SUPPLEMENTARY DATA

- *Oral glucose tolerance test* (*OGTT) with frequent sampling*

Subjects underwent OGTT with frequent sampling before and after achievement of the weight loss goal, after at least 48 hours since the last administration of liraglutide for those in the liraglutide arm. Patients were instructed to consume a weight maintaining diet containing 200-250 g of carbohydrate per day for at least 3 days before the OGTT. Patients were admitted to the Clinical Research Center (CRC) at 8 am after 10-12 hours overnight fast. For the post-weight loss OGTT, liraglutide was withheld two evenings before the OGTT such that the last dose was administered 48 h earlier. A catheter was inserted into an antecubital vein and another catheter was inserted retrogradely into a wrist vein for blood sampling. Each study lasted 130 minutes (-10' to 120'). At time 0', subjects ingested a 75-g glucose solution over 5'. Blood samples was collected at -10', 0', 15', 30', 45', 60', 90', 120' to measure plasma glucose and serum C-peptide and insulin (baseline samples and +30') concentrations.

Insulin sensitivity was obtained using the Matsuda index, a simple index which represents a composite of both hepatic and peripheral tissue sensitivity to insulin, and has been demonstrated to be highly correlated with the rate of whole-body glucose disposal during the euglycemic insulin clam (1). The following formula was employed: 10,000/square root (fasting plasma glucose* fasting plasma insulin) *(Mean OGTT glucose concentration *mean OGTT insulin concentration).

Beta cell secretion during 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 (2). C-peptide kinetics was assumed to be known in each subject according to a two-compartmental model previously proposed (3). Individual parameters were calculated from population data (4), according to sex, age, body surface area and presence/absence of obesity and type 2 diabetes.

SUPPLEMENTARY DATA

The model of C-peptide kinetics and secretion has been resolved using the SAAM II 2.3 software (TEG, Charlottesville, VA, USA), leading to unique identification of OGTT beta index (log units), a compound parameter of beta cell function during OGTT.

The OGTT beta index is derived from a very parsimonious description of beta cell response to oral glucose, developed for tests in which sampling number and frequency is limited (2). Glucose-stimulated insulin secretion (GSIS) during the OGTT is superimposed on basal insulin secretion rate (B-ISR; derived from basal C-peptide concentration and from the parameters of C-peptide kinetics calculated from population data as in (4)) and is described as follows:

$$GSIS(t) = \tau^{-1} \cdot X(t)$$

$$\frac{dX(t)}{dt} = -\tau^{-1} \cdot X(t) + \sigma \cdot [G(t) - \theta]$$

$$X[0] = 0$$

where GSIS(t) (units: pmol·min^{-1}) is C-peptide (insulin) secretion rate in response to glucose, τ (units: min) is the time constant with which C-peptide (insulin) made available for secretion is released into the bloodstream, X (units: pmol) is the amount of C-peptide (insulin) made available for secretion, σ (units: pmol·L·min^{-1}·mmol^{-1}) is the slope of the line relating glucose stimulus to the amount of insulin made available for secretion, G (units: mmol·L^{-1}) is the prevailing plasma glucose concentration, and θ (units: mmol·L^{-1}) is the apparent glucose concentration threshold above which insulin secretion is stimulated by plasma glucose.

$$\text{OGTT beta index} = \log\left[\sigma \cdot (11 - \theta) \cdot BSA^{-1} / (e + \tau)\right]$$

where 11 is the extreme nondiabetic glucose (mmol·L^{-1}) value at 120' during a standard OGTT, BSA (units: m^2) is the body surface area and e is the basis of natural logarithms, which was chosen to mitigate the disproportionate impact that τ values lower than 1 would exert on global beta index.

SUPPLEMENTARY DATA

Area under the insulin curve (AUC_{ins}) and area under the glucose curve (AUC_{gluc}) during the OGTT were calculated using the trapezoidal rule.

- *Magnetic resonance imaging (MRI) Quantification of Visceral and Subcutaneous Fat*

MR images were obtained with a Achieva Philips 1.5 Tesla body scanner, which was available at the Institute for Advanced Biomedical Technologies (ITAB), a neuroscience and imaging research center within the University of Chieti. A spin-echo sequence with a 500-ms repetition time and 20-ms echo time was used for all acquisitions. To plan the data acquisition, a transverse and sagittal image of the abdomen region were taken to identify the intervertebral space between the lumbar fourth (L4) and fifth (L5) vertebrae. Transverse slices (10 mm thick) were then acquired every 50 mm, beginning at the L4-L5 space and continuing toward the feet. The optimal threshold for adipose tissue was 110 (on a scale of 256). Calculation of adipose tissue area and volume was performed as previously described (5).

Analytical measurements

*Biological material collection***.** At admission to the study and after the achievement of the weight loss goal, venous blood samples were collected and frozen at -20°C for subsequent biochemical measurements.

Biochemical measurements

Plasma glucose concentration was measured by the glucose oxidase method and serum insulin and C-peptide levels by immunochemiluminometric assays. Serum high sensitivity-C-reactive protein (hs-CRP) concentrations were measured using highly sensitive immunoassay. The HbA_{1c} level was determined by automated high-performance liquid chromatography (HPLC) (6).

SUPPLEMENTARY DATA

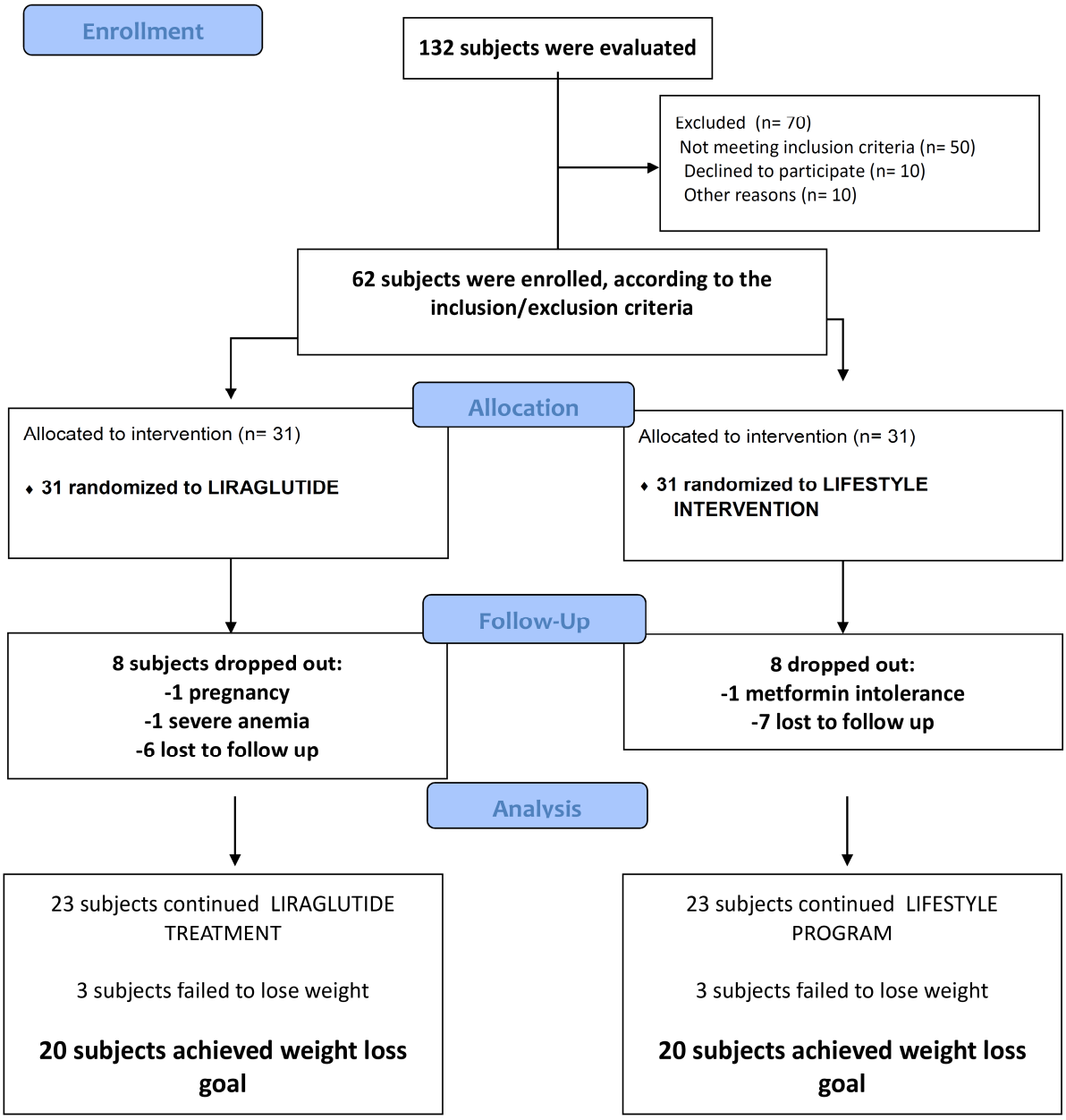
Serum IGF-I was measured with a specific radioimmunoassay kit (Mediagnost, Tubingen, Germany), which uses an excess of IGF-II to eliminate interferences by IGFBPs. The intra-assay CV was 6.7%, the interassay CV was 6.8%, and the sensitivity limit was 0.09 ng/mL. IGF-II was measured using a specific immunoassay kit (Mediagnost) with an interassay CV of <7.2%, an intra-assay CV of <6.6%, and a sensitivity limit of 0.02 ng/mL. IGFBP-3 was determined with a specific immunoassay kit (Mediagnost) with an intra-assay CV of <4.51%, an interassay CV of <6.3%, and a sensitivity limit of 0.1 ng/mL.

References

1. 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
2. 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.
3. Polonsky KS, Licinio-Paixao J, Given BD, et al. Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. J Clin Invest 1986;77:98-105
4. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41:368-377
5. Ross R, Léger L, Morris D, de Guise J, Guardo R. Quantification of adipose tissue by MRI: relationship with anthropometric variables. J Appl Physiol 1992;72:787-795
6. 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

SUPPLEMENTARY DATA

Supplementary Table S1. Flow diagram of the study



SUPPLEMENTARY DATA

Supplementary Table S2. Baseline characteristics of completers as compared to non-completers

Variable	Completers (n=40)	Non-completers (n=22)	p-value*
Age (years)	53.0 (49.0-60.2)	47.5 (42.2-57.5)	0.054
Gender (male), n (%)	21 (52.5)	4 (18.2)	0.014
BMI (kg/m^2)	36.6 (32.7-40.3)	40.8 (34.9-44.2)	0.064
Type 2 diabetes, n (%)	17 (42.5)	6 (27.3)	0.281
IGT/IFG, n (%)	24 (60)	17(77.3)	0.281
Waist (cm)	113.5 (104.0-124.2)	122.0 (107.0-127.2)	0.276
WHR	0.96 (0.90-1.01)	0.91 (0.86-0.97)	0.052
Systolic BP (mmHg)	139.0 (124.7-148.7)	138.0 (125.5-150.2)	0.959
Diastolic BP (mmHg)	80.0 (75.0-85.0)	80.0 (72.2-88.5)	0.796
Smoke, n (%)	4 (10)	3 (13.6)	0.691
Hypertension, n (%)	29 (72.5)	11(50)	0.099
Dyslipidemia, n (%)	19 (47.5)	9 (40.9)	0.790
MS (NCEPT-ATP III), n (%)	18 (45.0)	13 (59.1)	0.426
MS (IDF), n (%)	19 (47.5)	13 (59.1)	0.434
CVD, n (%)	6 (15)	0 (0)	0.081
Previous MI, or revascularization, n (%)	1 (2.5)	0 (0)	1.00
Previous TIA/stroke, or revascularization, n (%)	2 (5)	0 (0)	0.535
PAD, n (%)	1 (2.5)	0 (0)	1.00
Carotid stenosis, n (%)	4 (10)	0 (0)	0.287
Microvascular disease, n (%)	0 (0)	0 (0)	-
Total cholesterol (mmol/L)	4.4 (3.7-4.9)	4.3 (3.7-5.3)	0.691
HDL cholesterol (mmol/L)	1.1 (1.0-1.4)	1.1 (1.0-1.4)	0.768
Triglycerides (mmol/L)	1.3 (0.8-1.6)	1.5 (1.1-1.8)	0.088
Amylase (U/L)	60.0 (53.2-74.0)	57.0 (45.0-71.0)	0.279
Lipase (U/L)	111.0 (66.5-152.7)	85.0 (59.5-109.0)	0.071
Fasting plasma glucose (mmol/L)	5.3 (5.0-5.8)	5.3 (4.7-5.9)	0.735
1-hour-post load plasma glucose (mmol/L)	10.2 (9.0-11.3)	10.1 (7.2-11.2)	0.485
2-hour-post load plasma glucose (mmol/L)	8.5 (7.3-10.4)	8.7 (7.2-11.2)	0.933
HbA1c (%)	6.05 (5.62-6.50)	6.0 (5.70-6.5)	0.768
HbA1c (mmol/mol)	43 (38-48)	42 (39-48)	0.768
Fasting plasma insulin (uU/ml)	11.60 (8.87-20.92)	13.9 (6.7-24.5)	0.687
1-hour post load plasma insulin (uU/ml)	78.95 (39.35-106.17)	86.4 (49.5-132.6)	0.263

SUPPLEMENTARY DATA

2-hour post load plasma insulin (uU/ml)	76.3 (50.02-112.62)	94.9 (57.4-164.5)	0.160
Creatinine (µmol/L)	68.1(59.2-79.6)	55.7(55.7-72.5)	0.305
Total bilirubin (µmol/L)	10 (7-15)	9 (7-14)	0.400
hs-C-reactive protein (nmol/L)	28.6 (19.0-57.1)	42.8 (20.9-73.3)	0.350
AST (U/L)	32.0 (25.0-39.0)	26.0 (19.7-34.2)	0.071
ALT (U/L)	43.5 (35.2-61.2)	39.0 (28.7-48.2)	0.109
Metformin	40 (100)	22 (100)	-
ACE-I, n (%)	7 (17.5)	1 (4.5)	0.240
ARBs, n (%)	13 (32.5)	7 (31.8)	1.00
Diuretics, n (%)	12 (30)	6 (27.2)	1.00
Beta-blockers, n (%)	11(27.5)	7 (31.8)	0.775
CCA, n (%)	1 (2.5)	3 (13.6)	0.124
Statins, n (%)	7 (17.5)	2 (9.1)	0.471
Fibrates, n (%)	0 (0)	0 (0)	-
PUFA, n (%)	1 (2.5)	0 (0)	1.00
Proton Pump Inhibitors, n (%)	6 (15)	5 (22.7)	0.499
ASA, n (%)	4 (10)	1 (4.5)	0.647
IGF-I (ng/ml)	88.9 (65.2-114.4)	84.7 (69.7-104.2)	0.638
IGF-II (ng/ml)	631.6 (527.5-745.7)	702.0 (574.1-801.7)	0.143
IGFBP-3 (ng/ml)	2018.0 (1607.0-2351.0)	2113.4 (1696.8-2451.0)	0.466
SAT (cm^2)	402.9 (298.7-489.8)	515.3 (380.8-604.2)	0.015
VAT (cm^2)	287.8 (247.5-337.67)	285.36 (187.92-330.73)	0.449
Beta Index ($pmol \cdot min^{-2} \cdot m^{-2}$ BSA)	3.80 (2.75-5.13)	4.90 (3.78-5.48)	0.064

Abbreviations: BMI = body mass index, BP = blood pressure, IGT= impaired glucose tolerance, IFG= impaired fasting glucose, WHR= waist-to-hip ratio, MS= metabolic syndrome, NCEP-ATP III = National Cholesterol Education Program-Adult Treatment Panel III, IDF= International Diabetes Federation, hs= high sensitivity, ALT = alanine aminotransferase; AST = aspartate aminotransferase, CVD= cardiovascular disease, MI= myocardial infarction, TIA= transient ischemic attack, PAD= peripheral artery disease, ACE-I= ACE-inhibitors, ARBs= angiotensin receptor blockers, CCA= calcium channel antagonists, ASA= acetylsalicylic acid, IGF-I = insulin-like growth factor, IGFBP3= Insulin Like Growth Factor Binding Protein 3, SAT= subcutaneous adipose tissue, VAT= visceral adipose tissue.

By Mann–Whitney or chi-square, as appropriate. Data are expressed as medians and interquartile range unless otherwise indicated.