Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Clinical Science

Basal and stimulated calcitonin levels in patients with type 2 diabetes did not change during 1 year of Liraglutide treatment

Maria Elena Lunati[a,b], *Valeria Grancini*[a,b], *Carla Colombo*[a,b], *Eva Palmieri*[a], *Veronica Resi*[a], *Michela Perrino*[a,b], *Emanuela Orsi*[a,*], *Laura Fugazzola*[a,c,**]

[a] *Endocrine Unit, Fondazione IRCCS Ca' Granda, Milan, Italy*
[b] *Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy*
[c] *Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy*

ARTICLE INFO

Article history:
Received 10 June 2015
Accepted 9 September 2015

Keywords:
Calcitonin
Liraglutide
Calcium
Stimulation

ABSTRACT

Background and aims. The administration of Liraglutide, a long-acting GLP-1 receptor (GLP-1R) agonist, is associated with C-cell adenomas and carcinomas in rats. In humans, GLP-1R is highly expressed in C-cells hyperplasia (CCH) and in medullary thyroid cancer (MTC), though no changes in basal serum calcitonin (bCT) levels were recorded in type 2 diabetic (T2DM) patients treated with Liraglutide. To diagnose the possible development of CCH during Liraglutide treatment, we evaluated CT levels stimulated by calcium test (sCT).

Materials and methods. bCT and sCT and metabolic and anthropometric parameters were evaluated in 26 T2DM patients at baseline and at 1, 3, 6 and 12 months of treatment.

Results. In all patients, bCT remained within the normal range during the entire study period. In females and males, the higher sCT values were reached after 3 months and 1 month, respectively, with a progressive reduction at 6–12 months. The greater decrease of HbA1c values was reached at 3 months, while body weight and waist circumference decreased over the first 4 weeks of therapy. Lipase levels significantly increased, with a peak value at 1 month.

Conclusion. The chronic administration of Liraglutide did not lead to statistically significant variations in both bCT and sCT. Stimulated CT levels increased, though always below the normal range, during the first 1–3 months of treatment, and progressively decreased to baseline levels. This finding is consistent with the effects recorded at the glycometabolic level, and suggests the possible induction of a drug tolerance involving also the C cells and thus preventing CCH.

Abbreviations: GLP-1, Glucagon-like peptide 1; GLP-1R, GLP-1 receptor; CT, Calcitonin; bCT, basal Calcitonin; sCT, stimulated Calcitonin; T2DM, Type 2 diabetes mellitus; CCH, C-cells hyperplasia; MTC, Medullary thyroid cancer; OAD, Oral antidiabetics; FAT, Fat mass; FFM, Free fat mass; BMI, Body mass index; WC, Waist circumference; HbA1c, Glycosylated hemoglobin; FPG, Fasting plasma glucose; HDL, High density lipoprotein; LDL, Low density lipoprotein; TSH, Thyroid-stimulating hormone; FT4, Free triiodothyronine; FT3, Free thyroxine; AbTg, Antithyroglobulin antibody; AbTPO, Antithyroid peroxidase antibody; PTH, Parathyroid hormone; Ca, Calcium; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; HOMA-IR, Homeostatic model assessment-insulin resistance index; n, Number; SD, Standard deviation; ANOVA, One-way analysis of variance.

* *Correspondence to:* E. Orsi, Endocrine Unit, Padiglione Granelli, Fondazione IRCCS Ca' Granda, Via F. Sforza, 35, 20122 Milan Italy. Tel.: +39 0255033590; fax: +39 0250320605.
** *Correspondence to:* L. Fugazzola, Endocrine Unit, Padiglione Granelli, Fondazione IRCCS Ca' Granda, Via F. Sforza, 35, 20122 Milan Italy. Tel.: +39 0255033498; fax: +39 0250320605.
E-mail addresses: emanuela_orsi@yahoo.it (E. Orsi), laura.fugazzola@unimi.it (L. Fugazzola).

http://dx.doi.org/10.1016/j.metabol.2015.09.010
0026-0495/

1. Introduction

Among the novel drugs for type 2 diabetes mellitus (T2DM) treatment, the long-acting GLP-1 receptor agonist Liraglutide is widely used [1]. Glucagon-like peptide-1 (GLP-1) is an incretin hormone, secreted after meals by intestinal mucosal endocrine cells (L cells) [2]. It acts in a pleiotropic way that leads to a reduction of blood glucose concentration and caloric intake. The actions of GLP-1 are mediated by G-protein coupled receptors, highly expressed in the pancreas, intestine, stomach and nervous system, though receptors are also present in other tissues [3,4]. In pre-clinical trials, the expression of GLP-1 receptors (GLP-1R) has been also documented in rodents' thyroid glands [5], and GLP-1 receptor agonists were found to elicit calcitonin (CT) gene expression and CT release by parafollicular C cells in a dose-dependent manner. Liraglutide administration was found to associate in rats with the onset of C-cell adenomas and carcinomas, whereas in monkeys neither C cells hyperplasia (CCH) nor C cells tumors were observed [5]. In TT cells, derived from human medullary thyroid cancer (MTC), GLP-1 receptor agonists were found not to stimulate CT release. Moreover, the expression of GLP-1 receptors was very low with mRNA transcripts 14- to 21-fold lower than those recorded in the rat C-cells [5]. On the contrary, GLP-1R has been recently found to be highly expressed in human CCH and MTC [6]. In particular, GLP-1R immunoreactivity was detected in 91.6% of analyzed MTCs, and in the majority of them a high proportion (>70%) of neoplastic cells were stained for GLP-1R. GLP-1R immunoreactivity was always documented in CCH, either reactive or associated with *RET* germline mutations, while it was found only in a minority of normal C cells and papillary thyroid carcinoma cells, indicating that it is almost limited to diseased C-cells in humans, likely as a consequence of dysregulated cell cycle control, and may be aberrantly expressed in follicular cells [6].

To evaluate the possible effect of Liraglutide on the C cell compartment in humans and the related risk of CCH and MTC, basal serum CT (bCT) levels were previously monitored in T2DM patients and non-diabetic obese subjects treated for over 2 years [7], and no significant changes with any dose of the drug were observed. Nevertheless, since the best way to diagnose either CCH, which is a preneoplastic lesion, or microMTC is to evaluate stimulated CT (sCT), we performed the sequential evaluation of both bCT and sCT during Liraglutide treatment. The stimulation of CT has been obtained by means of the high-dose calcium test, which has been validated as the best test available to date [8].

2. Patients and methods

2.1. Study population

We enrolled 26 patients with type 2 diabetes (T2DM). Inclusion criteria were: (1) presence of T2DM, treated with oral antidiabetics (OAD)/insulin therapy; (2) age between 18 and 80 years; (3) HbA1c levels ranging 6.5–10.0% (48–86 mmol/mol, previous OAD monotherapy ≥3 months) or HbA1c ranging 6.5–9.0% (48–76 mmol/mol, previous combination therapy ≥3 months); (4) capability to perform self-blood glucose monitoring; and (5) no personal/familiar history of MTC or elevated bCT values.

At baseline all patients started therapy with once daily dose of Liraglutide (1.2 or 1.8 mg/day; Novo Nordisk A/S, Bagsvaerd, Denmark) injected subcutaneously. Treatment was given in combination with metformin. Metformin was maintained throughout the study, but could be added sulfonylurea (Gliclazide) if unacceptable glucose control.

Withdrawal criteria included: (1) insufficient glycemic control, defined as HbA1c levels ≥9% after 1 month of treatment; (2) severe hypoglycemia; and (3) gastro-intestinal intolerance and adverse effects, like acute pancreatitis and allergic reactions.

Each patient was evaluated at baseline and after 1, 3, 6, and 12 months of therapy with Liraglutide. All patients gave their informed consent to be enrolled in this study, which has been previously approved by the ethical committees of the institution involved (E.C.: 144/2013-788/2013).

2.2. Clinical parameters

Body weight and height were measured with scale and stadiometer whereas body mass index (BMI) was calculated as patient's weight (in kg) divided by patient height (in meters) squared. Waist circumference was taken at the umbilicus. Blood pressure was measured with a mercury sphygmomanometer after the patient had been lying supine for at least 5 min. Fat mass (FAT) and fat free mass (FFM) were measured using impedance method (Akern Quantum/S®).

2.3. Biochemical parameters and procedure

All patients were evaluated at any time for fasting plasma glucose (FPG), triglycerides, and total and HDL cholesterol, with calculation of low-density lipoprotein (LDL) cholesterol by the FriedWald formula, aspartate transaminase, alanine transaminase, γ-glutamyl transferase, total bilirubin, serum creatinine, urea, amylase and lipase values, PTH, serum calcium and phosphate, thyroid-stimulating hormone (TSH), free triiodothyronine (FT4), free thyroxine (FT3), antithyroglobulin antibody (AbTg) and antithyroid peroxidase antibody (AbTPO) levels. Hemoglobin A1c (HbA1c) was assessed by the use of a high performance liquid chromatography, National Glycohemoglobin Standardisation Program-certified and Diabetes Control and Complications Trial-standardized method. Patients were also evaluated for insulin and C-peptide levels. Insulin was analyzed by immunoenzymetric one-step assay (Medgenics Diagnostics, Belgium) and electrochemiluminescence immunoassay (Roche Diagnostics, Germany). Insulin resistance was then estimated by the homeostatic model assessment-insulin resistance (HOMA-IR) index (http://www.dtu.ox.ac.uk/homacalculator/index.php). CT was measured using a two-site automated chemiluminescent immunometric assay (Immulite 2000; Siemens Diagnostics, Deerfield, IL) in blood samples obtained before and at 2, 5, and 15 min from the end of the infusion of Ca by in-dwelling IV cannula. The assay has an analytical sensitivity of 2 pg/ml. Ca gluconate was administered IV at the dose of 25 mg/kg at 10 ml/min (2.3 mg or 0.12 mEq of elemental Ca), being adjusted body weight calculated (www.manuelsweb.com/IBW.htm for ideal body weight and adjusted body weight calculator) for each patient to avoid an over-dosage in obese. Thyroid ultrasound

evaluation was performed before and at 12 months after Liraglutide therapy.

2.4. Statistical analysis

Statistical analysis was performed with the statistical package SPSS for Windows version 20.0 (SPPS Inc. Chicago, IL). Data are expressed as mean ± standard deviation for continuous variables and as number of cases and percentage for categorical variables. Groups were compared using the Student's *t* test for parametric continuous variables, and the Mann–Whitney test for nonparametric continuous variables. Normality of distribution was preliminary assessed by the Kolmogorov–Smirnov test. One-way analysis of variance for repeated measures was used to analyze changes of variables examined. Values of $P < 0.05$ were considered statistically significant.

3. Results

Baseline characteristics of the study population are shown in Supplementary Table 1. Baseline therapy was: metformin monotherapy in 38.5% of patients (10/26), with mean daily dosage of 1750 ± 900 mg/day; combination treatments (metformin + sulfonylurea/metformin + DPP4i/metformin + acarbose) in 27% of patients (7/26); metformin + sulfonylurea + DPP4i in 7.7% of patients (2/26); metformin + long-acting insulin (glargine or detemir) in 15.3% of patients (4/26); and basal bolus insulin therapy ± metformin in 11.5% of patients (3/26). The patients enrolled were switched to once-daily dose of Liraglutide in combination with metformin due to either an insufficient glycometabolic control or a high BMI. In particular, Liraglutide was administered at the daily dose of 1.2 mg in 92.3% of patients (24/26) and at 1.8 mg/day in 7.7% of patients (2/26). On the other hand, metformin was given at the mean dosage of 1860 ± 850 mg/day. In 4 patients sulfonylurea (gliclazide, mean dosage: 200 ± 40 mg/day) was added as third drug in order to achieve an appropriate blood glucose control.

The completion of the 1 year treatment and follow-up was achieved in 77% of patients (20/26). Withdrawal rates were highest in the first 12-weeks trial: 4 patients withdrew after 3 months and 2 patients after 1 month. The main reason for withdrawal was gastro-intestinal intolerance (nausea, vomiting and diarrhea).

3.1. Efficacy

The highest reduction for both mean HbA1c and FPG levels was reached at 3 months (7.84 ± 1.3 *vs* 7.15 ± 1.14%, P = 0.02, and 148.3 ± 45.7 *vs* 129.4 ± 31.9 mg/dl, P = 0.07, respectively) (Fig. 1). Thereafter, a progressive increase occurred, and at 1 year follow-up no significant differences were found with respect to baseline values. Interestingly, the HbA1c target of <7% (<53 mmol/mol) recommended by the American Diabetes Association (ADA) was reached in 10/20 (50%) patients completing the follow-up. Both mean body weight and waist circumference decreased during the first 4 weeks of treatment and the decreased values were maintained over 12 months (baseline: 38.3 ± 8.2, 1 year: 36.8 ± 8.4 kg/m^2, P = 0.002, and baseline: 121.9 ± 13, 1 year: 114.8 ± 14.7 cm, P = 0.001, respectively) (Fig. 1). After 1 year, FAT values showed a slight decrease (38.2 ± 10.7 *vs* 37.8.4 ± 11.3 kg; P = 0.87, Student's *t* test), while FFM values increased (53.4 ± 9.1 *vs* 59.9 ± 15.5 kg; P = 0.19, Student's *t* test). The analysis of fasting insulin levels, HOMA-IR index, C-peptide, amylase, cholesterol, triglycerides, PTH, and serum calcium values did not show any statistically significant difference during follow-up (Supplementary Table 2), whereas lipase levels markedly increased, reaching the peak value during the first month of the trial (baseline *vs* 1 month: 42.3 ± 17.2 *vs* 89.0 ± 65.6 U/l; normal range: 13–60 U/l) (P = 0.001, Student's *t* test).

Hypoglycemia rate was very low in this study population, while nausea, vomiting, and diarrhea, known side effects of GLP-1 receptor agonists, increased during the first 4 weeks of Liraglutide treatment with a following decrease.

3.2. Calcium test and thyroid function

Among patients completing follow-up, 4/20 were affected with primary hypothyroidism due to Hashimoto's thyroiditis and were on levothyroxine replacement therapy, at the mean dosage of 1.05 mcg/kg/day. TSH, FT3 and FT4 levels remained in the normal range during the entire follow-up (Supplementary Table 2).

All patients had bCT levels <10 pg/ml during the entire follow-up. In all patients, a response to high-dose calcium administration was found, being the peak levels always below the normal range, which was defined according to the current indications [8,9]. Although not statistically significant, the mean peak response, obtained at 2 minutes after Ca infusion, was higher after 4 weeks of Liraglutide treatment than at baseline, with a progressive reduction after 6 months therapy. Delta CT values in all subjects were higher in the first 1 month of Liraglutide therapy, compared to baseline values, though without a statistically significant difference (Table 1). Considering genders separately, the highest peak response value of sCT was reached after 3 months in females (baseline *vs* 3 months: 36.1 ± 42.6 *vs* 39.1 ± 52.2 pg/ml), and after 1 month of therapy in males (baseline *vs* 1 month: 33.8 ± 21.9 *vs* 45.4 ± 35.4 pg/ml) (Supplementary Table 3). No statistically significant differences were found among genders in either bCT or sCT levels. Of note, patients on DPP4i at baseline did not differ from other patients in terms of CT response. At baseline, thyroid ultrasound evaluation showed a normal gland in 11/20 patients (55%), a benign multinodular goiter in 6/20 patients (30%) and an autoimmune thyroiditis in 3/20 (15%). No differences were recorded at thyroid ultrasound after one year of Liraglutide therapy.

4. Discussion

The administration of Liraglutide, a long-acting GLP-1R agonist, has been found to associate in rats with the onset of C-cell adenomas and carcinomas [5]. The mechanism underlying this phenomenon has been hypothesized to be related to the continuous stimulation of C-cells by this GLP-1R agonist [5]. On the other hand, though GLP-1R is highly expressed in human C-cell hyperplasia (CCH) and medullary thyroid cancer (MTC) [6], C-cells diseases have been never

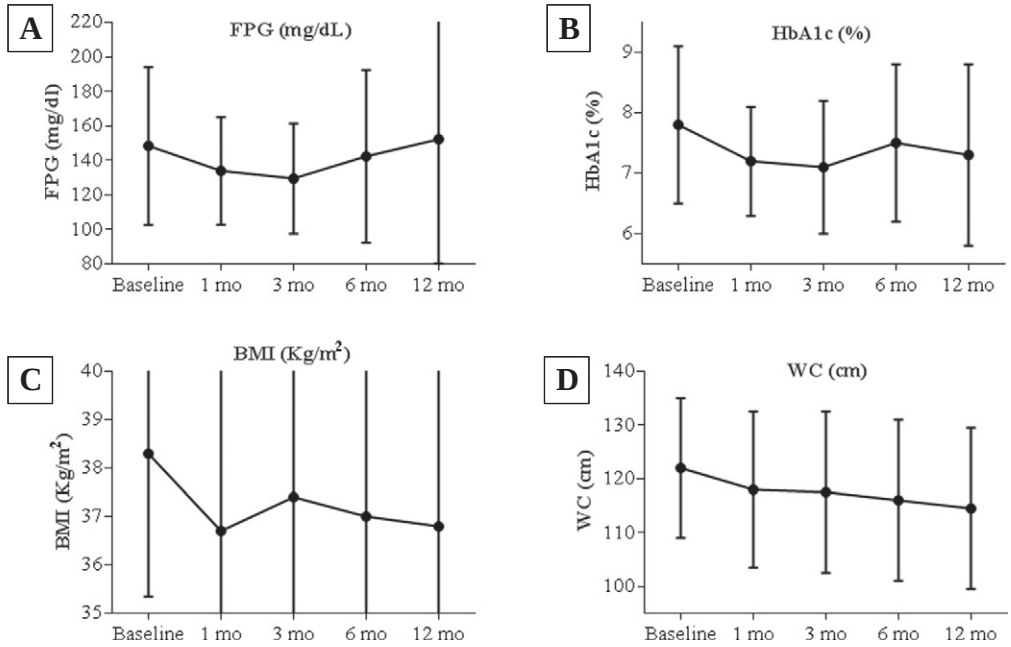


Fig. 1 – Mean ± SD fasting plasma glucose (FPG) (panel A), HbA1c (glycosylated hemoglobin) (panel B), BMI (body mass index) (panel C), and WC (waist circumference) (panel D) values over 1 year follow-up (n = 20).

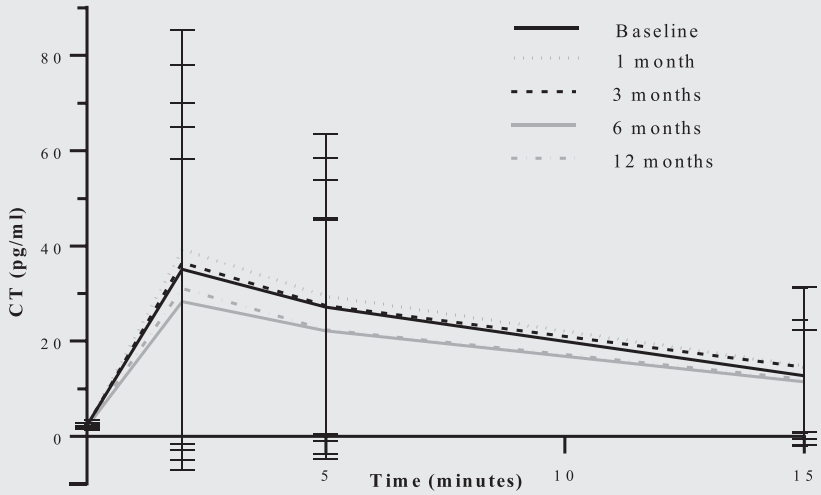
observed in patients exposed to GLP-1R agonist. A possible explanation of this discrepancy could be related to GLP-1R expression in normal thyroid tissues, which has been found to be higher in rodents than in humans, though data are scarce and discordant mostly because they have been obtained with poorly specific antibodies [10]. In this context, it has been speculated that if normal thyroid tissue does not express GLP-1R, then no risk can be postulated for a GLP-1R agonist treatment [10]. On the other hand, if thyroid cells express GLP-1R, those subjects harboring a higher expression could be at risk to develop CCH or even MTC [11]. The present study shows that the chronic administration of Liraglutide does not lead to clinically significant variations in both basal and stimulated CT levels. In particular, bCT levels were normal, i.e. <10 ng/ml, at baseline in all patients, affected with T2DM and obesity. It is worth to note that in a recent trial performed in an extremely large series of obese (mean BMI 32.5 ± 6.3 kg/m^2) and diabetic patients, bCT levels >10 ng/l were found in 14.6% of men and in 0.96% of women, and were significantly associated with a higher BMI and a reduced glomerular filtration rate (GFR) [12]. All the patients included in the present series had normal creatinine levels, indicating that the supraphysiological CT levels found in the above reported study could be related to the renal impairment, as already reported in the literature [13].

Basal CT levels remained within the normal range, <10 ng/ml, during the entire study period without significant differences between baseline and the 4 determinations obtained during the follow-up. Consistently, previous results obtained in a large group of patients treated with Liraglutide [7], showed at week 26 and 52 bCT levels always lying within the normal range, though significantly higher with respect to the control group. The main weakness of the present study is related to the sample size, which is smaller than that required to detect possible differences. Nevertheless, the major clinical and economic effort done to submit all the included patients to 5 Ca tests each limited the extension of the enrollment. On the other hand, the evaluation of CT levels during Liraglutide treatment not only basally, but after high-dose calcium load too, is an original finding and represents the main strength of the study. Ca load is the most potent and better tolerated stimulus for CT secretion and is a reliable tool to diagnose CCH, which is considered as a preneoplastic lesion. Interestingly, though without a statistically significant difference, the mean peak response, obtained at 2 minutes after Ca infusion, was higher after 4 weeks of Liraglutide treatment than at baseline, with a progressive reduction after 6 months therapy. Nevertheless, the peak levels were always below the normal range in both genders, according to the normal values previously established in healthy volunteers [8]. Moreover, it is worth to note that in no patient sCT levels exceeded the threshold levels that we previously demonstrated to be able to separate between non-C cells disease and CCH/MTC [9].

The highest levels of CT stimulation were obtained in both genders during the first 1–3 months of treatment, in parallel with the beneficial effects recorded at the glycometabolic level and the intensity of side effects. Indeed, treatment with Liraglutide provided FPG control and a significant body weight reduction particularly during the first months of treatment with a slight reduction of efficacy, associated to worst glycemic control and BMI increasing, starting from the 6th month of treatment. As expected from the efficacy studies [14], HbA1c reduction was around 0.5% at 1 year. Still, side effects increased during the first 4 weeks of Liraglutide treatment with a following decrease, and lipase values exceeded by 2-fold the baseline value in the first month of treatment, and then tended to decrease. Interestingly, it was recently demonstrated in healthy volunteers submitted to intravenous infusion of GLP-1 that the GLP-1-induced delay in

Table 1 – Mean CT values (pg/ml) at calcium test and delta CT levels in all patients (n = 20).

	Baseline	1 month	3 months	6 months	12 months	P
Basal CT	2.3 ± 1.13	2.24 ± 0.6	2.25 ± 0.64	2.05 ± 0.23	2.1 ± 0.45	NS
2 min	35.1 ± 35.1	39.2 ± 46.1	36.5 ± 41.5	28.3 ± 30	30.8 ± 33.9	NS
5 min	27.1 ± 26.6	29.5 ± 34.2	27.4 ± 31.1	22.1 ± 23.2	22.4 ± 23.5	NS
15 min	12.7 ± 11.8	14.7 ± 16.5	14.5 ± 16.7	11.4 ± 10.9	12.0 ± 12.6	NS
Delta CT	32.8 ± 34.7	37.0 ± 45.6	34.2 ± 41.3	26.3 ± 30.0	28.7 ± 33.8	NS
TV (ml)	11.4 ± 5.9	–	–	–	11.6 ± 4.8	NS



Legend: min = minutes; TV: thyroid volume.

gastric emptying progressively reduces with time. Two mechanisms conferring the induction of tolerance against the GLP-1 effects on gastric emptying were hypothesized, tachyphylaxis at the level of vagal nervous activation or downregulation or desensitization of the GLP-1 receptor in response to chronically elevated GLP-1 levels [15]. Accordingly, and based on the present findings, we suggest that Liraglutide induces a proliferation of C-cells during the first 1–3 months of treatment. We are tempting to speculate that, afterwards, a drug tolerance could occur, by the above reported mechanisms, which involves also the parafollicular C cells and in some way "protects" them from the chronic stimulation, thus preventing CCH. Thus, the translational potential of our finding is that Liraglutide does not lead to clinically significant variations in both bCT and sCT, and can therefore be safely administered at the above reported doses. Nevertheless, the long-term consequences of sustained GLP-1 receptor activation in the human C-cells remain unknown and deserve further investigation. Moreover, it should be noted that Liraglutide has been recently proposed for obesity treatment to be administered at the daily dosage of 2.4/3 mg [16]. With those higher doses the weight loss has been found to be significant and long-lasting. The results reported here indicate that further studies are warranted to evaluate the possible occurrence of CCH in obese patients chronically treated with high dose of Liraglutide. Basal CT should be measured in those patients, and specific monitoring should be offered to subjects with bCT > 10 pg/ml, which are expected to harbor reactive CCH [9]. Finally, the chronic administration of this drug must probably be avoided in *RET* mutations carriers.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2015.09.010.

Author Contributions

MEL, EO, and LF contributed substantially to the conception and design; all authors participated in acquisition, analysis or interpretation of the data, in the drafting of the article or critical revision and in final approval of the version to be published; all authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

Conflict of Interest

The authors declare no conflict of interest/financial disclosure statement.

REFERENCES

[1] Chia CW, Egan JM. Incretin-based therapies in type 2 diabetes mellitus. J Clin Endocrinol Metab 2008;93:3703–16.
[2] Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007;132:2131–57.
[3] Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. Endocrinology 1996;137:2968–78.

[4] Körner M, Stöckli M, Waser B, Reubi JC. GLP-1 receptor expression in human tumors and human normal tissues: potential for in vivo targeting. J Nucl Med 2007;48:736–43.
[5] Bjerre Knudsen L, Madsen LW, Andersen S, et al. Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. See comment in PubMed Commons below Endocrinology 2010; 151:1473–86.
[6] Gier B, Butler PC, Lai CK, et al. Glucagon like peptide-1 receptor expression in the human thyroid gland. J Clin Endocrinol Metab 2012;97:121–31.
[7] Hegedüs L, Moses AC, Zdravkovic M, et al. 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. J Clin Endocrinol Metab 2011;96:853–60.
[8] Doyle P, Düren C, Nerlich K, et al. Potency and tolerance of calcitonin stimulation with high-dose calcium versus pentagastrin in normal adults. Clin Endocrinol Metab 2009; 94:2970–4.
[9] Mian C, Perrino M, Colombo C, et al. Refining calcium test for the diagnosis of medullary thyroid cancer: cutoffs, procedures, and safety. J Clin Endocrinol Metab 2014;99:1656–64.
[10] Gagel RF. Activation of G-protein-coupled receptors and thyroid malignant tumors: the jury is still out. Endocr Pract 2011;17:957–9.
[11] Sternthal E. Glucagon-like peptide-1 receptor expression in the thyroid gland: a case of "hide-and-see"? Endocr Pract 2011;17:955–6.
[12] Daniels GH, Hegedüs L, Marso SP, et al. LEADER 2: baseline calcitonin in 9340 people with type 2 diabetes enrolled in the Liraglutide Effect and Action in Diabetes: evaluation of cardiovascular outcome Results (LEADER) trial: preliminary observations. Diabetes Obes Metab 2015;17:477–86.
[13] Kloos RT, Eng C, Evans DB, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid 2009;19:565–612.
[14] Nauck M, Frid A, Hermansen K, et al. Long-term efficacy and safety comparison of liraglutide, glimepiride and placebo, all in combination with metformin in type 2 diabetes: 2 year results from the LEAD-2 study. Diabetes Obes Metab 2013;15:204–12.
[15] Nauck MA, Kemmeries G, Holst JJ, et al. Rapid tachyphylaxis of the glucagon-like peptide 1–induced deceleration of gastric emptying in humans. Diabetes 2011;60:1561–5.
[16] Astrup A, Carraro R, Finer N, et al. Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. Int J Obes 2012;36:843–54.