The effects of 13 wk of liraglutide treatment on endocrine and exocrine pancreas in male and female ZDF rats: a quantitative and qualitative analysis revealing no evidence of drug-induced pancreatitis

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Vrang N, Jelsing J, Simonsen L, Jensen AE, Thorup I, Søeborg H, Knudsen LB. The effects of 13 wk of liraglutide treatment on endocrine and exocrine pancreas in male and female ZDF rats: a quantitative and qualitative analysis revealing no evidence of drug-induced pancreatitis. Am J Physiol Endocrinol Metab 303: E253–E264, 2012. First published May 15, 2012; doi:10.1152/ajpendo.00182.2012.—A possible association between glucagon-like peptide-1 (GLP-1) analogs and incidences of pancreatitis has been suggested based on clinical studies. In male and female diabetic Zucker diabetic fatty (ZDF) rats, we investigated the effects of continuous administration of liraglutide and exenatide on biochemical [lipase, pancreatic amylase (P-amylase)] and histopathological markers of pancreatitis. Male and female ZDF rats were dosed for 13 wk with liraglutide (0.4 or 1.0 mg·kg


-1·day


-1) or exenatide (0.25 mg·kg


-1·day


-1·sc once daily) or exenatide (0.25 mg·kg


-1·day


-1·sc, Alzet osmotic minipumps). P-amylase and lipase plasma activity were measured, and an extended histopathological and stereological (specific cell mass and proliferation rate) evaluation of the exocrine and the endocrine pancreas was performed. Expectedly, liraglutide and exenatide lowered blood glucose and Hb A1c in male and female ZDF rats, whereas β-cell mass and proliferation rate were increased with greatly improved blood glucose control. Whereas neither analog affected lipase activity, small increases in P-amylase activity were observed in animals treated with liraglutide and exenatide. However, concurrent or permanent increases in lipase and P-amylase activity were never observed. Triglycerides were lowered by both GLP-1 analogs. The qualitative histopathological findings did not reveal adverse effects of liraglutide. The findings were mainly minimal in severity and focal in distribution. Similarly, the quantitative stereological analyses revealed no effects of liraglutide or exenatide on overall pancreas weight or exocrine and duct cell mass or proliferation. The present study demonstrates that, in overtly diabetic male and female ZDF rats, prolonged exposure to GLP-1 receptor agonists does not affect biochemical or histopathological markers of pancreatitis, and whereas both exenatide and liraglutide increase β-cell mass, they have no effect on the exocrine pancreas. However, clinical outcome studies and studies using primate tissues and/or studies in nonhuman primates are needed to further assess human risk.

glucagon-like peptide-1 receptor agonists; exenatide; β-cell mass; stereology

GLUCAGON-LIKE-PEPTIDE 1 (GLP-1) receptor agonists are emerging as an important drug class for the treatment of type 2 diabetes (11). Although the GLP-1 receptor agonists are generally well tolerated (4, 12), postmarketing case reports of possible exenatide-associated acute pancreatitis (1, 2, 8, 40) led the FDA to issue postmarketing safety warnings to exenatide and liraglutide and the dipeptidyl peptidase IV inhibitor sitagliptin (41–43). Because of the low number of documented pancreatitis events even in epidemiological studies, it has been difficult to confirm a causal relationship between exenatide treatment and pancreatitis.

Acute pancreatitis is a clinical condition with a variable severity ranging from mild to life-threatening. The diagnosis is based on a number of clinical signs and laboratory findings, where abdominal pain coupled with increases in lipase and pancreatic amylase (P-amylase) are among the most common findings (17). It is believed that the pancreatic acinar cells play an important role in the pathogenesis of acute pancreatitis and that autodigestion and efflux of digestive enzymes into the interstitial space are the primary events leading to exaggerated inflammation, edema, and necrosis (23). Recent epidemiological studies have demonstrated that although both obesity and diabetes increase the risk of acute pancreatitis (5, 18), exenatide treatment does not appear to increase the risk of acute pancreatitis in these patient populations (9, 10, 18). However, a study analyzing the FDA Adverse Event Reporting System database showed a significantly increased incidence of pancreatitis with exenatide and sitagliptin (13). It should be noted that the individual reports in the database are unvalidated.

Preclinical studies have indicated the presence of a functional GLP-1 receptor on acinar cells (15, 26, 32, 34) and on pancreatic ductal cells (44, 45). Koehler et al. (24) demonstrated an increased pancreas weight but also increased expression of pancreatic anti-inflammatory proteins in mice treated with exendin-4 or liraglutide, hereby indicating a GLP-1 receptor-mediated proliferation of the pancreas. No effects of exendin-4 on chemically (caerulein) induced pancreatitis were observed (24), in agreement with other recent studies (38). In contrast to these studies, chronic treatment with exendin-4 has been reported to increase the signs of pancreatitis in rats with increased scores of inflammation and pyknotic nuclei and increased lipase (but not amylase) (29). Similarly, it has been suggested that elevated GLP-1 levels were responsible for sitagliptin-associated ductal metaplasia and increased duct cell proliferation in a transgenic rat model of type 2 diabetes (28). However, in the latter study, treatment with a GLP-1 receptor agonist was not investigated, nor were plasma levels of GLP-1 measured (28).
Since GLP-1 receptor-mediated β-cell neogenesis has been observed in diabetic rodents (44, 45), and since pancreatitis is more frequently encountered in diabetic than in healthy humans, we used the diabetic Zucker diabetic fatty (ZDF) rat as a model to uncover potential side effects of exenatide and liraglutide. Furthermore, whereas a number of studies have used stereological methods to quantify changes in endocrine pancreas mass (β/non-β-cell mass) following GLP-1 receptor stimulation (19, 33, 36), none of the available studies investigating effects of GLP-1-based drugs on the exocrine pancreas have used stereological quantification (24, 28, 29, 38). Therefore, we applied a combination of histopathological and stereological methods to describe not only qualitative but also quantitative changes in the endocrine and exocrine pancreas following 13 wk of dosing with exenatide or liraglutide to diabetic ZDF rats.

**METHODS**

**Animals.** 144 obese fa/fa (72 males, 72 females) and 24 lean Fa/− (12 males, 12 females) ZDF rats were purchased from Charles River Laboratories. Female (5–7 wk of age on arrival) and male rats (6–7 wk of age on arrival) were housed two per cage at a target temperature of 22 ± 2°C, relative humidity 50 ± 10%, with a 12:12-h light-dark cycle. Animals were provided with bedding, nesting, and a hide for environmental enrichment. Animals had free access to diet and tap water. Male rats were fed Purina 5008 (Brogaarden) during the entire study period, whereas female rats were fed the high-fat diet RD12468 (Research Diets, New Jersey) for 6 wk and then switched to Purina 5008 for the remainder of the study. All animal experiments were conducted in accordance with the internationally accepted Principles of Laboratory Animal Care (National Institutes of Health publication no. 85-23, revised 1985) and in compliance with personal animal licenses for J. Jelsing (2008/561-1565) issued by the Danish Committee for Animal Research.

**Compounds.** Six milligrams per milliliter of liraglutide (Victoza; Novo Nordisk) was diluted in isotonic buffered solution. Exenatide minipump phase dose formulations were prepared by dissolving exendin-4 (Bachem) in sodium acetate buffer, pH 4.5 (50 mM sodium acetate; 0.05%/Tween-80, 2% glycerol). During the subcutaneous (sc) dose titration period, exenatide dose formulations were diluted in exendin-4 (Bachem) in sodium acetate buffer, pH 4.5 (50 mM sodium acetate; 0.05%/Tween-80, 2% glycerol). During the subcutaneous (sc) dose titration period, exenatide dose formulations were diluted in exendin-4 (Bachem) in sodium acetate buffer, pH 4.5 (50 mM sodium acetate; 0.05%/Tween-80, 2% glycerol). During the subcutaneous (sc) dose titration period, exenatide dose formulations were diluted in exendin-4 (Bachem) in sodium acetate buffer, pH 4.5 (50 mM sodium acetate; 0.05%/Tween-80, 2% glycerol). During the subcutaneous (sc) dose titration period, exenatide dose formulations were diluted in exendin-4 (Bachem) in sodium acetate buffer, pH 4.5 (50 mM sodium acetate; 0.05%/Tween-80, 2% glycerol)).

**Stereological analyses.** Estimates of total mass were determined using the Cavalieri principle of uniform random systematic sectioning in combination with point counting (21). Exocrine and endocrine cell populations were identified by use of immunohistochemical and histochemical staining procedures (Fig. 1). CK20, an epithelial marker that is known to be expressed in rat pancreatic cells of the ductal system from centroacinar cells to main ducts (3), were used to identify intercalated duct cells from exocrine acinar cells (Fig. 1). No differentiation was made between centroacinar cells and main ducts since the latter contribute little to the overall duct cell mass (Fig. 1). The stereological estimations were performed using the newCAST system (Visiopharm, Copenhagen, Denmark) on digital slides scanned with a 40×20 objective on a Hamamatsu Nanozoomer 2.0 HT. Cell masses were estimated by point counting, using a grid system where all points hitting the structure of interest were counted (Fig.1). The estimation of proliferating cells was based on a modification of the unbiased three-dimensional counting rules. Hence, potential changes in specific cell proliferation were assessed by counting Ki67-labeled profiles using a two-dimensional unbiased counting frame, hereby providing an estimate of Ki67-labeled nuclei per area reference volume (Fig. 1). The Ki67 ratio was calculated as the Ki67-labeled nuclei per area reference volume multiplied by specific cell mass estimates to compensate for the differences in total cell mass between experimental groups.

**Statistical analyses.** In-life data were analyzed by one-way ANOVA models, with change from baseline value as response and with baseline value as a covariate. In addition to the normal active drug-to-vehicle group comparisons, the sc diabetic vehicle group was compared with the lean vehicle group and the diabetic baseline group. Statistical analyses were performed using SAS 9.2. Each sex was analyzed separately, and P < 0.05 was considered statistically significant.
RESULTS

Body weight and food and water intake. Both liraglutide and exenatide treatment initially reduced body weight gain (Fig. 2, A and B). In females, liraglutide-treated animals had increased body weight toward the end of the treatment period, whereas the body weight of the exenatide group was unchanged (Fig. 2B). Both liraglutide and exenatide markedly reduced food and water intake (Fig. 2, C–F).

Blood glucose, Hb A1c, and triglyceride levels. Blood glucose was lowered significantly in all treated animals (Fig. 3, A and B). In females fed the high-fat diet, mean blood glucose levels were ∼17 mmol/l at the time of initiation of treatment. Blood glucose levels continued to rise in vehicle-dosed animals to ∼25 mmol/l while on the high-fat diet. When animals were switched back to chow diet (Purina 5008), blood glucose levels dropped to mean stable blood glucose level ∼20 mmol/l (Fig. 3B). Both liraglutide and exenatide treatment caused a greater drop in blood glucose in females than in males (Fig. 3, A vs. B). Exenatide treatment reduced blood glucose to a greater extent than liraglutide in males (Fig. 3A), whereas liraglutide treatment was more efficacious in females (Fig. 3B). In both male and female rats, the effect of exenatide on blood glucose was not fully apparent until the continuous dosing regimen was initiated (Fig. 3, A and B).

Hb A1c levels were improved significantly in both liraglutide- and exenatide-treated animals at the end of the treatment period (Table 1 and Fig. 3, C and D).

Triglyceride levels in male animals were reduced significantly in the low- and high-dose liraglutide groups, whereas no significant effects of exenatide treatment were observed (Table 1). In females, exenatide treatment reduced triglyceride levels significantly, whereas no significant effect of liraglutide treatment was observed (Table 1).

Biochemical markers of pancreatitis. Neither liraglutide nor exenatide treatment increased plasma activity of lipase (Fig. 4, A and D). Actually, the lipase activity in liraglutide- and exenatide-treated animals tended to be lower than in vehicle animals (Fig. 4, A and D). In females treated with both low- and high-dose liraglutide, lipase activity was significantly lower than in vehicle animals at the end of treatment (Table 1). In male rats, P-amylase activity remained constant throughout the study period (Fig. 4B). However, when adjusting for baseline, end-of-treatment P-amylase activities were significantly higher in all treated groups than in the corresponding vehicle groups (Table 1 and Fig. 4B). In females, P-amylase activity was increased in treated rats (Fig. 4E). The increase in P-amylase in female rats was most pronounced on study day 29 immediately following the diet change on day 28 (Fig. 4E). No animals displayed permanent elevations of lipase or P-amylase, and more importantly, no animals displayed simultaneous elevations of lipase and P-amylase (Fig. 4, C and F).

Mortality. A total of nine rats (4 males and 5 females) died or were euthanized prematurely. The four male animals were from the pump vehicle group, and three deaths were related to a single erroneous dosing of liraglutide (0.5 mg/kg), which would be expected to lead to dehydration since GLP-1 acutely has powerful effects on water intake and diuresis (37). The death of the fourth male animal was related to a persistent pump infection. Two female animals euthanized prematurely were from the pump vehicle group, whereas three female animals were from the exenatide group. Three of these deaths...
Fig. 2. Body weight (A and B), food intake (C and D), and water intake (E and F) in male (A, C, and E) and female (B, D, and F) Zucker diabetic fatty (ZDF) rats during the 13-wk treatment period. Exenatide pumps were changed every 4 wk as the times indicated in the graphs. The shift from high-fat diet to Purina diet of female ZDF rats is likewise indicated in the graphs. Data are means ± SE; n = 8–12. ▲ on dotted lines, group 1 vehicle subcutaneous (sc) once daily (QD); ▲ on solid lines, group 2 liraglutide 0.4 mg·kg⁻¹·day⁻¹; △ on solid lines, liraglutide 1.0 mg·kg⁻¹·day⁻¹; ■ on dotted lines, group 4 pump vehicle; ■ on solid lines, group 5 exenatide 0.25 mg·kg⁻¹·day⁻¹; ● on dotted lines, group 7 lean vehicle sc QD.
were related to infections around the pump or associated with pump change, whereas two euthanizations (1 pump vehicle-treated and 1 exenatide-treated animal) were due to general lethargy and weight loss.

Qualitative histopathological evaluation of pancreas. In one prematurely euthanized female dosed with exenatide, moderate apoptosis-like necrosis, minimal inflammatory infiltration, and slight hemorrhage/edema were observed in the pancreas. This

Table 1. Terminal Hb A1c, lipase, P-amylase, and triglyceride levels and estimated treatment differences

<table>
<thead>
<tr>
<th></th>
<th>Vehicle sc</th>
<th>Liraglutide (Treatment Differences)</th>
<th>Vehicle Pump</th>
<th>Exenatide, 0.25 mg/kg (Treatment Differences)</th>
<th>Lean Vehicle sc</th>
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</thead>
<tbody>
<tr>
<td><strong>Hb A1c, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>8.1 ± 0.3</td>
<td>7.3 ± 0.5 (−0.8)§</td>
<td>6.9 ± 0.4 (−1.2)§</td>
<td>8.4 ± 0.4</td>
<td>6.7 ± 0.5 (−1.8)§</td>
</tr>
<tr>
<td>Lipase, U/l</td>
<td>16.0 ± 1.1</td>
<td>16.0 ± 2.5 (−0.2)</td>
<td>14.9 ± 1.0 (−1.0)</td>
<td>16.0 ± 1.1</td>
<td>14.9 ± 1.2 (−1.0)</td>
</tr>
<tr>
<td>P-amylase males, U/l</td>
<td>1,969 ± 178</td>
<td>2,313 ± 180 (+363)†</td>
<td>2,272 ± 244 (+301)§</td>
<td>2,126 ± 250</td>
<td>2,699 ± 282 (+497)§</td>
</tr>
<tr>
<td>Triglyceride males, mmol/l</td>
<td>9.1 ± 2.4</td>
<td>6.2 ± 1.5 (−2.9)§</td>
<td>5.0 ± 1.0 (−4.1)§</td>
<td>8.9 ± 3.2</td>
<td>7.4 ± 2.0 (−1.5)</td>
</tr>
<tr>
<td>Hb A1c, females, %</td>
<td>7.4 ± 0.8</td>
<td>5.1 ± 1.4 (−2.3)§</td>
<td>4.1 ± 0.3 (−3.2)§</td>
<td>7.2 ± 0.7</td>
<td>5.4 ± 1.1 (−1.9)§</td>
</tr>
<tr>
<td>Lipase females, U/l</td>
<td>14.0 ± 1.1</td>
<td>12.2 ± 1.6 (−1.8)†</td>
<td>10.7 ± 1.2 (−3.2)§</td>
<td>16.6 ± 1.4</td>
<td>17.0 ± 2.5 (−0.6)</td>
</tr>
<tr>
<td>P-amylase females, U/l</td>
<td>1,630 ± 366</td>
<td>1,976 ± 218 (+353)†</td>
<td>2,127 ± 206 (+497)§</td>
<td>1,727 ± 196</td>
<td>1,989 ± 238 (+259)*</td>
</tr>
<tr>
<td>Triglyceride females, mmol/l</td>
<td>8.7 ± 3.7</td>
<td>7.7 ± 2.9 (−1.0)</td>
<td>10.0 ± 2.1 (1.1)</td>
<td>12.0 ± 5.2</td>
<td>8.1 ± 3.2 (−4.0)§</td>
</tr>
</tbody>
</table>

Values are means ± SD; all statistical estimates of treatment differences are performed with baseline as covariate. P-amylase, pancreatic amylase; sc, subcutaneous. Treatment differences are estimated as compared with the corresponding vehicle group (sc/pump). *P < 0.05; †P < 0.01; ‡P < 0.001; §P < 0.0001.
correlated with an edematous and hemorrhagic pancreas seen at necropsy. Furthermore, at necropsy, the distal intestines appeared dark and hemorrhagic, in line with the clinical observation prior to euthanization that this animal suffered from acute generalized disease.

The main histopathological pancreas findings from the terminally euthanized animals are compiled in Table 2. In general, all histopathological changes were low in incidence and severity (Fig. 5). Most of the findings were given the minimal score and were focal in distribution. The highest incidences of pathological lesions were observed in the lean vehicle group (males) and the diabetic baseline groups (both sexes), with only focal acinar cell hyperplasia being more frequently observed in the exenatide-treated male rats. In females, minimal “acinar cell atrophy/metaplasia/duct proliferation” was seen with slightly higher incidence in the liraglutide-treated groups compared with the liraglutide vehicle group. However, the incidences were similar to or below those seen in female lean and diabetic baseline animals (Table 2).

Quantitative stereological evaluation of pancreas and pancreas weight. Liraglutide and exenatide treatment did not affect pancreas weight in male ZDF rats (Fig. 6A). In females, liraglutide decreased pancreas weight significantly, whereas no effect of exenatide was observed (Fig. 6B). The stereologically quantified masses of exocrine acinar and duct cells in males and females are shown in Fig. 6, C–F. Neither duct nor acinar cell mass was altered significantly by treatment. In contrast, a significant effect of both phenotype and age was apparent when the control groups were compared (Fig. 6, C–F). The assessment of specific cell proliferation in males showed no significant changes in duct or acinar cell proliferation in liraglutide-treated rats, whereas the acinar cell proliferation rate was slightly increased in the exenatide group (Fig. 7, A and C). In females, no significant effects of treatment were seen in duct or acinar cell proliferation (Fig. 7, B and D). Significant alterations in cellular proliferation rates were related to control groups.

Stereological estimates of β-cell and non-β-cell mass are shown in Fig. 8. In males, exenatide treatment led to a significantly higher β-cell mass, whereas no effect on β-cell mass of liraglutide treatment was observed (Fig. 8A). A significantly higher β-cell mass was observed in male lean ZDF rats and in the diabetic rats euthanized at baseline compared with the sc vehicle-dosed diabetic rats (Fig. 8A). In females, both liraglu-
tide- and exenatide-treated rats had a significantly higher β-cell mass (Fig. 8B). As seen in males, β-cell mass in the baseline group was high (Fig. 8B). In both males and females, β-cell proliferation rates were higher in groups with a high β-cell mass (Fig. 8, C and D). The quantitative analyses of endocrine non-β-cell mass revealed no effects of treatment (Fig. 8, E and F).

**DISCUSSION**

In the current study, we examined the effects of liraglutide and exenatide on biochemical and histological markers of pancreatitis when administered in high doses to both male and female diabetic ZDF rats for 13 wk. The data from the current study are the first to report long-term antidiabetic efficacy data in the female ZDF rat. The glucose-lowering effects of both liraglutide and exenatide were more potent in females than in males. In addition, the female rats revealed a dose response effect of liraglutide that was not apparent in the males. One possible explanation for this apparent discrepancy relates to the differential diabetes syndromes in male and female ZDF rats. Whereas males develop diabetes spontaneously on the Purina 5008 diet, females become diabetic only if fed a specialized high-fat diet (39). Pilot studies performed in our laboratory (data not shown) revealed an increased mortality when female ZDF rats were fed the high-fat diet (RD12468) for more than 8 wk, which was probably related to high blood glucose levels (>30 mM), kidney stones, and nephritis. Therefore, we induced diabetes by feeding the female ZDF rats the RD12468 diet for 6 wk and then switching them to Purina 5008.

Mean lipase activities of liraglutide- and exenatide-treated rats were relatively stable during the study period, except for an increase in exenatide-treated female rats at the last time point. Notably, there was a general tendency for lipase activity to be significantly lower than the corresponding vehicle groups. P-amylase activities were generally higher in the liraglutide- and exenatide-treated animals. In males, P-amylase did not change markedly during the study period, whereas in females P-amylase was increased in liraglutide- and exenatide-

Table 2. Summary of selected histopathological findings in the exocrine pancreas in terminally euthanized animals

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Vehicle sc</th>
<th>0.4 mg/kg</th>
<th>1.0 mg/kg</th>
<th>Vehicle Pump</th>
<th>Exenatide, 0.25 mg/kg</th>
<th>Lean Vehicle sc</th>
<th>ZDF Baseline</th>
</tr>
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<tbody>
<tr>
<td>Males#</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals examined</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Acinar cell atrophy/metaplasia/ductal proliferation§</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Acinar cell atrophy†</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Inflammatory cell infiltration†</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Acinar cell hyperplasia, focal</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Females#</td>
<td></td>
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<tr>
<td>No. of animals examined</td>
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<td>10</td>
<td>9</td>
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<td>Inflammatory cell infiltration†</td>
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<td>1</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>3</td>
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</tr>
</tbody>
</table>

ZDF, Zucker diabetic fatty. #The lesions were mostly of minimal grade and focal in distribution. §Areas showing reduced nos. of acinar cells and increased nos. of duct cells due to acinar cell atrophy, acinar cell metaplasia, and/or duct proliferation. †Areas where only the acinar cells are affected. TThe inflammatory cells were located in the interstitium between the acini, and in most cases the acinar cells were unaffected. In some cases, the inflammatory cells were seen to exist concurrently with acinar cell atrophy/metaplasia/ductal proliferation and acinar cell atrophy.

![Fig. 5. Pancreas histopathology. Photomicrographs from a male (A) and female ZDF rat (C) terminated at baseline and a male (B) and female ZDF rat (D) treated with liraglutide for 13 wk (1 mg·kg⁻¹·day⁻¹). Small areas of focal acinar cell atrophy/metaplasia/ductal proliferation are evident in both groups (magnification of insert shown in lower right corner).](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00182.2012)
treated groups, most markedly at day 29, immediately following the diet change to Purina 5008 on day 28. One possible explanation for the selective elevation of P-amylase activity could be a differential excretion and reabsorption of lipase and amylase in the kidneys. In rats, both lipase and amylase are excreted by the kidneys, but the reabsorption of lipase is higher than that of amylase (22, 27). Furthermore, it has been shown that urinary excretions of amylase in rats with normal kidney function are directly proportional to the glomerular filtration rate (16). Hence, although a certain degree of tubular reabsorption occurs, osmotic diuresis can increase amylase excretion (16). In support of the explanation for the paradoxical elevations in P-amylase, lipase and amylase were never simultaneously elevated, and no treatment-related pathological or stereological indications of acute pancreatitis were identified in the postmortem pancreatic analyses. Finally, it should be noted that the elevations in P-amylase observed here (maximally 50%) were of a considerably lower magnitude than that observed in rat models of chemically induced pancreatitis, where 200–300% elevations in P-amylase and lipase were seen (38).

Fig. 6. Absolute pancreas weight (A and B), duct cell mass (C and D), and acinar cell mass (E and F) in male (A, C, and E) and female ZDF rats (B, D, and F). Bars represent means ± SE; n = 8–12. *P < 0.05 compared with the corresponding vehicle (Veh) group. The diabetic baseline group is compared with the subcutaneous (s.c) Veh group. Lira, liraglutide; Exe, exenatide.
Our data are in agreement with a study using a shorter treatment period (38) as well as data from healthy Sprague-Dawley (SPD) rats treated for 12 wk with exenatide (20). Exenatide has actually been shown to exert slight but significant protection against chemically induced pancreatitis in diabetic db/db mice, SPD rats, and ZDF rats (38). In one study, exenatide administration for 75 days to normoglycaemic male SPD rats induced minor elevations (64%) in lipase activity but a nonsignificant decrease in amylase (29).

There was no indication from the histopathological or stereological evaluation of the exocrine pancreas that 13 wk of liraglutide or exenatide treatment induced pancreatitis in the ZDF rat. Neither exenatide nor liraglutide increased pancreas weight.

In the current study, the histopathological analysis focused on morphological changes in the exocrine pancreas. In one decedent female dosed with exenatide, necrosis in the pancreas was observed. This necrosis was described as apoptosis-like due to the condensed acinar cells and the minimal inflammatory cell infiltration. The mild degree of inflammatory cell infiltration and the apoptosis-like necrosis were not considered to support the diagnosis of an acute pancreatitis. Furthermore, macroscopic findings in the intestine indicated that this animal suffered from acute generalized disease. In all other animals, the histopathological evaluation of the exocrine pancreas showed that the lean vehicle and baseline groups had the highest incidences of “acinar cell atrophy/metaplasia/duct proliferation,” “acinar cell atrophy,” and “inflammatory cell infiltration.” Slightly higher incidences of acinar cell atrophy/metaplasia/duct proliferation were seen in female ZDF rats treated with liraglutide compared with the vehicle group. However, the incidences were similar to or below those seen in the lean vehicle group and the diabetic baseline group. A similar difference was not seen in males where the highest incidence of acinar cell atrophy/metaplasia/duct cell proliferation was seen in the lean vehicle group. Thus, this finding is not likely to be related to liraglutide treatment. Although our histopathological findings are in good agreement with pancreatic histopathological data recently reported from a two-year rat carcinogenicity study conducted as part of the development program for liraglutide (30) and studies examining the effects of exenatide on caerulein-induced pancreatitis (38), it should be noted that one study reported increased inflammation and pyknotic nuclei scores in the pancreas following exenatide administration for 75 days (29). Unfortunately, the article did not report the number of cases from their vehicle- and exenatide-treated SPD rats displaying morphological changes but rather group-based scores of pyknotic nuclei and inflammation, making direct

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**Fig. 7.** Duct cell proliferation (A and B) and acinar cell proliferation (C and D) in male (A and C) and female ZDF rats (B and D). Bars represent means ± SE; n = 8–12. *P < 0.05 compared with the corresponding Veh group. The diabetic baseline group is compared with the sc Veh group.
comparisons between that study and other reports difficult (29).
Our data indicating that prolonged GLP-1 receptor stimulation
does not induce pancreatitis are also in agreement with pan-
creas histopathological findings in healthy male SPD rats
treated once daily (10 μg/kg) for 12 wk (20). However, Gier et
al. (20) also examined duct cell proliferation, with a particular
emphasis on the recently identified so-called periductal glands
(35). Interestingly, exenatide treatment appeared to increase
not only the number of periductal glands but also the prolifer-
aton of duct cells in these glands and in glands around the
large collecting ducts (20). Although the physiological and
pathophysiological role of these mucin-producing glands is

Fig. 8. β-cell mass (A and B), β-cell proliferation (C and D) and non-β-cell mass (E and F) in male (A, C, and E) and female ZDF rats (B, D, and F). Bars represent means ± SE; n = 8–12. *P < 0.05 compared with the corresponding Veh group. The diabetic baseline group is compared with the sc Veh group.
LIRAGLUTIDE DOES NOT INDUCE PANCREATITIS IN THE ZDF RAT

still rather speculative, gland number has been reported to be increased during chronic pancreatitis in mice, and it has been speculated that this mucinous metaplasia could be the precursor of pancreatic epithelial neoplasia (35). In support of these findings in rats, Gier et al. (20) found increased chronic pancreatitis and pancreatic epithelial neoplasia in a chronic pancreatitis/pancreas cancer progression mouse model (PDX-1/Kras). However, it should be noted that although Gier et al. (20) counted a lot of cells, they did not apply stereological sampling or counting principles. This could potentially introduce sampling bias, which is critical when hyperplastic/hypertrophic structures (like periductal glands; see Ref. 35) are to be quantified. Despite the methodological issues, the findings by Gier et al. (20) are intriguing, and it will be important not only to further explore the role of the periductal glands in pancreas physiology/pathology but also to investigate in greater detail, and with stereological methods, the effects of GLP-1 receptor agonists on periductal gland mass and proliferation in healthy and diabetic animal models. It should be noted that in the current study we found no evidence for an increased proliferation in duct cells following either exenatide or liraglutide treatment. On the other hand, because the mass of the collecting ducts (including the periductal glands that also express CK20) makes up only a fraction of total duct cell mass, we cannot exclude a potentially increased proliferation in this particular cell type.

In the current study, using quantitative stereological analyses of the exocrine pancreas, we found no clear treatment effects on exocrine acinar or duct cell mass or proliferation. This is interesting in view of some studies suggesting that increased duct cell proliferation may be one of the mechanisms for GLP-1-induced pancreatitis and/or pancreas cancer (6, 20). This study is the first to provide true quantitative estimates of acinar and duct cell mass and proliferation using stereology. Therefore, from the current data there is no indication that exenatide, dosed continuously, or liraglutide should increase exocrine cell proliferation or exocrine cell mass.

Our finding that neither exenatide nor liraglutide increases duct cell proliferation is also interesting in light of the proposed role of duct cells as β-cell precursors. Whereas previous studies have indicated that GLP-1 and exenatide increase duct cell proliferation (possibly giving rise to novel β-cells of ductal origin) (44, 45), we failed to show this association in the current study. In males, we found significant long-term effects of liraglutide and exenatide on glucose homeostasis, but only in exenatide-treated rats was a higher β-cell mass observed. In females, both liraglutide and exenatide led to a higher β-cell mass. Although these data could indicate GLP-1 receptor-mediated β-cell mass increase, the strong coupling in both males and females between the effect on blood glucose and β-cell mass could indicate that better glucose control rather than GLP-1 receptor stimulation per se is what regulates β-cell mass in the long run. Interestingly, Ki67 immunostaining revealed that the highest degree of proliferation in both duct cells and β-cells was found in the diabetic baseline group that was euthanized at a time when β-cell mass was rapidly expanding. This could indicate that new β-cells could be of both β-cell and duct cell origin, in line with previous reports (7, 25, 44, 45).

In conclusion, the present study showed that continuous exposure to the GLP-1 receptor agonists liraglutide (dosed once daily) or exenatide (dosed continuously by pump) to male and female diabetic ZDF rats for 13 wk did not induce pancreatitis. Plasma markers that often increase in connection with pancreatitis, P-amylase and lipase, did not indicate any signs of change. The histopathological evaluation did not indicate any adverse treatment-related lesions in the pancreas. The quantitative stereology revealed no treatment-induced increases in overall pancreas mass, exocrine cell mass, or exocrine cell proliferation rate. Although these data indicate that there is not a straightforward pharmacological effect of GLP-1 receptor agonists on rat duct cells or acinar cells, they cannot rule out a human risk. The outcome studies for the GLP-1 analogs and dipeptidyl peptidase IV inhibitors will be important, but more studies are also needed with primate tissue, including human and/or in vivo studies in nonhuman primates.

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AUTHOR CONTRIBUTIONS


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