



Effects of Liraglutide on Weight Loss, Fat Distribution, and β -Cell Function in Obese Subjects With Prediabetes or Early Type 2 Diabetes

Diabetes Care 2017;40:1556–1564 | <https://doi.org/10.2337/dc17-0589>

Francesca Santilli,¹ Paola G. Simeone,¹ Maria T. Guagnano,¹ Marika Leo,¹ Marica T. Maccarone,² Augusto Di Castelnuovo,³ Cristina Sborgia,¹ Riccardo C. Bonadonna,⁴ Ermanno Angelucci,⁵ Virginia Federico,⁶ Stefano Cianfarani,^{7,8} Lamberto Manzoli,⁹ Giovanni Davi,^{1†} Armando Tartaro,² and Agostino Consoli¹

OBJECTIVE

Obesity is associated with an increased risk of type 2 diabetes and cardiovascular complications. The risk depends significantly on adipose tissue distribution. Liraglutide, a glucagon-like peptide 1 analog, is associated with weight loss, improved glycemic control, and reduced cardiovascular risk. We determined whether an equal degree of weight loss by liraglutide or lifestyle changes has a different impact on subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) in obese subjects with prediabetes or early type 2 diabetes.

RESEARCH DESIGN AND METHODS

Sixty-two metformin-treated obese subjects with prediabetes or newly diagnosed type 2 diabetes, were randomized to liraglutide (1.8 mg/day) or lifestyle counseling. Changes in SAT and VAT levels (determined by abdominal MRI), insulin sensitivity (according to the Matsuda index), and β -cell function (β -index) were assessed during a multiple-sampling oral glucose tolerance test; and circulating levels of IGF-I and IGF-II were assessed before and after a comparable weight loss (7% of initial body weight).

RESULTS

After comparable weight loss, achieved by 20 patients per arm, and superimposable glycemic control, as reflected by HbA_{1c} level ($P = 0.60$), reduction in VAT was significantly higher in the liraglutide arm than in the lifestyle arm ($P = 0.028$), in parallel with a greater improvement in β -index ($P = 0.021$). No differences were observed in SAT reduction ($P = 0.64$). IGF-II serum levels were significantly increased ($P = 0.024$) only with liraglutide administration, and the increase in IGF-II levels correlated with both a decrease in VAT ($\rho = -0.435$, $P = 0.056$) and an increase in the β -index ($\rho = 0.55$, $P = 0.012$).

CONCLUSIONS

Liraglutide effects on visceral obesity and β -cell function might provide a rationale for using this molecule in obese subjects in an early phase of glucose metabolism dysregulation natural history.

¹Department of Medicine and Aging, and Center of Aging Science and Translational Medicine (CESI-Met), “G. d’Annunzio” University of Chieti-Pescara, Chieti, Italy

²Department of Neuroscience, Imaging and Clinical Sciences, “G. d’Annunzio” University of Chieti-Pescara, Chieti, Italy

³Department of Epidemiology and Prevention, Istituto di Ricovero e Cura a Carattere Scientifico Istituto Neurologico Mediterraneo, Pozzilli, Italy

⁴Department of Clinical and Experimental Medicine, University of Parma, and Division of Endocrinology, Azienda Ospedaliera-Universitaria di Parma, Parma, Italy

⁵Department of Clinica Medica, “SS. Annunziata” Chieti Hospital, Chieti, Italy

⁶Department of Clinical Pathology, “SS. Annunziata” Chieti Hospital, Chieti, Italy

⁷Dipartimento Pediatrico Universitario Ospedaliero, “Bambino Gesù” Children’s Hospital—Tor Vergata University, Rome, Italy

⁸Department of Women’s and Children’s Health, Karolinska Institutet, Stockholm, Sweden

⁹Department of Medicine Sciences, University of Ferrara, Ferrara, Italy

Corresponding author: Francesca Santilli, f.santilli@unich.it

Received 22 March 2017 and accepted 21 August 2017.

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc17-0589/-/DC1>.

†Deceased.

© 2017 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at <http://www.diabetesjournals.org/content/license>.

Obesity predisposes to individuals to several chronic diseases (1), including type 2 diabetes and its complications. Not all obese subjects, however, share the same risk of the development of type 2 diabetes (2). Although BMI per se is also associated with an increased risk of the development of type 2 diabetes, this depends significantly on adipose tissue body distribution. Indeed, it has been shown that in obese adults visceral adipose tissue (VAT) and insulin resistance are independently associated with incident prediabetes and type 2 diabetes, but this is not the case for general adiposity or subcutaneous adipose tissue (SAT) (3). However, whether excess visceral adiposity is causally related to metabolic abnormalities and β -cell function decline is still an open question.

Glucagon-like peptide 1 (GLP-1) is an incretin hormone capable of inducing insulin secretion and reducing glucagon secretion in a glucose-dependent manner (4). GLP-1 enhances glucose-induced insulin synthesis and secretion upon binding to GLP-1 receptors in β -cells, thus increasing β -cell sensitivity to glucose (5,6). In addition, GLP-1 delays gastric emptying and induces satiety, leading to decreased energy intake and weight reduction. GLP-1 analogs with receptor agonist (RA) activity superimposable on the native hormone but with a circulating half-life compatible to clinical use, like liraglutide, have become available for type 2 diabetes treatment. Treatment with these agents is associated with improved glucose control and weight loss (7).

It has been proposed that the activation of the IGF-I receptor expression and signaling, modulated by IGF-II synthesis and secretion by the β -cells, might play a role in the actions exerted by incretin hormones on β -cells (8). In particular, GLP-1 increases the activity of an IGF-II/IGF-I receptor autocrine loop (8), and this might contribute to protecting β -cells against apoptosis. Interestingly, adipocytes from both SAT and VAT express both IGF-II and IGF-II receptors (9), thus suggesting a possible role of IGF-II in the modulation of body fat distribution. The IGF system might thus be involved in the modulation of incretin effects on both weight loss and β -cell function.

Hypocaloric diet and lifestyle counseling are also associated with weight loss (10). It is unknown, however, whether

achieving an equal degree of weight loss by intervention on caloric intake and physical activity or by using GLP-1 RA has a different impact on the relative reduction in SAT and VAT and, thus, whether a "healthier weight loss modality" might exist (11). Thus, in the current study we assessed modifications in SAT and VAT in obese subjects with impaired glucose tolerance (IGT), impaired fasting glucose (IFG) levels, or early type 2 diabetes, who achieved a modest and comparable weight loss (7% of initial body weight) induced by either liraglutide treatment or lifestyle counseling. We also evaluated whether modifications in SAT and VAT distribution achieved with either treatment were associated with changes in insulin sensitivity, as assessed by the Matsuda index, and/or in β -cell function. This might provide additional insight into the relationship between visceral fat inflammation and β -cell function and into additional potential mechanisms for the favorable effects of liraglutide on glycemic control and cardiovascular risk (12).

RESEARCH DESIGN AND METHODS

Subjects and Study Design

This study was a longitudinal, randomized, controlled, parallel-arm study. Each patient provided written informed consent to participate and the protocol was approved by the Ethics Committee of the University of Chieti. Subjects were enrolled at the Obesity and Diabetes Clinics of Chieti University Hospital. All study visits and procedures took place at the Clinical Research Center within the CeSI-Met (Center on Aging Sciences and Translational Medicine) at the University of Chieti.

This study was performed under the Good Clinical Practice regulations (Good Clinical Practice for Trial on Medicinal Product—CPMP/European Commission—July 1990; Decreto Ministeriale 27.4.1992—Ministero della Sanità) and the Declaration of Helsinki (Hong Kong 1989). In addition, by signing the present protocol, participants in the study committed themselves to adhere to local legal requirements.

Eligibility Criteria

We enrolled subjects with a BMI ≥ 30 , who had received a diagnosis of IGT, IFG, or type 2 diabetes < 12 months previously according to American Diabetes Association Guidelines (13). At the time of study enrollment, all patients were

treated with diet therapy plus metformin at the highest tolerated dose (up to 3,000 mg/day).

Exclusion criteria included type 1 diabetes; BMI < 30 ; diabetes diagnosed > 12 months previously; treatment with any other diabetes drug, other than metformin, within the last 3 months; uncontrolled hypertension (systolic/diastolic blood pressure $> 160/90$ mmHg); significant comorbidities such as kidney disease with a glomerular filtration rate < 60 ml or liver disease (aspartate aminotransferase [AST] or alanine aminotransferase [ALT] levels twice above the upper normal range); pregnancy or lactation; female of child-bearing potential not using adequate contraceptive methods while sexually active; any contraindication to liraglutide (known or suspected hypersensitivity to liraglutide or related products, previous acute pancreatitis or chronic pancreatitis, inflammatory bowel disease, gastrointestinal surgery including gastric bypass, New York Heart Association heart failure class III-IV); personal history or family history of medullary thyroid carcinoma or personal history of multiple endocrine neoplasia type 2; claustrophobia; metal implants or other contraindications for MRI; and recent participation in other research projects within the last 3 months or participation in two or more projects in 1 year.

Randomization and Allocation

After a baseline evaluation, patients were randomized in a 1:1 ratio to receive liraglutide or lifestyle counseling. The computer-generated random allocation sequence was prepared by the trial statistician in blocks of four participants. Based on the order of inclusion in the study, subjects were assigned a consecutive random number and then were assigned to one of the two treatment groups.

Study medication was supplied to the research pharmacy by Novo Nordisk as liraglutide 6.0 mg/mL in 3-mL prefilled pen injectors. Liraglutide treatment was administered daily by subcutaneous injection at bedtime with an initial dose of 0.6 mg/day (first week) and titrated over a 3-week period to doses of 1.2 mg daily (second week) and 1.8 mg daily (third week) based on the clinical response and side effects. The nonattainment of the 1.8-mg dose level did not constitute a withdrawal criterion.

Both groups received lifestyle and diabetes education as part of the standard care. Patients in the liraglutide arm were invited to continue with their usual dietary habits and physical activity.

Outcome and Study Visits

Participants stayed on the assigned treatment until they lost 7% of their initial body weight (calculated on the basis of body weight at baseline visit at the time of randomization). We chose the loss of 7% of initial body weight as the target weight loss based on previous reports where such a weight loss was associated with improved metabolic outcomes (14).

Patients not achieving this amount of weight loss within 15 months after randomization discontinued treatment, and their data (as well as those of subjects who dropped out of the study) were not included in the calculations. After signing the informed consent form, subjects were evaluated at baseline and after reaching the prespecified weight loss. Both visits included the following: clinical evaluation; and abdominal MRI for adipose tissue quantification and VAT and SAT (15) assessment. At each visit, blood samples were obtained after a 12-h overnight fast for the measurement of plasma glucose and plasma insulin levels, and circulating levels of hs-CRP. Right after each visit, a frequent-sampling oral glucose tolerance test (OGTT) was performed for the assessment of insulin sensitivity (Matsuda index) (16), and β -cell function (17) (β -index).

While receiving treatment, patients were seen every 3 weeks at the Clinical Research Center to reinforce their motivation to achieve the weight loss goal and to monitor compliance with liraglutide therapy (missed doses counting) or lifestyle changes (see below). On each occasion, participants underwent a physical examination and were carefully monitored for adverse events.

Lifestyle Intervention Program

Participants were encouraged to achieve the target weight loss within the first 6 months after randomization, since previous behavioral weight loss studies suggested that in most individuals maximum weight loss is achieved within the first 20–24 weeks of a lifestyle intervention (18). Standard lifestyle recommendations were provided in written form and during periodic 20- to 30-min individual sessions

focused on the importance of a healthy lifestyle. Patients met the nutritionists on staff once a week for the first 4 weeks, then once every 2 weeks for the following 20 weeks, and finally once a month (until the achievement of the weight loss goal). Participants were encouraged to follow the Food Guide Pyramid and the equivalent of a National Cholesterol Education Program Step 1 diet, to reduce their weight (healthy low-calorie, low-fat diet) and to increase their physical activity (moderate intensity, such as brisk walking, for at least 150 min/week, to approximate at least 700 kcal/week expenditure).

OGTT With Frequent Sampling

Subjects underwent OGTTs with frequent sampling before and after achievement of the weight loss target, at least 48 h after the last administration of liraglutide for those in the liraglutide arm. Patients were admitted to the Clinical Research Center at 8:00 A.M. after a 10- to 12-h overnight fast. A catheter was inserted into an antecubital vein, and another catheter was inserted retrogradely into a wrist vein for blood sampling. At time 0 minutes, subjects ingested a 75-g glucose solution over 5 min. Blood samples were collected at -10 , 0, 15, 30, 45, 60, 90, and 120 min to measure plasma glucose, serum C-peptide, and insulin (baseline samples and 30 min) concentrations.

Insulin sensitivity was calculated using the Matsuda index, which represents a composite of both hepatic and peripheral tissue sensitivity to insulin, as previously described (16).

β -Cell secretion during an OGTT was estimated by applying a minimal model of glucose-induced insulin secretion to the glucose and C-peptide curves of each subject, as previously described in detail (17).

MRI Quantification of Visceral and Subcutaneous Fat

Magnetic resonance images were obtained with a Philips 1.5-Tesla Achieva body scanner, available at the Institute for Advanced Biomedical Technologies within the University of Chieti. Calculation of adipose tissue area and volume was performed as previously described (15).

Analytical Measurements

Biological Material Collection

At baseline and after achievement of the weight loss goal, venous blood samples

were collected and frozen at -20°C for subsequent biochemical measurements.

Biochemical Measurements

Plasma glucose concentrations were measured by the glucose oxidase method, and serum insulin and C-peptide levels were measured by immunochemiluminometric assays. Serum hs-CRP concentrations were measured using a highly sensitive immunoassay. HbA_{1c} levels were determined by automated high-performance liquid chromatography (19).

Serum IGF-I, IGF-II, and insulin-like growth factor binding protein 3 (IGFBP-3) were measured with specific radioimmunoassay kits (Mediagnost, Tubingen, Germany), as previously described (20).

More detailed methods are available in the Supplementary Data.

Statistical Analysis

The primary outcome was the change in VAT (pretreatment – posttreatment) after achievement of the weight loss target. Continuous secondary outcomes were changes in levels of SAT, CRP, Matsuda index, hs-CRP, glycemic control measures, including HbA_{1c}, fasting glucose, 2-h post-load glucose, and β -cell function, as assessed by β -index.

Twenty patients were to be studied in each treatment group for a two-tailed α value of 0.05 and a power of 90% to detect, at the end of the treatment period, a mean difference in the VAT areas of at least 1 SD (of the distribution of VAT changes) between liraglutide and lifestyle intervention. Allowing for a 35% dropout rate or lack of achievement of the weight loss target, we estimated that ~ 30 patients for each treatment arm should have been enrolled and randomized.

The Kolmogorov-Smirnov test and examination of residual distribution were used to determine whether each variable had a normal distribution. When necessary, natural-log transformation was used to normalize the data or appropriate nonparametric tests were used (Mann-Whitney U test, Spearman correlation coefficient). Comparisons of baseline data between groups were performed by χ^2 tests, Fisher exact tests, unpaired Student t tests, or Mann-Whitney U tests.

For the primary analysis, we used a linear mixed-effects model for repeated measures over time, with change in VAT as the dependent variable, study group and time-by-group interaction as fixed effects, time-to-weight loss (month) as the fixed-effect

covariate, and patients and error as random effects. Within the mixed model, we obtained least-squares estimates of the treatment differences and SEs, and estimated 95% CIs and *P* values for the two prespecified intergroup contrasts (liraglutide and lifestyle intervention) for baseline and end of study within each group.

For other continuous variables (such as insulin sensitivity, β -cell function), we used the same procedure as in the primary analysis.

P values <0.05 were considered to be statistically significant. Data analysis was generated using SAS/STAT software, version 9.4 of the SAS System for Windows 2009 (SAS Institute Inc., Cary, NC).

RESULTS

Between June 2012 and September 2013, 132 patients were assessed for eligibility in the outpatient Diabetes Clinic and Obesity Centre of Chieti University Hospital. Among these, 70 were excluded (53 did not meet the eligibility criteria, 10 refused to participate, 5 had claustrophobia, and 2 were wearing a pacemaker). A total of 62 patients was randomized to one of the two treatment groups—31 were assigned to the liraglutide group and 31 were assigned to the lifestyle changes group—and monitored until achievement of the weight loss goal. Patients were recruited from October 2013 to July 2015.

Final outcome status was ascertained for 40 patients (20 per arm).

Twenty-two subjects were excluded from the analysis. Of those subjects, 6 did not achieve the weight loss target within the allowed 15-month period and 16 were lost to follow-up (13 for unwillingness to continue the study, 1 due to metformin intolerance, 1 due to pregnancy, and 1 due to severe anemia) (Supplementary Table 1, see flowchart). No serious adverse event occurred during the treatment period. Minor gastrointestinal side effects were generally well tolerated. All patients were able to tolerate the maximum dosage of liraglutide (1.8 mg once daily), except one patient who continued with a dose of 1.2 mg/day because of persistent nausea.

Completers were comparable to non-completers with regard to baseline characteristics (Supplementary Table 2).

Table 1 shows the demographic and clinical characteristics of the 40 patients

who completed the study. At baseline, the only significant between-group differences were triglyceride levels (greater in the liraglutide group, *P* = 0.026) and VAT (slightly greater in the liraglutide arm, *P* = 0.046) (Table 1).

Effects of Liraglutide and Lifestyle Interventions

Weight Loss

The amount of weight loss (as a percentage of the baseline body weight) was prespecified per protocol; thus, it was the same in both groups. In absolute amount, total weight loss was of 7.79 kg in the liraglutide group and 7.20 kg in the lifestyle intervention group (*P* > 0.05). The median time necessary to achieve the prespecified amount of weight loss was not different between the two treatment arms (4 months [interquartile range (IQR) 3.25–6 months] and 4 months [IQR 3–5.75 months] in the liraglutide and lifestyle intervention arms, respectively).

Reduction of VAT and SAT

After comparable weight loss, reduction in VAT was significantly higher in the liraglutide arm (−15.3%) than in the lifestyle arm (−9.0%) (between-group *P* = 0.028) (Fig. 1A and Table 2). On the contrary, no differences between the two groups were observed in SAT reduction (between-group *P* = 0.64) (Fig. 1B and Table 2).

Glucose Control

An improvement in glycemic control was observed in both groups. HbA_{1c} was comparably reduced in both arms (*P* = 0.60) (from 43 to 38 mmol/mol [6.1–5.6%] in the lifestyle arm and from 42 to 38 mmol/mol (5.95–5.65%) in the liraglutide arm) (Fig. 2A and Table 2). A tendency toward improvement in fasting blood glucose level and glucose tolerance was observed in both groups. However, the OGTT 1-h and 2-h plasma glucose values decreased significantly only in the liraglutide group (Fig. 2B and Table 2).

Insulin Sensitivity and β -Cell Function

As expected, a trend toward improved insulin sensitivity, as assessed by the Matsuda index (Fig. 2C and Table 2), was observed in both groups after weight loss. This improvement reached statistical significance only in the lifestyle intervention group; however, no significant between-group difference was observed relative to this parameter.

β -Cell function, as assessed by β -index, improved in the liraglutide group (1.36 pmol \cdot min^{−2} \cdot m^{−2} body surface area [BSA], *P* = 0.001) but not in the lifestyle group (0.46 pmol \cdot min^{−2} \cdot m^{−2} BSA, *P* = 0.30) (between-group *P* = 0.021) (Fig. 2D and Table 2).

IGF-II

At baseline, VAT was inversely related to IGF-II in the whole cohort (*n* = 62; data not shown).

IGF-II serum levels were significantly increased (*P* = 0.024), whereas IGFBP-3 levels were significantly reduced (*P* = 0.015) after liraglutide-induced weight loss but not after lifestyle intervention-associated weight loss (Table 2). In the liraglutide arm, but not in the lifestyle intervention arm, a significant correlation was observed between IGF-II increase and both VAT decrease (ρ = −0.435, *P* = 0.056) and β -index increase (ρ = 0.55, *P* = 0.012) (data not shown).

The circulating IGF-I level was not affected by either intervention (Table 2).

CONCLUSIONS

Weight loss is associated with several metabolic benefits. However, because of different metabolic characteristics of VAT and SAT, it is believed that the loss of VAT, as opposed to SAT, is more beneficial. Treatment with GLP-1 RAs of both subjects with type 2 diabetes and obese subjects is associated with weight loss (7).

The current study demonstrates that, for the same degree of weight loss, treatment with a GLP-1 RA is more effective than a standardized lifestyle intervention protocol in reducing visceral fat.

Previous studies on the effect of GLP-1 RAs on adipose tissue distribution have yielded conflicting results, mostly because of heterogeneity of the length of the intervention and the methods used to assess adipose tissue areas (21–23). In the only study directly comparing liraglutide treatment to lifestyle intervention effects (24), the two treatment arms were not comparable in terms of the weight loss achieved but only relative to the duration of the intervention. In our study, the impact on visceral fat loss was independent both of the length of drug exposure (3–12 months) and of overall weight loss per se. Thus, it is conceivable that specific properties of liraglutide (or in general of GLP-1 RAs) might be responsible

Table 1—Baseline characteristics of obese patients randomized to liraglutide- or lifestyle-induced weight loss

Variable	Pre-liraglutide therapy (n = 20)	Pre-lifestyle change (n = 20)	P value*
Age (years)	55.5 (48.2–63.7)	52.2 (50.2–57.2)	0.48
Male sex, n (%)	11 (55)	10 (50)	1.00
BMI (kg/m ²)	36.7 (34.7–40.9)	35.0 (31.3–40.3)	0.24
Type 2 diabetes, n (%)	10 (50)	7 (35)	0.52
IGT/IFG, n (%)	10 (50)	13 (65)	0.52
Waist (cm)	116.5 (112.0–128.5)	110.0 (100.4–119.2)	0.04
WHR	0.9 (0.9–1.0)	0.9 (0.9–1.0)	0.32
Systolic BP (mmHg)	144.5 (130–153)	134.0 (122.2–143.2)	0.14
Diastolic BP (mmHg)	83.0 (78.0–87.5)	80.0 (70.0–83.7)	0.31
Smoke, n (%)	4 (20)	0 (0)	0.11
Hypertension, n (%)	17 (85)	12 (60)	0.16
Dyslipidemia, n (%)	9 (45)	10 (50)	1.00
MS (NCEP-ATP III), n (%)	10 (50)	8 (40)	0.75
MS (IDF), n (%)	10 (50)	9 (45)	1.00
CVD, n (%)	1 (5)	5 (25)	0.18
Previous MI or revascularization, n (%)	0 (0)	1 (5)	1.00
Previous TIA/stroke or revascularization, n (%)	1 (5)	1 (5)	1.00
PAD, n (%)	1 (5)	0 (0)	1.00
Carotid stenosis, n (%)	0 (0)	4 (20)	0.11
Microvascular disease, n (%)	0 (0)	0 (0)	
Total cholesterol (mmol/L)	4.4 (3.6–5.0)	4.4 (3.8–4.6)	0.34
HDL cholesterol (mmol/L)	1.2 (1.0–1.4)	1.1 (1.0–1.4)	0.67
Triglycerides (mmol/L)	1.4 (0.9–2.2)	1.0 (0.8–1.3)	0.026
Amylase (units/L)	56.5 (53.5–70.75)	62.5 (52.5–77.2)	0.58
Lipase (units/L)	105.0 (66.2–117.5)	134.5 (66.5–173.2)	0.15
Fasting plasma glucose (mmol/L)	5.2 (4.9–5.9)	5.3 (5.0–5.7)	0.99
1-h postload plasma glucose (mmol/L)	10.6 (9.2–11.4)	10.6 (8.6–11.2)	0.16
2-h postload plasma glucose (mmol/L)	8.7 (7.9–10.7)	8.1 (6.3–10.2)	0.17
HbA _{1c} (%)	5.95 (5.62–6.70)	6.1 (5.6–6.5)	0.86
HbA _{1c} (mmol/mol)	42 (38–50)	43 (38–48)	0.86
Fasting plasma insulin (μ U/mL)	13.35 (9.62–20.92)	10.7 (7.5–21.7)	0.39
1-h postload plasma insulin (μ U/mL)	72.75 (31.7–102.82)	78.9 (55.5–140.0)	0.13
2-h postload plasma insulin (μ U/mL)	76.9 (42.87–100.75)	76.3 (55.3–123.3)	0.17
Creatinine (μ mol/L)	61.9 (53.0–70.7)	70.7 (61.9–79.6)	0.089
Total bilirubin (μ mol/L)	10 (7–15)	10 (7–14)	0.90
hs-CRP (nmol/L)	38.1 (28.6–85.7)	28.6 (9.5–47.6)	0.35
AST (units/L)	29.0 (24.2–39)	33.0 (27.5–43.5)	0.32
ALT (units/L)	41.0 (36.2–46.5)	50.0 (33.2–66.5)	0.39
Metformin, n (%)	20 (100)	20 (100)	1.00
ACE-Is, n (%)	4 (20)	3 (15)	1.00
ARBs, n (%)	7 (35)	6 (30)	1.00
Diuretics, n (%)	7 (35)	5 (25)	0.73
β -Blockers, n (%)	7 (35)	4 (20)	0.48
CCA, n (%)	0 (0)	1 (5)	1.00
Statins, n (%)	2 (10)	5 (25)	0.41
Fibrates, n (%)	0 (0)	0 (0)	
PUFA, n (%)	1 (5)	0 (0)	1.00
Proton pump inhibitors, n (%)	3 (15)	3 (15)	1.00
ASA, n (%)	1 (5)	3 (15)	0.60
IGF-I (ng/mL)	84.9 (56.7–110.0)	100.9 (80.4–124.2)	0.096
IGF-II (ng/mL)	558.4 (520.8–651.8)	682.7 (616.3–796.9)	0.015
IGFBP-3 (ng/mL)	1,769.0 (1,355.8–2,170.0)	2,222.7 (1,832.9–2,614.5)	0.017
SAT (cm ²)	434.1 (317.9–527.2)	374.9 (254.2–455.3)	0.31
VAT (cm ²)	324.2 (257.0–386.9)	254.5 (180.2–318.9)	0.046
β -Index (pmol \cdot min ⁻² \cdot m ⁻² BSA)	3.41 (2.58–5.05)	4.34 (3.06–5.29)	0.14

ACE-I, ACE inhibitor; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; BP, blood pressure; CCA, calcium channel antagonist; CVD, cardiovascular disease; IDF, International Diabetes Federation; MI, myocardial infarction; MS, metabolic syndrome; NCEP-ATP III, National Cholesterol Education Program-Adult Treatment Panel III; PAD, peripheral artery disease; TIA, transient ischemic attack; WHR, waist-to-hip ratio. Data are expressed as the median (IQR), unless otherwise indicated. *Determined by Mann-Whitney or χ^2 test, as appropriate.

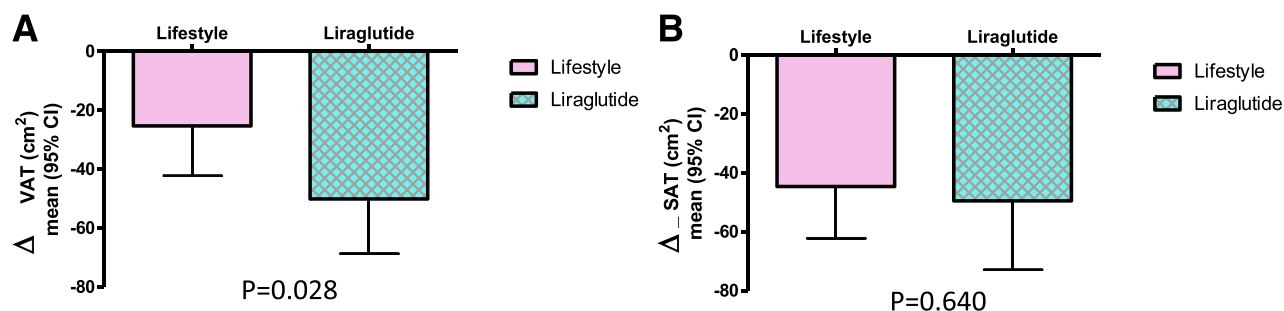


Figure 1—Effects of liraglutide- or lifestyle-induced weight loss on adipose tissue body distribution. Changes in VAT (A) and SAT (B) after liraglutide- or lifestyle-induced weight loss, in obese subjects with prediabetes and early type 2 diabetes. *P* values (lifestyle vs. liraglutide) are for comparison of changes between groups.

for the more pronounced visceral fat loss observed in the liraglutide arm.

A GLP-1–specific receptor, structurally and/or functionally distinct from that expressed in the pancreas, has been identified in adipose tissue, and its mRNA and protein levels are increased in VAT from severely insulin-resistant, morbidly obese patients (25,26). GLP-1 promotes preadipocyte differentiation (27), reduces the expression of adipogenic and lipogenic genes, and enhances the expression of lipolytic markers in human adipose tissue explants, with distinct effects on VAT and SAT (28). Some of the molecular mechanisms described in these preclinical studies might explain the liraglutide effects on VAT that we observed. Consistently, liraglutide treatment is associated with a rapid and substantial reduction of epicardial adipose tissue, an emerging cardiovascular risk factor reflecting organ-specific visceral fat, which correlates with atherosclerosis and coronary artery disease (29). In addition, it has been recently described (30), in rodents as well as in humans, that liraglutide-induced weight loss might be mediated by the activation of adipose-resident invariant natural killer cells, which in turn drive the production of fibroblast growth factor 21 by adipocytes, with subsequent thermogenic browning of white fat. These observations, coupled with our results, might point to the adipose tissue as a novel target organ for GLP-1 agonists.

We also observed that liraglutide treatment was associated with an improvement in β -cell function significantly greater than that observed in subjects achieving the same weight loss through lifestyle intervention (31).

In this regard, our findings are consistent with those of the Liraglutide and

β -cell RepAir (LIBRA) trial (32), where liraglutide treatment was associated with a significant and sustained enhancement in β -cell function in individuals with early type 2 diabetes. In the LIBRA trial (33), the increase in the β -cell secretory capacity could have been attributable to weight reduction and/or lowering of blood glucose level rather than to a direct liraglutide effect. In our study, subjects in the two treatment arms achieved the same percentage reduction in body weight and superimposable HbA_{1c} values. Thus, the observed liraglutide effects on β -cell function are presumably independent of weight loss and/or glycemic control (34). In addition, patients in both arms were already in good glycemic control before randomization, as reflected by baseline HbA_{1c} values (median 6.05 [IQR 5.62–6.50]), and >50% of subjects did not have overt diabetes but were affected just by prediabetes, thus limiting the role of baseline glucotoxicity in inducing β -cell dysfunction. Moreover, we tried to be as accurate as possible in estimating β -cell function in our subjects by applying a minimal model of glucose-induced insulin secretion to the post-OGTT glucose and C-peptide curves of each subject (17). Finally, since our study also involved subjects with prediabetes, one might speculate, on the basis of our results, that early treatment with a GLP-1 RA could be helpful in contrasting and eventually reversing the β -cell deterioration, potentially leading to overt diabetes even in patients with IFG and/or IGT.

Supporting this hypothesis, liraglutide modulates 1-h postload hyperglycemia during an OGTT, which is associated with a higher risk of the development of type 2 diabetes and cardiovascular disease in individuals with prediabetes (35).

We also speculated that the effects of liraglutide on both visceral fat and β -cell function might be intertwined and challenged the hypothesis that the IGF system may be implicated as a mechanistic link. Interestingly, GLP-1 protects β -cells against apoptosis by increasing the activity of an IGF-II/IGF-I receptor autocrine loop (8), which in turn is dependent on IGF-II synthesis and secretion by the β -cells (36). The autocrine action of IGF-II also regulates adult β -cell mass and function (37). β -cell–specific inactivation of IGF-II in mice was associated with impaired glucose-stimulated insulin secretion and circulating levels of IGF-II below the detection limit of the ELISA (37), suggesting that serum IGF-II levels largely reflect the autocrine synthesis and secretion by β -cells.

In our study, IGF-II circulating concentrations significantly increased only in the liraglutide arm, and this increase was related to the positive changes in the β -index. It is tempting to establish a parallel between the elevation in serum IGF-II mirroring the liraglutide mediated autocrine IGF-II synthesis and the possible positive GLP-1analogue effects on β -cell proliferation, eventually reflected by the increase in β -index.

We also observed that at baseline IGF-II was inversely related to VAT and that in the liraglutide arm the IGF-II increase paralleled the decrease in VAT area. Since both IGF-II and IGF-II receptors are expressed by adipocytes (9), circulating IGF-II might be a possible link between VAT decrease and β -cell function improvement during liraglutide treatment. Thus, one could speculate that the observed effect of liraglutide on β -cell function might be associated in part with the effect of the drug on visceral fat loss, through an adipoinsular axis where the

Table 2—Clinical, biochemical, and imaging parameters of obese patients before and after liraglutide- or lifestyle-induced weight loss

Variable	Pre-liraglutide therapy	Post-liraglutide therapy	P value*	Pre-lifestyle change	Post-lifestyle change	P value*
BMI (kg/m ²)	36.7 (34.7–40.9)	33.9 (31.4–37.9)	<0.001	35.0 (31.3–40.3)	32.5 (29.0–37.1)	<0.001
Waist (cm)	116.5 (112.0–128.5)	110.0 (104.2–120.7)	<0.001	110.0 (100.4–119.2)	106.0 (97.2–112.7)	0.001
WHR	0.97 (0.92–1.04)	0.98 (0.92–1.00)	0.59	0.9 (0.9–1)	0.9 (0.9–1)	0.78
Systolic BP (mmHg)	144 (130–153)	133 (122–144)	0.029	134 (122–143)	133 (125–143)	0.68
Diastolic BP (mmHg)	83 (78–87)	78 (69–83)	0.079	80 (70–84)	80 (77–86)	0.48
Total cholesterol (mmol/L)	4.4 (3.6–5.02)	3.9 (3.4–4.6)	<0.001	4.4 (3.8–4.6)	4.2 (3.7–4.6)	0.51
HDL cholesterol (mmol/L)	1.2 (1.0–1.4)	1.15 (1.0–1.4)	0.085	1.1 (1.0–1.4)	1.2 (0.9–1.3)	0.40
Triglycerides (mmol/L)	1.4 (0.9–2.2)	1.5 (0.9–1.7)	0.29	1.0 (0.8–1.3)	0.5 (1.0–1.5)	0.31
Amylase (units/L)	56.5 (53.5–70.75)	67.5 (46.7–82.2)	0.65	62.5 (52.5–77.2)	74.5 (55.2–90.7)	0.046
Lipase (units/L)	105.0 (66.2–117.5)	132.0 (99.0–223.0)	0.004	134.5 (66.5–173.2)	118.5 (79.0–172.0)	0.88
Fasting plasma glucose (mmol/L)	5.2 (4.9–5.9)	4.9 (4.5–5.2)	0.001	5.3 (5.0–5.7)	4.9 (4.6–5.2)	0.057
1-h postload plasma glucose (mmol/L)	10.6 (9.2–11.4)	9.0 (7.1–10.0)	<0.001	10.0 (8.6–11.2)	8.7 (7.8–9.9)	0.097
2-h postload plasma glucose (mmol/L)	8.7 (7.9–10.7)	7.2 (5.1–9.9)	0.001	8.1 (6.3–10.1)	7.7 (5.7–10.4)	0.39
HbA _{1c} (%)	5.95 (5.62–6.70)	5.65 (5.40–5.97)	<0.001	6.1 (5.6–6.5)	5.6 (5.4–6.1)	0.001
HbA _{1c} (mmol/mol)	42 (38–50)	38 (36–42)	<0.001	43 (38–48)	38 (36–43)	0.001
Fasting plasma insulin (μ U/mL)	13.3 (9.6–20.9)	9.7 (6.7–15.1)	0.015	10.7 (7.5–21.7)	8.9 (6.3–11.1)	0.001
Creatinine (μ mol/L)	61.6 (52.8–70.4)	61.6 (52.8–79.2)	0.80	70.4 (61.6–79.2)	70.4 (61.6–79.2)	0.36
Total bilirubin (μ mol/L)	10 (7–15)	10 (9–14)	0.88	10 (7–14)	10 (7–14)	0.81
hs-CRP (nmol/L)	38.1 (28.6–85.7)	28.6 (9.5–57.1)	0.004	28.6 (9.5–47.6)	28.6 (19.0–28.6)	0.018
AST (units/L)	29.0 (24.2–39)	22.0 (19.2–25.5)	<0.001	33.0 (27.5–43.5)	24.0 (20.0–33.7)	<0.001
ALT (units/L)	41.0 (36.2–46.5)	31.0 (26.2–37.0)	<0.001	50.0 (33.2–66.5)	35.0 (28.2–49.0)	0.001
IGF-I (ng/mL)	84.9 (56.7–110.0)	97.6 (65.0–120.0)	0.12	100.9 (80.4–124.2)	101.7 (86.6–128.7)	0.28
IGF-II (ng/mL)	558.4 (520.8–651.8)	610.7 (543.0–724.5)	0.024	682.7 (616.3–796.9)	708.9 (633.0–818.4)	0.17
IGFBP-3 (ng/mL)	1,769.0 (1,355.8–2,170.0)	1,510.0 (1,176.0–1,940.1)	0.015	2,222.7 (1,833.0–2,614.5)	1,972.7 (1,547.3–2,606.6)	0.100
SAT (cm ²)	434.1 (317.9–527.2)	421.0 (258.2–481.8)	0.001	374.9 (254.2–455.3)	294.3 (210.6–403.8)	<0.001
VAT (cm ²)	324.2 (257.0–386.9)	274.6 (183.9–321.2)	<0.001	254.5 (180.2–318.9)	231.6 (171.3–290.7)	0.017
β -Index (pmol \cdot min ⁻² \cdot m ⁻² BSA)	3.41 (2.58–5.05)	4.77 (3.39–5.50)	0.001	4.34 (3.06–5.29)	4.80 (3.55–5.24)	0.30

BP, blood pressure; WHR, waist-to-hip ratio. Data are expressed as the median (IQR), unless otherwise indicated. *P values for comparison before vs. after treatment within groups.

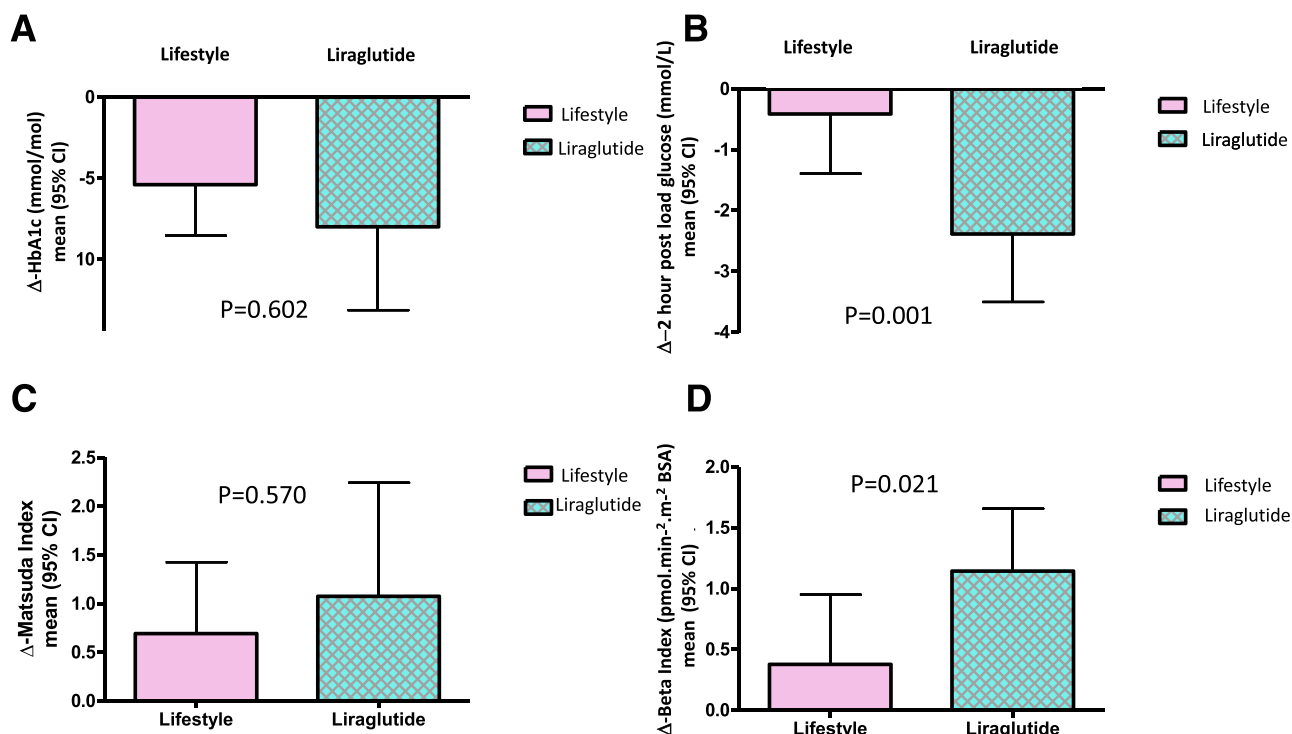


Figure 2—Effects of liraglutide- or lifestyle-induced weight loss on glycemic control, insulin sensitivity, and β -cell function. Changes in HbA_{1c} (A), 2-h postload plasma glucose (B), Matsuda index (C), and β -index (D) after liraglutide- or lifestyle-induced weight loss in obese subjects with prediabetes and early type 2 diabetes. *P* values (lifestyle vs. liraglutide) are for comparison of changes between groups.

IGF system plays a role. However, although our data do suggest an effect of liraglutide on IGF-II increase, our findings remain largely correlational, and need to be confirmed by more in-depth studies specifically designed to test the hypothesis that the IGF system is indeed involved in mediating liraglutide effects on visceral fat and β -cell function.

A potential limitation of our study, beside the relatively limited sample size, which might have prevented the detection of additional differences between the two groups, is the high rate of study withdrawals. This was largely anticipated, given the complexity of the study design and the requirement of obtaining at least a minimum prespecified weight loss, and was therefore factored in the a priori sample size calculation and subsequent randomization of an excess of subjects. However, study withdrawals were not related to liraglutide side effects, because they were mostly dependent on the nonattainment of the weight loss goal or on metformin intolerance. More importantly, completers and noncompleters did not differ in their baseline characteristics (Supplementary Table 2), thus largely ensuring against a possible selection bias. Still, we cannot exclude that the

per protocol nature of the analysis might somehow weaken our conclusions. Another limitation is the lack of postintervention follow-up, which prevented assessment of the durability of our findings, in particular on adipose tissue distribution. Finally, the reduction in VAT associated with liraglutide treatment was definitely significant but limited in magnitude. Its clinical significance, if any, remains to be fully established.

In conclusion, in a group of obese subjects with prediabetes or early type 2 diabetes randomized to therapy with liraglutide or lifestyle changes to achieve comparable weight loss, we observed significantly enhanced abdominal visceral fat loss and improved β -cell function with liraglutide. Both interventions were equally effective on glycemic control, although improvement in glucose tolerance seemed more pronounced after liraglutide treatment. The liraglutide effects on visceral obesity and β -cell function might provide a rationale for its use in obese subjects in an early phase of the natural history of glucose metabolism dysregulation.

Acknowledgments. The authors thank Drs. Maria Golato (Department of Clinical Pathology, “SS.

Annunziata” Chieti Hospital), Daniela Germani (Department of Systems Medicine, Tor Vergata University), and Domenico De Cesare (Atherosclerosis Prevention Center, “G. d’Annunzio” University of Chieti-Pescara) for help in the biochemical measurements; Dr. Maria Linda Boselli (Division of Endocrinology and Metabolic Diseases, University of Verona) for her contribution in the analysis of the data; and Alessia Quirino (Department of Clinica Medica, “SS. Annunziata” Chieti Hospital) for patient education.

Funding. This study was supported by a grant from the Italian Ministry of University and Research (PRIN no. 2010J3PMZ to F.S.).

Duality of Interest. A.C. received lecture fees and fees for serving on advisory boards from Novo Nordisk, Eli Lilly, AstraZeneca, Sanofi, Merck Sharp & Dohme, and Takeda and grant support to his institution from Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. F.S. contributed to the study design and protocol, the analysis plan, the supervision of the analysis, study implementation, data acquisition and interpretation, statistical analyses, writing of the manuscript, and critical revision and final approval of the manuscript. P.G.S. contributed to study implementation, data acquisition and interpretation, and writing of the manuscript. M.T.G., M.L., M.T.M., C.S., R.C.B., E.A., V.F., S.C., G.D., and A.T. contributed to study implementation, data acquisition and interpretation, and final approval of the manuscript. A.D.C. and L.M. contributed to the analysis plan, the supervision of the analysis, statistical analyses, and final approval of the manuscript. A.C. contributed to the study design and protocol, the analysis plan, the supervision

of the analysis, data interpretation, writing of the manuscript, and critical revision and final approval of the manuscript. F.S. and A.C. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in poster form at the 75th Scientific Sessions of the American Diabetes Association, Boston, MA, 5–9 June 2015, and at the 51st Annual Meeting of the European Association for the Study of Diabetes (EASD), Stockholm, Sweden, 14–18 September 2015.

References

- Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. *N Engl J Med* 2017;376:254–266
- Kim SH, Després JP, Koh KK. Obesity and cardiovascular disease: friend or foe? *Eur Heart J* 2016;37:3560–3568
- Neeland IJ, Turer AT, Ayers CR, et al. Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. *JAMA* 2012;308:1150–1159
- Nauck MA, Meier JJ. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. *Lancet Diabetes Endocrinol* 2016;4:525–536
- Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006;368:1696–1705
- Yusta B, Baggio LL, Estall JL, et al. GLP-1 receptor activation improves beta cell function and survival following induction of endoplasmic reticulum stress. *Cell Metab* 2006;4:391–406
- Vilsbøll T, Zdravkovic M, Le-Thi T, et al. Liraglutide, a long-acting human glucagon-like peptide-1 analog, given as monotherapy significantly improves glycemic control and lowers body weight without risk of hypoglycemia in patients with type 2 diabetes. *Diabetes Care* 2007;30:1608–1610
- Cornu M, Yang J-Y, Jaccard E, Poussin C, Widmann C, Thorens B. Glucagon-like peptide-1 protects β -cells against apoptosis by increasing the activity of an Igf-2/Igf-1 receptor autocrine loop. *Diabetes* 2009;58:1816–1825
- Sinha MK, Buchanan C, Raineri-Maldonado C, et al. IGF-II receptors and IGF-II-stimulated glucose transport in human fat cells. *Am J Physiol* 1990;258:E534–E542
- Pi-Sunyer X, Astrup A, Fujioka K, et al.; SCALE Obesity and Prediabetes NN8022-1839 Study Group. A randomized, controlled trial of 3.0 mg of liraglutide in weight management. *N Engl J Med* 2015;373:11–22
- Després JP. Obesity and cardiovascular disease: weight loss is not the only target. *Can J Cardiol* 2015;31:216–222
- Marso SP, Daniels GH, Brown-Frandsen K, et al.; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2016;375:311–322
- American Diabetes Association. Classification and diagnosis of diabetes. Sec. 2. In *Standards of Medical Care in Diabetes—2015*. *Diabetes Care* 2015;38(Suppl. 1):S8–S16
- Knowler WC, Barrett-Connor E, Fowler SE, et al.; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346:393–403
- Ross R, Léger L, Morris D, de Guise J, Guardo R. Quantification of adipose tissue by MRI: relationship with anthropometric variables. *J Appl Physiol* (1985) 1992;72:787–795
- Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462–1470
- Cretti A, Lehtovirta M, Bonora E, et al. Assessment of beta-cell function during the oral glucose tolerance test by a minimal model of insulin secretion. *Eur J Clin Invest* 2001;31:405–416
- Jeffery RW, Wing RR, Mayer RR. Are smaller weight losses or more achievable weight loss goals better in the long term for obese patients? *J Consult Clin Psychol* 1998;66:641–645
- Khuu HM, Robinson CA, Goolsby K, Hardy RW, Konrad RJ. Evaluation of a fully automated high-performance liquid chromatography assay for hemoglobin A1c. *Arch Pathol Lab Med* 1999;123:763–767
- Cianfarani S, Inzaghi E, Alisi A, Germani D, Puglianiello A, Nobili V. Insulin-like growth factor-I and -II levels are associated with the progression of nonalcoholic fatty liver disease in obese children. *J Pediatr* 2014;165:92–98
- Bouchi R, Nakano Y, Fukuda T, et al. Reduction of visceral fat by liraglutide is associated with ameliorations of hepatic steatosis, albuminuria, and micro-inflammation in type 2 diabetic patients with insulin treatment: a randomized control trial. *Endocr J* 2017;64:269–281
- Li CJ, Yu Q, Yu P, et al. Changes in liraglutide-induced body composition are related to modifications in plasma cardiac natriuretic peptides levels in obese type 2 diabetic patients. *Cardiovasc Diabetol* 2014;13:36
- Morano S, Romagnoli E, Filardi T, et al. Short-term effects of glucagon-like peptide 1 (GLP-1) receptor agonists on fat distribution in patients with type 2 diabetes mellitus: an ultrasonography study. *Acta Diabetol* 2015;52:727–732
- Pastel E, McCulloch LJ, Ward R, et al. GLP-1 analogue-induced weight loss does not improve obesity-induced AT dysfunction. *Clin Sci (Lond)* 2017;131:343–353
- Montrose-Rafizadeh C, Yang H, Wang Y, Roth J, Montrose MH, Adams LG. Novel signal transduction and peptide specificity of glucagon-like peptide receptor in 3T3-L1 adipocytes. *J Cell Physiol* 1997;172:275–283
- Vendrell J, El Bekay R, Peral B, et al. Study of the potential association of adipose tissue GLP-1 receptor with obesity and insulin resistance. *Endocrinology* 2011;152:4072–4079
- Challa TD, Beaton N, Arnold M, Rudofsky G, Langhans W, Wolfrum C. Regulation of adipocyte formation by GLP-1/GLP-1R signaling. *J Biol Chem* 2012;287:6421–6430
- El Bekay R, Coin-Aragüez L, Fernández-García D, et al. Effects of glucagon-like peptide-1 on the differentiation and metabolism of human adipocytes. *Br J Pharmacol* 2016;173:1820–1834
- Iacobellis G, Mohseni M, Bianco SD, Banga PK. Liraglutide causes large and rapid epicardial fat reduction. *Obesity (Silver Spring)* 2017;25:311–316
- Lynch L, Hogan AE, Duquette D, et al. iNKT cells induce FGF21 for thermogenesis and are required for maximal weight loss in GLP1 therapy. *Cell Metab* 2016;24:510–519
- Færch K, Johansen NB, Witte DR, Lauritzen T, Jørgensen ME, Vistisen D. Relationship between insulin resistance and β -cell dysfunction in subphenotypes of prediabetes and type 2 diabetes. *J Clin Endocrinol Metab* 2015;100:707–716
- Retnakaran R, Kramer CK, Choi H, Swaminathan B, Zinman B. Liraglutide and the preservation of pancreatic β -cell function in early type 2 diabetes: the LIBRA trial. *Diabetes Care* 2014;37:3270–3278
- Arnolds S, Sawicki PT. Comment on Retnakaran et al. Liraglutide and the preservation of pancreatic β -cell function in early type 2 diabetes: the LIBRA trial. *Diabetes Care* 2014;37:3270–3278. *Diabetes Care* 2015;38:e25
- Ferrannini E, Camastra S, Gastaldelli A, et al. Beta-cell function in obesity: effects of weight loss. *Diabetes* 2004;53(Suppl. 3):S26–S33
- Fiorentino TV, Sesti F, Andreozzi F, et al. One-hour post-load hyperglycemia combined with HbA1c identifies pre-diabetic individuals with a higher cardio-metabolic risk burden. *Atherosclerosis* 2016;253:61–69
- Cornu M, Modi H, Kawamori D, Kulkarni RN, Joffraud M, Thorens B. Glucagon-like peptide-1 increases beta-cell glucose competence and proliferation by translational induction of insulin-like growth factor-1 receptor expression. *J Biol Chem* 2010;285:10538–10545
- Modi H, Jacovetti C, Tarussio D, et al. Auto-crine action of IGF2 regulates adult β -cell mass and function. *Diabetes* 2015;64:4148–4157