired GLP-1/protein ratio in each intestinal segment averaged, in female rats, 53.1 ± 3.1% ($n = 46$; $p < 0.001$) of the corresponding mean value found in male animals (100.0 ± 10.0%; $n = 34$). In our prior study conducted in BB rats *(2)*, we already observed that the GLP-1 content (ng/mg of protein) of the jejunum, ileum, and colon averaged, in female rats, 76.4 ± 5.2% ($n = 36$) of the mean corresponding values found in males (100.0 ± 4.6%; $n = 34$; $p < 0.005$). The former percentage is virtually identical to that found in the present work in the control rats (73.5 ± 7.2%; $n = 9$). Thus,

Table 2
Intestinal Data

	Control		STZ (type 1)		STZ (type 2)	
	Male	Female	Male	Female	Male	Female
Jejunum						
Weight (g)	4.6 ± 0.1 (3)	3.4 ± 0.2 (3)	5.5 ± 0.4 (4)	4.9 ± 0.6 (4)	5.3 ± 0.2 (2)	3.5 ± 0.2 (6)
Length (cm)	49.0 ± 1.0 (3)	46.3 ± 2.0 (3)	49.0 ± 1.5 (4)	49.8 ± 2.9 (4)	46.0 ± 0.0 (2)	44.3 ± 1.1 (6)
Protein (mg)	56.9 ± 4.6 (3)	31.8 ± 3.1 (3)	59.2 ± 7.5 (4)	55.2 ± 6.7 (4)	48.1 ± 2.5 (2)	40.6 ± 2.9 (6)
GLP-1 (ng)	307 ± 88 (3)	169 ± 15 (3)	559 ± 78 (4)	285 ± 32 (4)	77 ± 8 (2)	151 ± 26 (6)
Paired GLP-1/protein content ratio (ng/mg protein)	5.4 ± 1.7 (3)	5.4 ± 0.4 (3)	9.7 ± 1.3 (4)	5.3 ± 0.6 (4)	1.6 ± 0.1 (2)	3.8 ± 0.7 (6)
Ileum						
Weight (g)	3.7 ± 0.1 (3)	2.8 ± 0.3 (3)	4.7 ± 0.4 (4)	3.9 ± 0.5 (4)	4.8 ± 0.6 (2)	3.1 ± 0.2 (6)
Length (cm)	46.0 ± 2.3 (3)	46.3 ± 2.6 (3)	51.3 ± 1.9 (4)	49.8 ± 3.8 (4)	49.0 ± 3.0 (2)	44.3 ± 2.1 (6)
Protein (mg)	36.3 ± 2.0 (3)	23.2 ± 3.5 (3)	41.8 ± 4.7 (4)	37.2 ± 6.4 (4)	43.2 ± 6.8 (2)	28.1 ± 1.4 (6)
GLP-1 (ng)	854 ± 82 (3)	361 ± 45 (3)	2043 ± 494 (4)	652 ± 106 (4)	175 ± 25 (2)	342 ± 68 (6)
Paired GLP-1/protein content ratio (ng/mg protein)	23.7 ± 3.0 (3)	15.7 ± 0.5 (3)	47.7 ± 7.5 (4)	19.0 ± 3.5 (4)	4.1 ± 0.1 (2)	12.7 ± 3.0 (6)
Colon						
Weight (g)	1.5 ± 0.1 (3)	1.2 ± 0.1 (3)	1.9 ± 0.3 (4)	1.6 ± 0.1 (4)	1.9 ± 0.2 (2)	1.2 ± 0.1 (6)
Length (cm)	13.2 ± 0.6 (3)	12.8 ± 0.8 (3)	14.8 ± 1.3 (4)	14.8 ± 0.5 (4)	16.0 ± 2.0 (2)	13.2 ± 0.6 (6)
Protein (mg)	13.8 ± 1.4 (3)	7.8 ± 0.2 (3)	16.2 ± 1.9 (4)	15.2 ± 1.8 (4)	16.3 ± 2.8 (2)	10.3 ± 0.5 (6)
GLP-1 (ng)	412 ± 43 (3)	133 ± 8 (3)	1359 ± 358 (4)	280 ± 32 (4)	267 ± 53 (2)	196 ± 22 (6)
GLP-1 (ng/mg protein)	31.3 ± 6.9 (3)	17.0 ± 1.3 (3)	83.9 ± 17.0 (4)	18.9 ± 1.7 (4)	16.3 ± 0.5 (2)	19.3 ± 2.7 (6)
Cecum						
Weight (g)	1.0 ± 0.1 (3)	0.7 ± 0.1 (3)	1.2 ± 0.1 (4)	1.0 ± 0.1 (4)	—	0.8 ± 0.1 (6)
Protein (mg)	12.4 ± 1.1 (3)	9.6 ± 0.9 (3)	15.0 ± 1.1 (4)	13.7 ± 0.5 (4)	—	9.0 ± 0.4 (6)
GLP-1 (ng)	203 ± 21 (3)	92 ± 2 (3)	286 ± 27 (4)	154 ± 9 (4)	—	87 ± 13 (6)
GLP-1 (ng/mg protein)	16.7 ± 1.9 (3)	9.8 ± 1.0 (3)	19.2 ± 1.7 (4)	11.3 ± 0.9 (4)	—	9.6 ± 1.3 (6)
Intestinal tract						
GLP-1 (ng)	1776 ± 163 (3)	755 ± 34 (3)	4247 ± 673 (4)	1372 ± 135 (4)	—	776 ± 115 (6)
Intestinal tract (except cecum)						
GLP-1 (ng)	1573 ± 182 (3)	663 ± 36 (3)	3711 ± 566 (4)	1217 ± 141 (4)	519 ± 37 (2)	689 ± 105 (6)

such a gender difference is observed in both control and diabetic animals in different strains of rats. Hence, it occurs independently of any gender difference that might exist in the response to STZ or other diabetogenic factors.

In type 1 diabetic rats, secondary to STZ administration, wet weight, protein content, and GLP-1 content of the jejunum, ileum, colon, and cecum were higher than in control animals. An increased content of GLP-1 and glucagon-like immunoreactive peptides (glicentin and oxyntomodulin) in the colon was already observed by Kreyman et al. *(8)* in adult rats, 2 mo after STZ administration. Likewise, Brubaker et al. *(9)* reported an increased ileal concentration of glucagon-like immunoreactive peptides, 15–22 d after administration of STZ to adult male Wistar rats.

In type 2 diabetic rats, wet weight, protein content, and GLP-1 content of the four intestinal segments were, as a rule, not significantly different from those found in control animals. If any, the trend was toward a lower GLP-1 content in the jejunum and ileum of type 2 diabetic rats. This contrasted with a trend toward a higher protein content of the intestinal tract in the same animals, this increase achieving statistical significance in the ileum and colon.

As far as GLP-1 content of the jejunum, ileum, and colon is concerned, the results in the present model of type 1 diabetes represent a mirror image of those recently collected in another animal model of type 1 diabetes, namely BBdp, compared with BBc rats *(2)*. These contrasting findings suggest that the situation found in BBdp rats cannot be attributed to

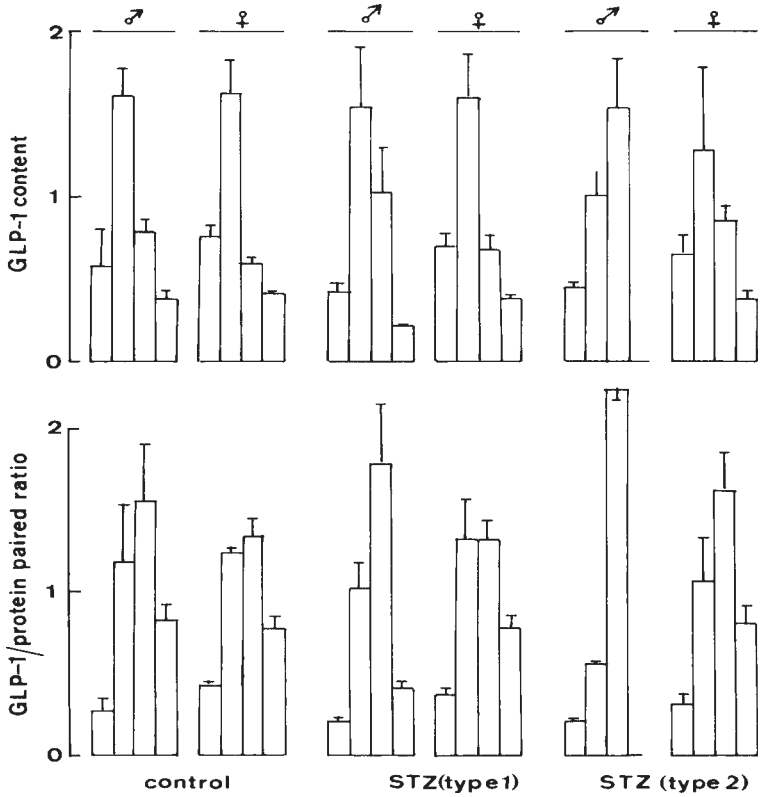

Fig. 2. GLP-1 content (ng) (**top**) and GLP-1/protein paired ratio (ng/mg) (**lower**) of the four segments of the intestinal tract (from left to right: jejunum, ileum, colon, and cecum) in male and female control animals and either type 1 or type 2 diabetic rats are expressed relative to the mean value derived, in each case, from the measurements made in the jejunum, ileum, and colon. Mean values (±SEM) refer to two to six individual measurements (see Table 2).

either hyperglycemia or hypoinsulinemia, even more so because the BBdp rats examined in our prior study failed to display hyperglycemia when killed 60–66 d after birth. Therefore, the present study reinforces the view that low GLP-1 content of the intestinal tract in BBdp rats reflects a proinflammatory state of the GI tract associated with compromised function and preceding the occurrence of pancreatic insulitis in these diabetes-prone animals *(2)*.

The present findings in type 2 diabetic rats further support the view that glucose intolerance and relative insulinopenia, both of prolonged duration (as opposed to only 1 wk in type 1 STZ rats), may only play, at the most, a minor contributive role in the occurrence of a decreased GLP-1 content of the intestinal tract. It should be stressed, however, that hyperglycemia and hypoinsulinemia were much more marked in the type 1 diabetic rats than in the type 2 diabetic animals. Our results are compatible, therefore, with the view that these variables have a direct effect on increasing GLP-1 content of the intestinal tract, separate from whether the diabetes model reflects a type 1 or type 2 disease state or duration of the glucose intolerance and insulinopenia.

In conclusion, selected animal models of type 1 and type 2 diabetes, as well as distinct experimental models of type 1 diabetes, may affect in opposite manner the GLP-1 content of the intestinal tract, pointing to both a multifactorial regulation of this biologic variable and the existence of distinct roles for such a variable in the pathogeny of the diabetic state.

Materials and Methods

Animals

Male and female inbred Wistar rats from our local colony (Madrid, Spain) were kept on a standard pellet diet (UAR, Panlab, Barcelona, Spain) and tap water ad libitum up to the time of sacrifice. Type 1 diabetic rats received, at adult age (about 200 g of body wt) a single dose of STZ (60 μg/g of body wt; Sigma-Aldrich Quimica S.A., Madrid, Spain) dissolved in saline and administered intraperitoneally *(6)*. Seven days later, the animals with a glucosuria in excess of 5.6 mmol/L (Aution Sticks; Menarini, Florence, Italy) were selected for the study. Type 2 diabetic rats received a single dose of STZ (100 μg/g of body wt) dissolved in a citrate buffer (0.05 mol/L, pH 4.5) and administered intraperitoneally on the day of birth *(7)*. At the age of 9–12 wk (10.7 ± 0.4 wk; $n = 8$), those rats with a glucose disappearance constant below 2.5×10^{-2}/min during an IVGTT (0.5 mg of D-glucose/g of body wt injected over 30 s) were selected for

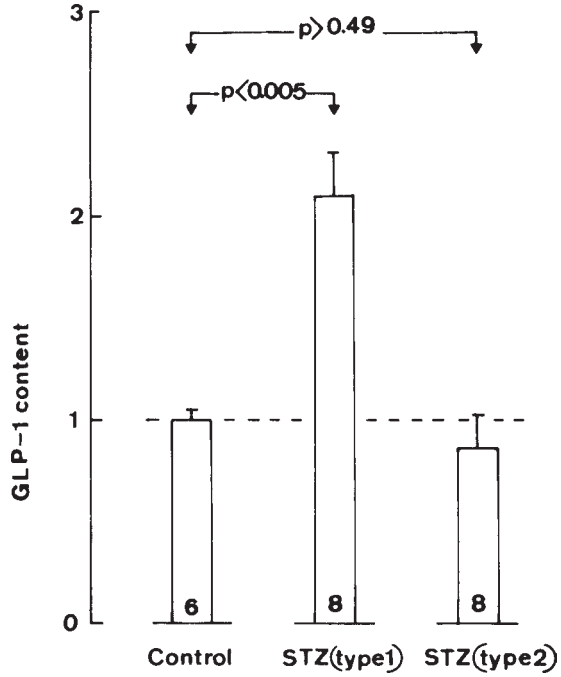

Fig. 3. GLP-1 content (ng) of the intestinal tract (excluding the cecum) in control rats and type 1 or type 2 diabetic animals is expressed relative to the mean value found in control rats of the same sex. Mean values (±SEM) refer to the number of individual observations indicated at the bottom of each column. Also shown are the statistical significances of the differences between the mean value found in control rats (dotted horizontal line) and those recorded in the diabetic animals.

further study *(10)*. Control and diabetic rats were kept concurrently and sacrificed at comparable ages. The desired glucose outcomes were met in virtually all type 1 diabetic rats injected at the adult age with STZ, and in 26 of 30 type 2 diabetic rats injected with STZ during the neonatal period. All measurements in the biologic samples were made without knowledge of the identity of the animal group.

Biologic Samples

Blood samples were collected from the severed tail for measurement of D-glucose by the glucose oxidase method *(11)*, in blood (Glucocard Memory Strips; Menarini) and also in plasma (Glucose analyzer 2; Beckman, Galway, Ireland), and for that of plasma insulin by radioimmunoassay (RIA) *(12)* using rat insulin (Linco, St. Charles, MO) as standard and a guinea pig antiinsulin serum (GP-25) developed in our laboratory. The entire small and large intestine was washed with cold 0.9% saline and trimmed free of all mesenteric tissues. The small intestine, from the Treitz's ligament up to the ileocecal opening, was divided into two equal parts; the proximal segment was designated as the jejunum, and the distal one as the ileum. The lengths of the jejunum, ileum, and colon were measured. Gut segments were then frozen in liquid nitrogen and stored at −80ºC until GLP-1 extraction.

GLP-1 Extraction

The method was based on that described by Luttichau et al. *(13)*, with some modifications. The frozen tissue was quickly minced in boiling water (5 mL/g of wet tissue) and further boiled for 5 min. Then glacial acetic acid was added to reach a final concentration of 0.5 mol/L, and the mixture was homogenized and centrifuged at 10,000*g* for 20 min, at 4ºC. An aliquot volume of the supernatant (extract) was taken for protein content determination *(14)*, and the rest was lyophilized and redissolved in the GLP-1 assay buffer.

GLP-1 Assay

The extract samples, or GLP-$1^{7\text{-}36\ \text{amide}}$ standard, were incubated for 4 d at 4ºC in 0.4 mL of 0.2 mol/L of glycine, pH 8.8, containing 0.25% human serum albumin (HSA), 500 kallikrein inhibitor unit (KIU)/mL of Trasylol, and 1 fmol of mono-^{125}I-labeled tracer *(15)* in the absence (nonspecific binding) or presence of the anti-GLP-1 serum (2135, 1:50,000 final dilution). Separation of free from bound peptide was achieved by dextran-coated charcoal treatment (0.5 mL of 0.25–0.50% in 0.2 mol/L of glycine, pH 8.8) and subsequent centrifugation at 4ºC. The 2135 serum, a gift from Dr. J. J. Holst (Copenhagen), is a side-viewing antiserum that recognizes all molecules containing the central sequence of GLP-1 regardless of C- or N-terminal truncations or extensions; it fully reacts with GLP-$1^{7\text{-}36\ \text{amide}}$, GLP-$1^{7\text{-}37}$, GLP-$1^{1\text{-}36\ \text{amide}}$, and GLP-$1^{1\text{-}37}$, and 79% with GLP-$1^{9\text{-}36\ \text{amide}}$ *(16)*.

Pancreas Insulin Extraction

The method was based on that of Jackson et al. *(17)*. The pancreas (total tissue) was homogenized (Polytron) in 82% ethanol adjusted to pH 8.2 with phosphoric acid (2.5 mL/g of tissue) at 4ºC, and then sonicated. The mixture was kept overnight at 4ºC and then spun down (1800*g* for 15 min at 4ºC). The supernatant was collected and saved at 4ºC, and the pellet was subjected to a reextraction with 65% ethanol, pH 8.2 (1.25 mL/g of tissue), for 4–6 h at 4ºC and then again centrifuged. The new supernatant was mixed with the first one, and this mixture was used for insulin and protein measurement. The pellet was dissolved in 0.5 *N* KOH at 60ºC for protein content determination *(14)*. For insulin measurement (RIA), extract samples were previously dried with a Speed-vac and redissolved in the RIA buffer.

Statistical Analyses

All results are presented as mean values (±SEM) together with the number of individual determinations (*n*). Statistical analysis of results was conducted, as required, by either student's *t*-test or analysis of variance completed by Bonferroni multiple comparison test. The level of significance was set at 0.05.

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