GLP-1 and Calcitonin Concentration in Humans: Lack of Evidence of Calcitonin Release from Sequential Screening in over 5000 Subjects with Type 2 Diabetes or Nondiabetic Obese Subjects Treated with the Human GLP-1 Analog, Liraglutide

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Background: Serum calcitonin (CT) is a well-accepted marker of C-cell proliferation, particularly in medullary thyroid carcinoma. Chronic glucagon-like peptide-1 (GLP-1) receptor agonist administration in rodents has been associated with increased serum CT levels and C-cell tumor formation. There are no longitudinal studies measuring CT in humans without medullary thyroid carcinoma or a family history of medullary thyroid carcinoma and no published studies on the effect of GLP-1 receptor agonists on human serum CT concentrations.

Aim: The aim of the study was to determine serum CT response over time to the GLP-1 receptor agonist liraglutide in subjects with type 2 diabetes mellitus or nondiabetic obese subjects.

Methods: Unstimulated serum CT concentrations were measured at 3-month intervals for no more than 2 yr in a series of trials in over 5000 subjects receiving liraglutide or control therapy.

Results: Basal mean CT concentrations were at the low end of normal range in all treatment groups and remained low throughout the trials. At 2 yr, estimated geometric mean values were no greater than 1.0 ng/liter, well below upper normal ranges for males and females. Proportions of subjects whose CT levels increased above a clinically relevant cutoff of 20 ng/liter were very low in all groups. There was no consistent dose or time-dependent relationship and no consistent difference between treatment groups.

Conclusions: These data do not support an effect of GLP-1 receptor activation on serum CT levels in humans and suggest that findings previously reported in rodents may not apply to humans. However, the long-term consequences of GLP-1 receptor agonist treatment are a subject of further studies. (***J Clin Endocrinol Metab*** **96: 853–860, 2011)**

Serum calcitonin (CT) is an important biomarker for C-cell diseases such as medullary thyroid carcinoma (MTC) and hereditary C-cell hyperplasia (CCH) because of its sensitivity and specificity (1, 2). Although CT monitoring is routinely used in patients diagnosed with MTC, there are no longitudinal studies on serum CT levels in individuals without underlying thyroid disease.

Liraglutide is a human glucagon-like peptide-1 (GLP-1) analog designed as once-daily therapy for patients with type 2 diabetes mellitus. Preclinical studies of this drug in mice and rats demonstrated that liraglu-

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Abbreviations: CCH, C-cell hyperplasia; CT, calcitonin; GLP-1, glucagon-like peptide-1; H2, histamine-2; LLOQ, lower limit of quantification; MTC, medullary thyroid carcinoma; PPI, proton pump inhibitor; RMA, repeated measurement analysis; UNR, upper normal range.

tide activated GLP-1 receptors on the thyroid C-cells, causing the release of CT and a dose-dependent effect of liraglutide on C-cell pathology (3). In rats, liraglutide was associated in both genders with CCH, C-cell adenomas, and C-cell carcinomas. Of note, rats are susceptible to spontaneous development of C-cell lesions with age (4, 5). Very high doses of liraglutide (~45 times human exposure) also caused a small number of C-cell carcinomas in female mice.

Cynomolgus monkeys receiving high doses of liraglutide (~64-fold human exposure) did not demonstrate an increase in serum CT levels and did not develop C-cell pathology (3). In contrast to a rodent C-cell line (3), where GLP-1 receptor agonists stimulate both cAMP and CT secretion, liraglutide did not cause CT stimulation *in vitro* in a human C-cell line (TT). The TT cell line and sections of human thyroid tissue have been shown to have far fewer GLP-1 receptors than rodent C cells *in vitro* or *in vivo* (3). Because of these species differences, the relevance of rodent data for humans (6) remains unknown.

To examine the effect of liraglutide on serum CT in humans, serial CT measurements were incorporated in a series of clinical studies comparing the efficacy of liraglutide with placebo or a variety of standard therapies for over 5000 patients with type 2 diabetes mellitus or nondiabetic obese subjects. In addition, the pattern of serum CT measurements over a 2-yr period was explored. To date, there are no reports of screening programs assessing CT levels over time, either in healthy volunteers or in individuals with diabetes or obesity. This paper focuses on the results of the liraglutide CT screening program.

Subjects and Methods

Study design and subject inclusion

CT concentration was monitored at baseline and at 12-wk intervals thereafter in all subjects enrolled in six phase 3 clinical trials, each with a similar randomized and controlled trial design. All trials were performed in accordance with the Declaration of Helsinki, and Good Clinical Practice Guidelines. Each Liraglutide Effect and Action in Diabetes (LEAD) trial had core phases of 26- to 52-wk duration, and two trials had open-labeled extensions with data available for up to 104 wk. Key aspects of the core phases of the six trials have been published previously (7–12). CT concentrations also were monitored in two phase 3 trials with Japanese subjects with type 2 diabetes (trial IDs 1700 and 1701), and in one phase 2 trial with nondiabetic obese subjects (trial ID 1807)—similar to the LEAD trials, the core endpoints for these three trials are already or soon to be published (13–15). In all trials, subjects were randomized to receive liraglutide at doses ranging from 0.6–3.0 mg, active comparator and/or placebo. Table 1 summarizes these trials.

The primary endpoint in all diabetes trials was glycosylated hemoglobin (%), and due to ineffective therapy, the number of subjects exposed to placebo who completed the long-term trials diminished proportionately more than the number of subjects treated with liraglutide or active comparators. Subjects were allowed to take concomitant medications during the trials including histamine-2 (H2) receptor blockers and proton pump inhibitors (PPIs), which are known to increase CT levels (16).

Assay

Serum CT concentration was measured by a highly specific and sensitive chemiluminescent enzyme immunoassay (Immulite 2000; Siemens Medical Solutions Diagnostics, Deerfield, IL). For this assay, the upper normal range (UNR) was 5 ng/liter for females and 8.4 ng/liter for males, and the lower limit of quantification (LLOQ) was 0.7 ng/liter. In the Japanese trials, the UNRs were 4.43 and 5.87 ng/liter for females and males, respectively, with the LLOQ being 2.0 ng/liter.

TABLE 1. Trial details where CT concentrations were monitored

Trial ID: no.; first author, year (Ref.)	Core trial duration (wk)	Core trial + extension phase (wk)	Liraglutide doses tested (mg/d)	Randomized subjects (n)	Mean age (yr)	Mean weight (kg)	Active comparator added to pre/run-in treatment (dose)	Pre/run-in treatment to which placebo was added (dose)	Clinicaltrial.gov ID (extensions)
LEAD-1: 1436; Marre, 2009 (7)	26	N/A	0.6, 1.2, 1.8	1041	56.1	81.6	Rosiglitazone (2–4 mg)	Glimepiride (2–4 mg)	NCT00318422
LEAD-2: 1572; Nauck, 2009 (8)	26	104	0.6, 1.2, 1.8	1091	56.8	88.6	Glimepiride (4 mg)	Metformin (1500–2000 mg)	NCT00318461(NCT00395746)
LEAD-3: 1573; Garber, 2009 (9)	52	104	1.2, 1.8	746	53.0	92.6	Glimepiride (8 mg)	N/A	NCT00294723 (extension phase listed under same ID)
LEAD-4: 1574; Zinman, 2009 (10)	26	N/A	1.2, 1.8	533	55.1	96.3	N/A	Metformin (2000 mg) and rosiglitazone (8 mg)	NCT00333151
LEAD-5: 1697; Russell-Jones, 2009 (11)	26	N/A	1.8	581	57.5	85.4	Glargine (variable dose)	Metformin (2000 mg) and glimepiride (2–4 mg)	NCT00333151
LEAD-6: 1797; Buse, 2009 (12)	26	40	1.8	464	56.7	93.0	Exenatide (10 μg, twice daily)	Maximum tolerated dose of metformin and/or sulfonylurea	NCT00333151
1700; Kaku, 2010 (13)	24	48	0.9	411	58.4	66.1	Glibenclamide (1.25–2.5 mg)	N/A	NCT00393718
1701; Seino, 2010 (14)	24	48	0.6, 0.9	267	59.7	65.8	Glimepiride (≤2.5 mg)	Sulfonylurea	NCT00395746
1807; Astrup, 2009 (15)	20	52	1.2, 1.8, 2.4, 3.0	564	45.9	97.2	Orlistat (120 mg)		NCT00395746

N/A, Not available in given trial.

Statistical analysis and pooling

Effects of the various treatments on CT were assessed by evaluating estimated means of the basal levels (fasting CT) and outliers of the basal levels.

Geometric mean levels of CT

The statistical evaluation of treatment differences in CT was conducted by a repeated measurement analysis (RMA) for normal censored data, where the logarithm of CT was the (censored) response and the trial, time, treatment, gender, and treatment by time interaction were fixed effects. Subjects were entered as random effects. CT was included as a censored variable, because a large percentage of the CT values was below the LLOQ. Separate estimation of geometric means and pair-wise comparisons of treatment effect were made at all visits where CT was measured to enable an evaluation of the trends over time.

Pooled RMA were performed and presented for wk 26 (LEAD-1 to -5, including all data up to wk 26) and wk 52 and 104 (LEAD-2 and -3 including all data up to wk 104) by pooling treatment groups by dose of liraglutide, placebo, and active comparator. The LEAD-6 trial directly compared two GLP-1 receptor agonists (liraglutide and exenatide); CT concentration data from this trial were examined separately to investigate any treatment-specific effects. Trial 1807 included patients with obesity without diabetes mellitus who received higher doses of liraglutide (1.2–3 mg/d) for 52 wk. These data were also analyzed separately to investigate potential effects of higher liraglutide doses on CT concentration.

Outlier analysis

CT shift tables were assessed using two approaches with the following categories: one defined by the UNR in the assay [<UNR (UNR − 2UNR), and ≥2UNR], and one defined by CT values generally considered to clinically indicate underlying C-cell abnormalities (<20 ng/liter, 20 to <50 ng/liter, 50 to <100 ng/liter, and ≥100 ng/liter]. Serum CT concentration greater than 20 ng/liter is considered a potential marker for C-cell disease that warrants additional evaluation (17).

CT data from 26, 52, and 104 wk are presented; wk 20–28 and wk 48–52 were pooled and will be referred to as wk 26 and 52, respectively.

The following analysis sets were used:

- 26-wk safety analysis set: LEAD 1–6, 1700 and 1701 phase 3 trials, plus the obesity trial 1807 (liraglutide, n = 3551; placebo, n = 710; active comparator, n = 1412);
- 52-wk safety analysis set: LEAD-2 and LEAD-3, 1700, 1701, and 1807 (liraglutide, n = 1741; placebo, n = 216; active comparator, n = 630); and
- 104-wk safety analysis set: LEAD-2 and LEAD-3 (liraglutide, n = 839; placebo, n = 61; active comparator, n = 320).

Results

Baseline characteristics

Table 1 (7–12) summarizes demographic data from subjects included in the core phases of the six LEAD trials and trials 1700, 1701, and 1807 from which CT data were collected and analyzed. The estimated median baseline CT concentration across all subjects from the pooled RMA was at the very low end of the normal range (0.6 ng/liter).

Data from phase 3 trials—26-, 52-, and 104-wk data

Geometric mean levels of CT

At 26 wk, the estimated geometric mean CT concentrations were 0.96, 0.99, and 1.01 ng/liter for liraglutide 0.6, 1.2, and 1.8 mg, respectively. In the pooled active comparator group and the placebo group, CT concentrations were 0.95 and 0.90 ng/liter, respectively. At 26 wk, statistically significant treatment differences were apparent between liraglutide 1.8 mg and active comparator (1.01 *vs.* 0.95 ng/liter; $P = 0.0018$) and placebo (1.01 *vs.* 0.90 ng/liter; $P = 0.0281$), and between liraglutide 1.2 mg and placebo (0.99 *vs.* 0.90 ng/liter; $P = 0.0189$).

By wk 52, the mean absolute concentrations of CT were 0.76, 0.84, and 0.83 ng/liter for liraglutide 0.6, 1.2, and 1.8 mg, respectively. For the active comparator and placebo groups, mean CT concentrations were 0.77 and 0.66 ng/liter, respectively. Mean CT concentrations were statistically higher with liraglutide 1.8 mg ($P = 0.0472$) and 1.2 mg ($P = 0.0400$) compared with placebo but not compared with active comparator.

The results of serum CT levels over time for up to 104 wk for three doses of liraglutide, for placebo, and for active comparators are illustrated graphically in Fig. 1 (3). Subjects exposed to liraglutide for 104 wk had no clinically significant increases in serum CT concentration from wk 52, with estimated levels being 0.73, 0.79, and 0.76 ng/liter for liraglutide 0.6, 1.2, and 1.8 mg, respectively.

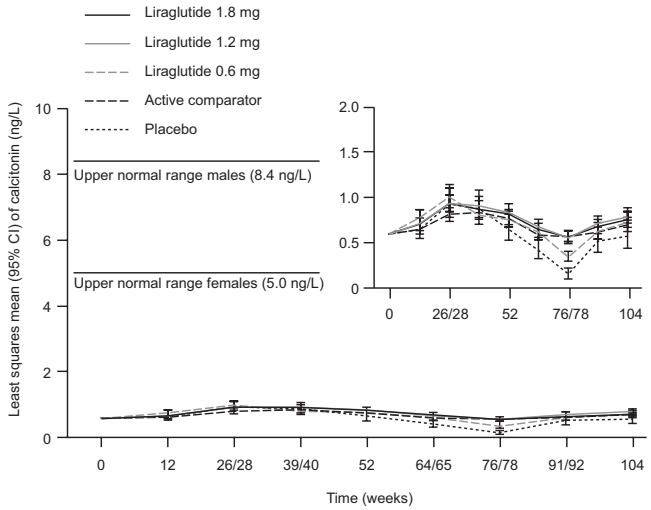

FIG. 1. Least squares mean CT concentration over 104 wk for liraglutide 0.6 mg (n = 242), 1.2 mg (n = 492), and 1.8 mg (n = 489); active comparator (n = 492); and placebo (n = 122). Patient CT data were pooled from LEAD-3 and LEAD-2. CI, Confidence interval. [Reprinted with permission from Bjerre Knudsen *et al.*: Endocrinology 151:1473–1486, 2010 (3). © The Endocrine Society.]

CT concentrations of 0.70 and 0.58 ng/liter were seen in subjects receiving active comparator and placebo, respectively. Statistical differences were found when comparing liraglutide 1.2 mg with placebo ($P = 0.0223$), but all other treatment group comparisons were found to be nonsignificant ($P > 0.05$). In all subjects including those exposed to liraglutide for up to 104 wk, CT concentrations were low and remained low throughout the trial period.

Outlier analysis

The majority of subjects did not switch category from baseline to wk 26, 52, and 104.

At wk 26, 52, and 104, respectively, 2.7, 2.3, and 3.1% of liraglutide-treated subjects shifted from baseline to a higher CT category (*i.e.* from below the UNR to above the UNR, from between UNR and 2 × UNR to above 2 × UNR). These percentages were very similar to those for subjects treated with an active comparator, where 2.2, 2.1, and 3.1% of subjects shifted to a higher CT category at wk 26, 52, and 104, respectively. For placebo, 1.7 and 1.4% of subjects shifted to a higher CT category at wk 26 and 52, whereas no patients shifted to a higher category at 104 wk.

Shift tables for subjects changing from lower to a higher category (from <20 ng/liter to above; from 20 to <50 ng/liter to above; and from 50 to <100 ng/liter to above) were comparable across the three groups over time. For liraglutide, 0.3, 0.2, and 0.1% switched to a higher category at wk 26, 52, and 104. The percentages were 0.3, 0.3, and 0.3% for the active comparator and 0.1, 0.0, and 0.0% for placebo.

During the trials, the proportions of subjects with CT concentration above 20 ng/liter remained below 2%, and there were no clear trends in increase or decrease in these proportions between weeks or between treatment groups (Table 2).

At baseline, 25 subjects had serum CT above 20 ng/liter; 14 were in the liraglutide group, nine received active comparator, and two were in the placebo group (Table 2). In the subset of liraglutide-treated subjects with baseline CT above 20 ng/liter (14 of 3551), there was no consistent pattern in the change of CT concentration over time. A total of 15 of the 25 subjects with baseline CT above 20 ng/liter withdrew from the trial; six (three subjects receiving liraglutide and three subjects receiving active comparator) withdrew because of increased CT levels. Two of these 25 subjects (one in the liraglutide group and one in the comparator group) were taking H2 receptor blockers or PPIs at baseline.

TABLE 2. Subjects with CT concentrations ≥20 ng/liter at baseline and at wk 26, 52, and 104 of trial treatment

	Liraglutide	Active comparators	Placebo
Baseline	14 (0.39)	9 (0.64)	2 (0.28)
Wk 26	30 (0.84)	12 (0.85)	3 (0.42)
Wk 52	13 (0.75)	13 (1.11)	0
Wk 104	14 (1.67)	6 (1.88)	0

Data are expressed as number (percentage).

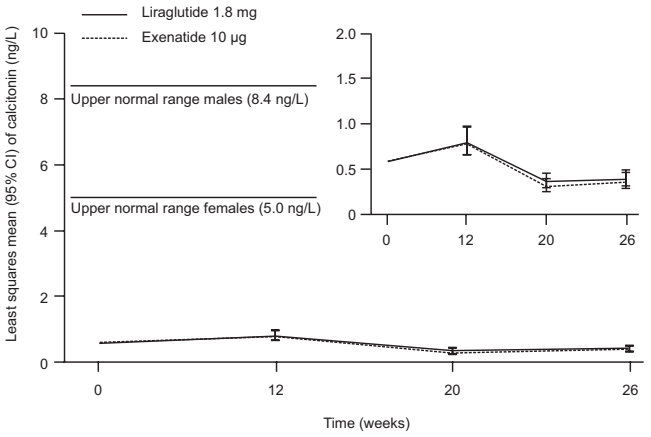

FIG. 2. Least squares mean CT concentration over 26 wk in LEAD-6 for liraglutide 1.8 mg (n = 233) and exenatide 10 μg (n = 231). CI, Confidence interval.

A total of 892 of 5673 (16%) subjects included in the 26-wk population were receiving H2 receptor blockers or PPIs, which have the potential to increase serum CT (611, 186, and 95 subjects in the liraglutide, active comparator, and placebo groups, respectively). Seven of the 14 liraglutide-treated subjects who had at least one postbaseline CT value above 20 ng/liter were taking these agents, whereas the number of subjects in the active comparator and placebo groups was one (of nine) and zero (of two), respectively.

Direct comparison of two GLP-1 receptor agonists

A trial studying the effect of liraglutide *vs.* exenatide (12) provided the opportunity to compare the CT response to liraglutide (1.8 mg once daily) with that of exenatide (10 μg twice daily), another GLP-1 analog. At 26 wk, the estimated geometric mean CT concentration in the liraglutide group (0.38 ng/liter) was indistinguishable from the exenatide group (0.36 ng/liter; $P = 0.5630$) (Fig. 2); 0.4% of liraglutide-treated and 1.2% of exenatide-treated subjects shifted to a higher CT category during the study (*i.e.* from below the UNR to above the UNR, from between UNR and 2 × UNR to above 2 × UNR).

Liraglutide at doses above 1.8 mg/d—clinical study in nondiabetic obese subjects

CT concentrations in obese subjects without diabetes receiving liraglutide (up to 3 mg daily) were compared with those receiving orlistat (120 mg three times daily) or placebo (Fig. 3). The estimated median serum CT concen-

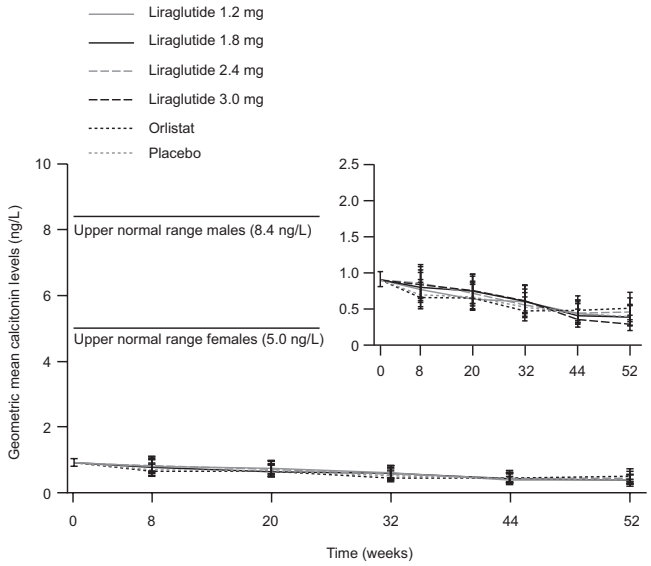

FIG. 3. Geometric mean CT levels over 52 wk in trial 1807 for liraglutide 1.2 mg (n = 95), 1.8 mg (n = 90), 2.4 mg (n = 93), and 3.0 mg (n = 93); orlistat (n = 122); and placebo (n = 98).

tration remained in the low end of the normal range over 52 wk in all treatment groups.

Clinical adverse events related to C cells

Six cases of C-cell pathology were identified during the course of these studies: four subjects receiving liraglutide (subjects 1–4) (0.11%), and two subjects in the active comparator group (0.14%) (subjects 5 and 6) (Table 3). Subjects 1 to 4 were all found to have CCH. Subject 5, in the active comparator group, had C-cell pathology that was consistent with CCH but was interpreted by the pathologist as "medullary carcinoma *in situ*." Subject 6, in the active comparator group, had MTC with a basal CT concentration above 1000 ng/liter. Subject 2 had a baseline CT concentration above the reference range but below 20 ng/liter, whereas subjects 1 and 4 had baseline CT concentrations above 20 ng/liter (21.5 and 22.3 ng/liter, respectively). Subject 3 had a normal baseline CT concentration but elevated CT after calcium stimulation at 52 wk. All six patients underwent thyroidectomy as a consequence of CT measurements. Since these analyses were performed, a seventh subject also has been identified as having C-cell pathology during a trial (18). During the trial, a male subject receiving liraglutide 1.2 mg plus metformin had an elevated CT concentration at baseline but below 20 ng/liter (11.84 ng/liter). A diagnosis of mild nodular hyperplasia/focal CCH was recorded. Although there were fluctuations in CT concentrations among these seven subjects during the trial periods, no consistent pattern was noted (data over time not shown).

TABLE 3. Human CCH reported in the liraglutide clinical development program

Subject no.	Gender	Reason for thyroidectomy	Treatment	Overall duration of treatment (d)	Duration of treatment at onset (d)	Pathology
1	Male	Elevated CT (21.5 ng/liter) reported at randomization	Liraglutide 0.6 mg + metformin	190	1	Bilateral nodular goiter, CCH
2	Male	Elevated CT reported at baseline (15.1 ng/liter) and 9 months after randomization (22.3 ng/liter)	Liraglutide 1.8 mg + metformin	363	280	Papillary microcarcinoma/physiological CCH/goiter/benign thyroid nodules
3	Female	Elevated stimulation test at 12-month visit (CT concentrations were 80.7 and 94 ng/liter at 5 and 10 min after stimulation, respectively). Baseline CT was normal[b]	Liraglutide 1.2 mg	484	356	Diffuse CCH
4	Male	Elevated basal CT at baseline (22.3 ng/liter)[a]	Liraglutide 1.8 mg	28	1	Bilateral neoplastic nodular CCH
5	Male	Elevated basal CT reported approximately 3 months after randomization (12.1 ng/liter)[a]	Glimepiride + metformin	370	87	Neoplastic CCH (MTC *in situ*)
6	Male	Elevated CT (1023 ng/liter) reported 2 months before randomization and remained elevated	Insulin glargine + metformin + glimepiride	145	−55	MTC/benign thyroid nodules
7[c]	Male	Elevated CT (11.84 ng/liter) recorded at baseline	Liraglutide 1.2 mg + metformin	164	1	Mild nodular hyperplasia/focal CCH (probably reactive)

[a] Reference range, 0.7–8.4 ng/liter. Calcium stimulation test, UNR 90 ng/liter for females and 130 ng/liter for male subjects.

[b] Subject had >10-fold elevation of CT in response to calcium stimulation prior to receiving liraglutide; however, the maximum CT level achieved was 21.2 ng/liter, which although at the 90th percentile for females in the study, is well below the cutoff of 90 ng/liter.

[c] A seventh case of C-cell pathology was reported during a trial that was not included in this original data analysis set.

Discussion

C-cell pathology in rodents was discovered during the preclinical testing for liraglutide (3) and exenatide (19), both GLP-1 receptor agonists. GLP-1 receptors are easily identified on thyroid slices from rodents and on rodent C-cell lines in tissue culture (3). Based on *in vitro* and *in vivo* rodent experiments, Bjerre Knudsen *et al.* (3) have proposed the following mechanism to explain C-cell pathology in rodents: GLP-1 receptor activation causes cAMP and CT synthesis and release from the rodent C cells. Over time, this continued stimulation may cause CCH and in some animals C-cell neoplasia, including adenomas and carcinomas.

In contrast, nonhuman primate and human thyroid glands and the human TT cell line express very low levels of C-cell GLP-1 receptors. The human TT cell line, unlike rat thyroid C-cell lines, does not respond to GLP-1 receptor agonists with an acute release of CT (3). Nonhuman primates do not develop C-cell pathology after up to 18 months of treatment with high doses of GLP-1 agonists. Conversely, some animal models may develop CCH after as few as 4 wk of exposure to oral vitamin D and calcium (20).

Serum CT concentration is a widely accepted and validated biomarker of pathological C-cell proliferation. CT is measured routinely as an important part of the follow-up of patients with known MTC and in some individuals with the genetic predisposition (mutated RET oncogene) to MTC (21). CCH is a premalignant lesion in patients with familial multiple endocrine neoplasia 2, but there are no data on progression of CCH in patients without RET oncogene mutations or familial MTC. Indeed, surgical and autopsy specimens suggest that CCH is relatively common in the normal population, up to 30% in studies by Guyétant *et al.* (22, 23). CT concentrations in the multiple endocrine neoplasia 2 population with CCH are well described, but in contrast, data on serum CT in patients with sporadic CCH are sparse.

This report provides data on the long-term CT response to liraglutide in subjects with type 2 diabetes and obese subjects without diabetes. In addition, it is the first longitudinal study of serum CT changes over time in individuals without known C-cell pathology. Although screening data are available on serum CT concentrations in populations with nodular thyroid disease, no large-scale, cross-sectional or longitudinal data on CT serum concentrations are available in individuals without known thyroid disease.

The baseline CT concentrations were low in subjects with diabetes and in those with obesity with estimated median values of 0.6 ng/liter (UNR females, 5 ng/liter; males, 8.4 ng/liter) for the LEAD trials. Baseline CT concentrations were above 20 ng/liter in 0.28–0.64%. In many screening studies, a CT concentration above 20 ng/liter is used to trigger further evaluation (17).

There was no consistent change in CT concentrations over time (up to 104 wk) with any dose of liraglutide or in the comparator groups. There were no differences between CT responses to liraglutide or exenatide, two distinct GLP-1 analogs. The CT concentration was statistically higher at wk 26 and 52 in liraglutide compared with comparators. However, these levels were well within the normal range, the geometric mean difference between groups was around 0.1 ng/liter, and there is no indication that these differences are of clinical significance. The response of CT to calcium stimulation (which magnifies the sensitivity of baseline CT measurements) was indistinguishable between liraglutide and comparators in human subjects (3).

There was no pattern of further CT increase in the population with baseline (before drug exposure) CT concentrations above 20 ng/liter, although 15 of these 25 subjects did withdraw from the studies in some cases for surgical intervention of moderately elevated CT levels. The proportion of subjects moving to a higher category of CT concentration was low and did not differ between treatment groups. Subjects receiving H2 receptor blockers or PPIs, agents known to increase serum CT concentration, did not have a further increase in CT concentrations with liraglutide.

The initial analysis revealed that six subjects were found to have histologically documented C-cell disease, with identical prevalence in the liraglutide and nonliraglutide (or comparator) groups: one case of MTC and five cases of CCH. The case of MTC occurred in a subject who was not treated with liraglutide. Four subjects had baseline CT elevations, whereas the other two with CCH developed increased CT concentrations during the trial, one who was treated with liraglutide and the other who was treated with active comparator. One additional case of CCH was also reported in a subject with elevated baseline CT concentrations, presenting a total of seven cases of histologically documented C-cell disease.

Based on these data, we find no evidence that liraglutide stimulates CT release from human C cells. By inference, we find no evidence that liraglutide is a potential cause of C-cell proliferation or C-cell pathology in humans based on the CT response pattern. However, we recognize that the longest duration of exposure, *i.e.* 2 yr, and the number of subjects exposed may not be sufficient to demonstrate clinically significant C-cell pathology. On the other hand, the fact that CT levels did not increase, even in subjects

with baseline CT elevations, suggests that the human C cell is poorly responsive to GLP-1 receptor agonists. This is distinctly different from the reported increase in CT levels in individuals treated with PPIs, an increase presumed to be secondary to a direct effect of gastrin on the C cell (16).

The strength of this study is the large number of CT measurements in over 5000 subjects over time, using a highly sensitive CT assay. There are, however, several potential limitations of this study. We have examined thyroid pathology in only a limited number of subjects demonstrating elevated or changing CT concentrations. This is also true of almost all studies using CT measurements in individuals with thyroid nodules. Although it is conceivable that liraglutide could cause C-cell proliferation without CT release, there are no data to support such a hypothesis; in rodents, CT release always accompanies C-cell pathology. It is possible that subjects with established MTC would have a different response to GLP-1 receptor agonists. This concern deserves further study, but we would propose that individuals with MTC or RET-positive family members of MTC kindreds not receive GLP-1 analogs.

Together, these data do not support any significant risk for the activation or growth of C cells in humans in response to GLP-1 receptor agonists including liraglutide over the 2-yr period that it has been studied.

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Disclosure Summary: L.H. has received consulting fees from and has served on an advisory council for Novo Nordisk A/S. A.C.M., M.Z., and T.L.T. are employees of and shareholders in Novo Nordisk. G.H.D. has acted as a consultant for Novo Nordisk, currently serves on a steering committee, has received honoraria as a consultant, and has provided Food and Drug Administration testimony as a consultant.

References

1. **Costante G, Durante C, Francis Z, Schlumberger M, Filetti S** 2009 Determination of calcitonin levels in C-cell disease: clinical interest and potential pitfalls. Nat Clin Pract Endocrinol Metab 5:35–44
2. **Leboulleux S, Baudin E, Travagli JP, Schlumberger M** 2004 Medullary thyroid carcinoma. Clin Endocrinol (Oxf) 61:299–310
3. **Bjerre Knudsen L, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ, Gotfredsen C, Egerod FL, Hegelund AC, Jacobsen H, Jacobsen SD, Moses AC, Mølck AM, Nielsen HS, Nowak J, Solberg H, Thi TD, Zdravkovic M** 2010 Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology 151:1473–1486
4. **Martín-Lacave I, Bernab R, Sampedro C, Conde E, Fernández-Santos JM, San Martín MV, Beato A, Galera-Davidson H** 1999 Correlation between gender and spontaneous C-cell tumors in the thyroid gland of the Wistar rat. Cell Tissue Res 297:451–457
5. **Capen CC, Martin SL** 1989 The effects of xenobiotics on the structure and function of thyroid follicular and C-cells. Toxicol Pathol 17:266–293
6. **Körner M, Stöckli M, Waser B, Reubi JC** 2007 GLP-1 receptor expression in human tumors and human normal tissues: potential for in vivo targeting. J Nucl Med 48:736–743
7. **Marre M, Shaw J, Brändle M, Bebakar WM, Kamaruddin NA, Strand J, Zdravkovic M, Le Thi TD, Colagiuri S** 2009 Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in patients with type 2 diabetes (LEAD-1 SU). Diabet Med 26:268–278
8. **Nauck M, Frid A, Hermansen K, Shah NS, Tankova T, Mitha IH, Zdravkovic M, Düring M, Matthews DR** 2009 Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin in type 2 diabetes. Diabetes Care 32:84–90
9. **Garber A, Henry R, Ratner R, Garcia-Hernandez PA, Rodriguez-Pattzi H, Olvera-Alvarez I, Hale PM, Zdravkovic M, Bode B** 2009 Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 mono): randomised, 52-week, phase III, double-blind, parallel-treatment trial. Lancet 373:473–481
10. **Zinman B, Gerich J, Buse JB, Lewin A, Schwartz S, Raskin P, Hale PM, Zdravkovic M, Blonde L** 2009 Efficacy and safety of the human GLP-1 analog liraglutide in combination with metformin and TZD in patients with type 2 diabetes mellitus (LEAD-4 Met+TZD). Diabetes Care 32:1224–1230
11. **Russell-Jones D, Vaag A, Schmitz O, Sethi BK, Lalic N, Antic S, Zdravkovic M, Ravn GM, Simó R** 2009 Liraglutide vs insulin glargine and placebo in combination with metformin and sulphonylurea therapy in type 2 diabetes mellitus: a randomised controlled trial (LEAD-5 met+SU). Diabetologia 52:2046–2055
12. **Buse JB, Rosenstock J, Sesti G, Schmidt WE, Montanya E, Brett JH, Zychma M, Blonde L** 2009 Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). Lancet 374:39–47
13. **Kaku K, Rasmussen MF, Clauson P, Seino Y** 2010 Improved glycaemic control with minimal hypoglycaemia and no weight gain with the once-daily human GLP-1 analogue liraglutide as add-on to sulphonylurea in Japanese patients with type 2 diabetes. Diabetes Obes Metab 12:341–347
14. **Seino Y, Rasmussen MF, Nishida T, Kaku K** 2010 Efficacy and safety of the once-daily human GLP-1 analogue, liraglutide, vs glibenclamide monotherapy in Japanese patients with type 2 diabetes. Curr Med Res Opin 26:1013–1022
15. **Astrup A, Rössner S, Van Gaal L, Rissanen A, Niskanen L, Al Hakim M, Madsen J, Rasmussen MF, Lean ME; NN8022–1807 Study Group** 2009 Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. Lancet 374:1606–1616
16. **Erdogan MF, Gursoy A, Kulaksizoglu M** 2006 Long-term effects of elevated gastrin levels on calcitonin secretion. J Endocrinol Invest 29:771–775
17. **Costante G, Meringolo D, Durante C, Bianchi D, Nocera M, Tumino S, Crocetti U, Attard M, Maranghi M, Torlontano M, Filetti S** 2007 Predictive value of serum calcitonin levels for preop-

erative diagnosis of medullary thyroid carcinoma in a cohort of 5817 consecutive patients with thyroid nodules. J Clin Endocrinol Metab 92:450–455

18. **Pratley RE, Nauck M, Bailey T, Montanya E, Cuddihy R, Filetti S, Thomsen AB, Søndergaard RE, Davies M; 1860-LIRA-DPP-4 Study Group** 2010 Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycaemic control with metformin: a 26-week, randomised, parallel-group, open-label trial. Lancet 375: 1447–1456
19. **Food and Drug Administration** 2005 Byetta (exenatide), pharmacology review 2005. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021773_Byetta_pharmr.PDF
20. **Okada H, Toyota N, Harimaya Y, Matsukawa K** 1991 Immunohistochemical alterations of C cells in sheep treated with vitamin D. J Comp Pathol 105:263–270
21. **American Thyroid Association Guidelines Task Force, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD, Schlumberger M, Wells Jr SA** 2009 Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid 19:565–612
22. **Guyétant S, Rousselet MC, Durigon M, Chappard D, Franc B, Guerin O, Saint-André JP** 1997 Sex-related C cell hyperplasia in the normal human thyroid: a quantitative autopsy study. J Clin Endocrinol Metab 82:42–47
23. **Guyétant S, Bléchet C, Saint-André JP** 2006 C-cell hyperplasia. Ann Endocrinol (Paris) 67:190–197