Study Title MULTICENTRE RANDOMIZED DOUBLE BLIND, CROSSOVER, PLACEBO-CONTROLLED CLINICAL TRIAL TO EVALUATE THE EFFECT OF LIRALUTIDE ON LUNG FUNCTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS (LIRALUNG STUDY).

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1. PROJECT OVERVIEW/SUMMARY

1.1. Background an hypothesis: Type 2 diabetes (T2DM) is related to reduced pulmonary function. As experimental studies with *glucagon-like peptide 1* (GLP-1) have shown an increase in pulmonary surfactant secretion, and the GLP-1 receptor has been found in significant amounts in the lung, it could be hypothesized that the treatment with liraglutide (a GL-1 agonist) will improve this reduced pulmonary function

1.2. Aim and specific objectives: The general aim of this project is to evaluate the effect of short (5-weeks) incretin-based therapy (liraglutide 1.8 mg once daily) compared to placebo on pulmonary function in type 2 diabetic patients. Our hypothesis is that liraglutide may ameliorate lung function independently of weight reduction.

The specific objectives are the following:

a.- To identify changes in the parameters related to respiratory function of 5-week liraglutide 1.8 mg once daily treatment compared to placebo in type 2 diabetic patients. For this purpose pulmonary volumes, expiratory flows, diffusion lung capacity for carbon monoxide, and exercise tolerance will be assessed.

b.- To study the effect on respiratory parameters during sleep of 5-week liraglutide 1.8 mg once daily treatment compared to placebo in type 2 diabetic patients. For this purpose a previously validated non-attended respiratory polygraphy will be performed, and the apnea-hypopnea index (AHI) and the cumulative percentage of time spent with oxygen saturations below 90% (CT90) will be evaluated.

c.- To determine whether the mechanism involved in the expected beneficial effect of liraglutide treatment on lung function could be attributed to their action on surfactant production. For this purpose changes from basal in serum levels of surfactant A protein, the major surfactant-associated protein, will be evaluated.

d.- To establish the effect of 5-week liraglutide 1.8 mg once daily treatment compared to placebo in type 2 diabetic patients on lung inflammation. For this purpose inflammation markers in exhaled breath concentrate (nitric oxide, pH, and concentration of nitrites, nitrates and isoprostane) will be measured.

1.3. Study design: This is a double-blind, randomized, crossover, placebo-controlled study, with two parallel groups (liraglutide once daily sc *vs*. placebo sc/once daily) of 18-weeks.

1.4. Study periods: (1) selection, **(2)** active treatment (7 weeks, 5 of them at 1.8 mg once daily), **(3)** wash-out (4 weeks), and **(4)** alternative treatment (7 weeks).

1.5. Sample size: We have estimated that a number of 60 patients at the end of the study will be sufficient to test our hypothesis. However, taking into account a drop-out ratio of 20%, the final number of patients that will be included in the study will be 76 (see details in point 9)

1.6. Population to be examined: Type 2 diabetic patients, treated with metformin (± sulfonylurea) at a stable dose for at least the previous 3 months, with haemoglobin A1c between 7.0 and 10.0%, BMI ≥ 30 Kg/m^2, and no known lung disease.

1.7. Participating institutions: 5 reference Spanish hospitals.

2. INTRODUCTION

2.1. Why this work is important?

There is growing evidence to suggest an association between type 2 diabetes and impaired pulmonary function. In this regard, several cross-sectional studies have appeared showing decreased indices of forced expiration, lung volume and diffusion capacity as the main lung dysfunctions detected in type 2 diabetic populations (*Barrett-Connor et al. Diabetes Care 1996; Davis et al. Diabetes Res Clin Pract 2000; Yeh et al. Diabetes Care 2008*). In fact, diabetes is frequently co-morbid with chronic obstructive pulmonary disease (*Mannino et al. Eur Respir J 2008*), and data from the Atherosclerosis Risk in Communities Study showed a faster pulmonary function decline in type 2 diabetic patients than in other participants (*Yeh et al. Diabetes Care 2008*). This is important because the reduction of FEV1 has been demonstrated an independent cause of mortality in diabetic patients (*Davis et al. Diabetes Care 2004*).

Interestingly, lung function measures start to decrease several years before the diagnosis of diabetes (*Davis et al. Diabetes Care 2004*). In this regard we have found that insulin resistance is an independent determinant of pulmonary function in non-diabetic morbidly obese women (*Lecube et al. Diabetes Metab Res Rev 2010*). In addition, our results suggest that the metabolic pathways related to insulin resistance are crucial in initiating lung abnormalities in type 2 diabetic patients.

The reasons for the association between respiratory disease and diabetes are unclear. However, the relationship between type 2 diabetes and muscle strength, the impairment in lung elastic properties, and the presence of a low-grade chronic inflammation state are involved. In supporting these findings, thickening of the alveolar epithelia and pulmonary capillary basal lamina, fibrosis, centrilobular emphysema, and pulmonary microangiopathy have been detected in autopsies of diabetic patients (*Nicolaie et al. Rom J Intern Med 2003*). In addition, defects in the bronchiolar surfactant layer, which is involved in maintaining airway stability and diameter, may also be considered a contributing factor to the impairment of airway calibre regulation in diabetic patients. When the alveolocapillary barrier is damaged, surfactant proteins leak into the bloodstream. A recent population-based random sample study has described how increased circulating levels of surfactant protein A, the major surfactant-associated protein, were associated with altered glucose tolerance and insulin resistance (*Fernández-Real et al. Diabetes Care 2008*). Therefore, surfactant defects in diabetic individuals may also lead to an increase in airway resistance and to a reduction in ventilatory patterns as observed in our studies. In addition, as experimental studies have shown that glucagon-like peptide 1 plays a role in the stimulation of surfactant production (*Benito et al. Endocrinology 1998; Vara et al. Am J Respir Crit Care Med 2001; Ahrén et al. Horm Metab Res 2004*), its underlying deficit in type 2 diabetes could also enhance the airway resistance observed in these patients. However, the beneficial effects on pulmonary function using incretin-based therapies remain to be elucidated.

2.2. What work has already been done?

Our group has recently shown that type 2 diabetes is an independent risk factor for severe nocturnal hypoxaemia in obese patients (*Lecube et al. PloS One 2009*). In addition, we have provided, for the first time, evidence that both insulin resistance and type 2 diabetes are risk factors for respiratory function impairment in morbidly obese women (*Lecube et al. Diabetologia 2010; Lecube et al. Diabetes Metab Res Rev 2010*). The two main abnormalities detected were an obstructive ventilatory pattern and an increase in residual volume. As a 10% decrease in forced expiratory volume in 1 s (FEV1) has been associated with a 12% increase in all-cause mortality in diabetic paients (*Davis et al. Diabetes Care 2004*), our results have serious implications for patients suffering from obesity and diabetes. Notably, we have also found a relationship between the degree of blood glucose control (fasting glucose and HbA_{1c}) and the impairment of pulmonary function tests. In addition, fasting glucose and HbA_{1c} contributed independently to lung volumes in multiple linear regression analysis. This finding strongly suggests that metabolic pathways related to hyperglycaemia are the main factor accounting for this impairment and points to the lung as a new target of long-term diabetic complications. Finally, we have recently provided first evidence that circulating levels of soluble TNF-α receptors type 1 (sTNF-R1) are related to reduced lung volumes and airflow limitation in morbidly obese patients, being the main abnormalities a significant negative correlation between sTNF-R1 and both FEV1 and forced vital capacity (FVC), as well as with the

maximum midexpiratory flow (*Lecube et al. Cytokine 2011*). This finding strongly suggests that inflammatory pathways related to obesity are involved in this impairment and provide a mechanistic support to previous studies showing an inverse relationship between FEV1 and anthropometric parameters.

3. STUDY HYPOTHESIS

Our hypothesis is that treatment with an incretin mimetic such as liraglutide may ameliorate lung function parameters in type 2 diabetics patients, independently of weight reduction. This hypothesis is based on the following factors:

a.- There is growing evidence to suggest an association between type 2 diabetes and impaired pulmonary function.

b.- In patients with type 2 diabetes, the incretin effect is severely reduced or absent, contributing to the reduced lung function parameters observed in type 2 diabetic patients.

c.- GLP-1 stimulates surfactant production in “in vitro” studies and, in consequence, the increase in surfactant production induced by liraglutide could be the main factor involved in the respiratory improvement.

4. PRIMARY OBJECTIVE AND SECONDARY OBJECTIVES

The main objectives of the project are the following:

4.1. Primary objective: To assess the effect of 5-week liraglutide 1.8 mg once daily treatment compared to placebo in type 2 diabetic patients on measurements of respiratory function.

4.2. Secondary objectives: To evaluate the effect of 5-week liraglutide 1.8 mg once daily treatment compared to placebo in type 2 diabetic patients on:

- the effect on respiratory parameters during sleep,
- changes from basal in serum levels of surfactant A protein, and
- inflammation markers in the exhaled air.

5. RESEARCH DESIGN

5.1 Setting

The clinical trial will be performed in 5 reference Spanish hospitals and will involve close collaboration between recognized endocrinologists and pneumologists.

These are the centres that will participate in this clinical trial and their main investigators, all from the Endocrinology and Nutrition Department:

. Hospital Universitari Arnau de Vilanova, Lleida, Spain (Dr. Albert Lecube, Ph.D. ,M.D.)

. Hospital Universitari Germans Trias i Pujol, Badalona, Spain (Dr. Didac Mauricio, Ph.D., M.D.)

. Hospital Universitari Vall d'Hebron, Barcelona, Spain (Dr. Rafael Simó, Ph.D., M.D.)

. Clínica Universitaria de Pamplona, Pamplona, Spain (Dr. Javier Salvador, Ph.D., M.D.)

. Hospital Universitario Virgen de la Victoria de Málaga, Málaga, Spain (Dr. Francisco Tinahones, Ph.D., M.D.)

.Back up centre: Hospital Universitario Virgen del Rocio de Sevilla, Sevilla, Spain (Dr. Pedro Pablo García Luna, Ph.D., M.D.)

5.2 Ethic considerations

The trial has been registered in the European Clinical Trial Database (EudraCT number: 2010-023518-29). Once accepted, the last version of the protocol will be newly submitted to the Investigational Ethics Committees of all the participating clinical centres. Investigators believe that no major ethical implications have to be considered in this clinical trial. However, patients diagnosed of severe sleep disorder breathing will be evaluated with the Pneumology Department in each center. Patients in whom treatment with continuous positive airway pressure should be considered mandatory will be withdrawn from the clinical trial. In addition, the trial will follow the Helsinki Declaration as well as the Good Clinical Practice Guidelines.

5.3 Study design

5.3.1. Study type: This is a phase IV double-blind, randomized, crossover, placebo-controlled study, with two parallel groups (1.8 mg of liraglutide once daily sc *vs* placebo once daily sc) for 14 weeks.

5.3.2. The consent process: The consent process will begin when a potential research patient is initially contacted. Prior to any study-related activity, including screening procedures, potential candidates to be recruited will receive extensive, both oral and written, information about the study, including the risk

associated with the study procedures and study-drug. In addition to signing the consent, the patient will enter the date of the signature on the consent document, to permit verification that consent was actually obtained before the patient began participation in the study. A copy of the consent document will be provided to the patient and the original signed consent document will be retained in the study records.

5.3.3. Screening phase (population to be examinated): The study will include type 2 diabetic patients, treated with metformin (± sulfonylurea) at a stable dose for at least the previous 3 months, with HbA1c between 7.0 and 10.0%, BMI ≥ 30 Kg/m^2, and no known lung disease. The patients included in the study will be recruited from the Endocrinology Departments of all the participating centers and will be followed-up by the endocrinologists and pneumologists in the investigating teams. The main exclusion criteria include type 1 diabetes mellitus, treatment with insulin, dipeptidyl peptidase-4 inhibitors and/or pioglitazone, history of smoking habit, chronic respiratory disease, asthma, cardiovascular disease, hearth failure, stroke, and/or chest wall disease.

5.3.4. Inclusion phase: After informed consent, the recruited patients will be transferred to Pneumology Departments where the following examinations will be performed: expiratory flows, pulmonary volumes, 6-minute walking test, non-attended respiratory polygraphy with oxygen saturation monitoring, inflammatory markers in exhaled air, and serum surfactant A protein measurement. After these evaluations patients will be randomly allocated in a 1:1 ratio to one of the two treatment groups.

5.3.5. Treatment groups:

Group A: (i) 7-week subcutaneous liraglutide treatment once daily, (ii) a 4-week wash-out period, and (iii) 7-week subcutaneous placebo once daily.

Group B: (i) 7-week subcutaneous placebo once daily, (ii) a 4-week wash-out period, and (iii) 7-week subcutaneous liraglutide treatment once daily.

5.3.6. Titration schedule: in order to minimize the loss of weight associated with incretin therapies and to achieve the optimal dose of liraglutide, active treatment will start at 0.6 mg once daily during the first week, 1.2 mg once daily during the second week, and 5 weeks at 1.8 mg once daily. Whether a dose of liraglutide is not well tolered, it will be reduced to the minimum tolerated dose. Placebo arm will perform the same scalation that active treated patients.

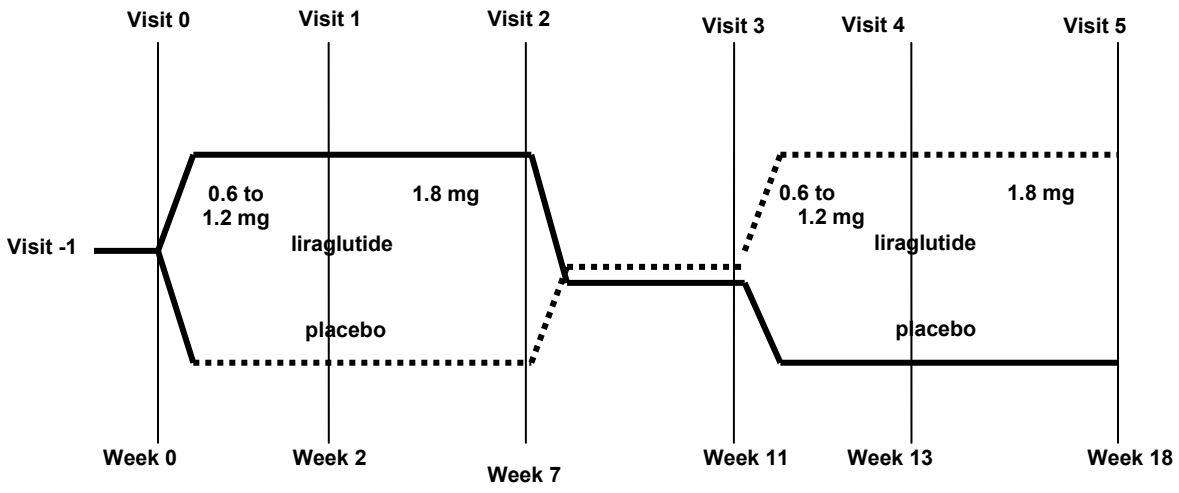
5.3.7. Treatment formulation: both liraglutide and placebo will be administered subcutaneous using a 3 mL pre-filled pen injector. Regarding liraglutide, one ml of solution will contain 6 mg of the product, so one pre-filled pen injector will contain 18 mg of liraglutide in 3 mL.

5.3.8. Sample size: we have estimated that a number of 76 screened patients at the end of the study will be enable to demonstrate our hypothesis (see below *sample size calculation*).

5.3.9. Baseline treatment: all type 2 diabetic patients who gave their written informed consent will continue with metformine (± sulfonylurea) at the same doses.

5.3.10. Chronogram: see next page

5.3.11. Visit schedule: see next page



	Screening	Basal	Week 2	Week 7	Week 11	Week 13	Week 18
Visit number	**-1**	**0**	**1**	**2**	**3**	**4**	**5**
Informed Consent	X	-	-	-	-	-	-
Inclusion/exclusion criteria	X	X	X	X	X	X	X
Randomization	-	X	-	-	-	-	-
Physical examination	X	X	X	X	X	X	X
Liraglutide/placebo dispensed	-	X	X	-	X	X	-
Drug accountability	-	-	X	X	-	X	X
Concomitant Medication	-	X	X	X	X	X	X
Expiratory flows	-	X	-	X	X	-	X
Pulmonary volumes	-	X	-	X	X	-	X
6-minute walking test	-	X	-	X	X	-	X
Respiratory polygraphy	-	X	-	X	X	-	X
Nocturnal Oxygen Saturation	-	X	-	X	X	-	X
Serum Surfactant A Protein	-	X	X	X	X	X	X
Inflamm. markers in exhaled air	-	X	X	X	X	X	X
Laboratory	-	X	X	X	X	X	X
Urine pregnacy test (if applicable)	X	-	-	-	X	-	-
Active collection of adverse events	X	X	X	X	X	X	X

6. INCLUSION/EXCLUSION CRITERIA

6.1. Inclusion criteria:

- Type 2 diabetic patients (≥ 18 years of age) with more than 5 years of evolution of the disease,
- treatment with metformin (± sulfonylurea) at a stable dose for at least the previous 3 months,
- HbA1c ≥ 7.0 and ≤ 10.0%,
- body mass index ≥ 30 Kg/m^2,
- no known lung disease,
- baseline reduction in FEV1 equal or higher than 10% in the percentage predicted value,
- provision of an informed consent form signed and personally dated by the patient.

6.2. Exclusion criteria:

- type 1 diabetes,
- treatment with insulin, dipeptidyl peptidase-4 inhibitors, and/or pioglitazone,
- history of smoking habit,
- chronic pulmonary obstructive disease,
- sleep-breathing disorders that require CPAP (continuous positive airway pressure) therapy,
- asthma,
- previous bariatric surgery,
- cardiovascular disease, heart failure, and/or stroke,

- chest wall diseases,
- serum creatinine > 1.7 mg/dl,
- abnormal *liver function test (ALT/AST levels greater than twice the upper limit of normal),*
- *history of acute or chronic pancreatitis,*
- *history or family history of medullary carcinoma or the thyroid or MEN 2,*
- female of child-bearing potential who is pregnant, breast-feeding or intends to become pregnant
- females of childbearing potential who are not using adequate contraceptive methods (as required by local law or practice).

6.3. Concomitant therapies: the next concomitant therapies will be included in the exclusion criteria because of their effects on pulmonary function or glucose metabolism. Patients enrolled in the study who initiate any of these concomitant treatments will be also excluded from the study:

- insulin
- antidiabetic agents others than metformin or sulfonylureas
- inhaled bronchodilator drugs or teophilin
- CPAP (continuous positive airway pressure) therapy
- oral corticosteroids.

7. PRIMARY AND SECONDARY MEASURES

All these measurements will be performed throught a forced spirometry (DATOSPIR touch, Sibel S.A.) and a non-attended ambulatory respiratory polygraphy ("Sleep&Go", Sibel S.A.) devices. Blood specimens will be recollected, processed and stored in each centre, and evaluations will be evaluated in a central laboratory.

7.1. Primary efficacy measure: Forced expiratory volume in 1 s (FEV1). We have selected this variable because in our previous studies the main abnormality detected in type 2 diabetic patients was a decrease in FEV 1 (*Lecube et al. Diabetologia 2010; Lecube et al. Diabetes Metab Res Rev 2010*). In this regard, it shoud be emphasized that a 10% decrease in FEV1 has been associated with a 12% increase in all cause mortality in diabetic patients (*Davis et al. Diabetes Care 2004*).

7.2. Secondary efficacy measures:

- Other parameters drown from a forced spirometry:

- Forced vital capacity (FVC)
- Maximum mid-expiratory flow (FEF_{25-75})
- FEV1 to FVC ratio

- Parameters from static pulmonary volume measurements:
    - Residual volume (RV)
    - Total lung capacity (TLC)
    - Residual functional capacity (RFC)
- Transfer factor of the lungs for carbon monoxide (TLCO)
- Data from the six-minute walking test
- Parameters from a previously validated non-attended respiratory polygraphy
    - apnea-hypopnea index (AHI)
    - cumulative percentage of time spent with oxygen saturations below 90% (CT90)
- Changes from basal in serum levels of surfactant A protein,
- Data from the study of inflammation markers in the exhaled breath concentrate: nitric oxide, pH, and concentration of nitrites, nitrates and isoprostane

7.3. Other variables to be evaluated:

- Anthropometric data: weight, height, waist circumference, and neck circumference
- Daytime sleepiness through the *Epworth Sleepiness Test*
- Fasting blood glucose, HbA1c, fructosamine, fasting insulin, and insulin resistance measured by HOMA-IR.

8. SAMPLE SIZE CALCULATION

The inclusion of 50 patients will achieve a 80% of statistical power (β=0.2) to detect a significant increase, due to the treatment, in the FEV1 levels of at least 20%, assuming a baseline expected values of FEV1 for untreated patients of 88.4±19.7 [as previously reported by Lecube et al (Diabetologia 2010)] and using and ANOVA test to specifically assess the effect in a cross-over design, setting the threshold for statistical significance at 5% (α=0.05, one sided). For this computation, the within mean square error of the ANOVA test for repeated measurements was calculated as $\sqrt{(2 \times (19.7^2)} = 27.86$. Sample size will be increased in 10 patients according to an expected drop-out rate of 20%. Hence, a total of 60 patients will be included in the cross-over randomized trial. In addition, in order to avoid unexpected changes in the final sample size, we will recruit only patients with a baseline decrease in FEV1 equal or higher than 10% in the percentage predicted value.

However, data regarding factors to be analysed in the present project (for example, levels of serum surfactant A protein and parameters measured in the exhaled air) are lacking and, therefore, to give ourselves a safety margin we have increased the sample size. Hence, we hope that the number of 76 patients will be sufficient to demonstrate our hypothesis.

9. STATISTICAL ANALYSIS

First, a homogeneity analysis will be undergone to compare patients in both groups (A and B) with regards to basal clinical and anthropometric variables. Mean (and standard deviation) and frequency (and percentage) will be used to describe quantitative and qualitative variables respectively, using Mann-Whitney test for categorical variables to assess the differences between groups. Second, the effect of the treatment will be evaluated using an ANOVA test with repeated measures, also adjusting for the potential carry-over effect and those potential confounders detected in the homogeneity analysis, using for this purposed mixed linear models. Third, similar analysis will be performed to assess the effect of the treatment on the secondary outcomes. All analysis will be obtained using R statistical package and threshold for statistical significance will be set at 0.05.

10. DATA MANAGEMENT

Data management is always the responsibility of the primary investigator. Data management will be delegated under an agreement of transfer of responsabilities to an external Contract Research Organisation (CRO). Appropiate measures, including encryption of data files containing person identifiable data, will be used to ensure confidentiality of patients data, when they are transmitted over open networks.

An electronic Case Report Form (eCRF) that accurately represents the protocol of the clinical trial will be designed and developed by the CRO to collect the specific data.

The primary investigators of each center will fulfill the sponsor responsibilities detailed in the Spanish legislation as well as in the Good Clinical Practices of the ICH. To prevent patient confidentiality each patient will be identified with a unique code, this will be the only identifiable information that will be recorded in the eCRF. No patient initials will be recorded.

Monitors designated by the CRO will be responsible for contacting and visiting the investigators for the purpose of inspecting the facilities and, upon request, inspecting the various records of the clinical study (eg, case report forms and other pertinent data) to provide that patient confidentially is respected. The monitor will also be responsible for verifying the eCRF at regular intervals throughout the study to verify adherence to the protocol: completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research.

11. TIMELINES

Overall study duration: 24 months

Treatment duration: 14 weeks

Enrollment period: 18 months

Anticipated date of first patient visit: January 18, 2016 (or as soon as contract will be signed)

Anticipated date that first patient enters treatment: January 25, 2016

Anticipated date that last patient enters trial: September 26, 2016

Anticipated date that last patient enters treatment: October 3, 2016

Anticipated date of the final last patient visit: January 16, 2017

Anticipated date of first manuscript submitted: May 1, 2017

12. TRIAL SUPPLIES

Trial supplies comprise trial products and auxiliary supplies:

12.1 Trial products: Trial products comprise investigational trial products. The following trial products for s.c. injection will be provided by Novo Nordisk:

. liraglutide 6 mg/mL, 3 mL pre-filled pen-injector

. liraglutide placebo 6 mg/mL, 3 mL pre-filled pen-injector.

Patients will be instructed when randonised and ready to start the trial in administration of s.c. injection of trial product in order to ensure patient's willingness and ability to self-inject.

Liraglutide, both active drug and placebo will be visually identical.

Novo Nordisk will supply trial product and DFU to the primary investigator located at Hospital Univesitari Arnau de Vilanova de Lleida (Spain). Therefore, primary investigator will ensure to supply with sufficient trial products to the four other sites.

12.2. Auxiliary supplies: Novo Nordisk will supply directions for use devices, whereas needles for pre-filled pen systems will be provided by the primary sponsor.

12.3 Labelling: Labelling of the investigational medicinal products will be in accordance with local law and trial requirements.

12.4. Storage: The investigator from each centre must ensure the availability of proper storage conditions, and record and evaluate the temperature. The investigator from each centre must inform Primary Investigator immediately if any trial product has been stored outside defined conditions (eg, outside temperature range).

Trial products stored outside the temperature range for more than fifteen minutes are not to be used and must be stored separately within allowed temperature range until after evaluation of condition. Evaluation will be performed by Novo Nordisk. Trial products that have been stored improperly must not be dispensed to any patient before it has been re-evaluated and approved for further use.

Returned trial products (unused, partly used or used including empty packaging material) must be stored separately fron non-allocated trial products.

The temperature during storage should be monitored by a calibrated, stationary and continuously recording system. A temperature log must be kept to document storage within the right temperature interval and storage facilities should be checked frequently.

Storage conditions: Not in use, liraglutide both active drug and placebo must be store in a refrigerator between 2ºC and 8ªC, and must not be exposed to excessive heat or direct sunlight

In-use conditions: Liraglutide both active drug and placebo must be stored below 30ºC or in a refrigerator (2ºC-8ºC), protected from light, not freezed, and used within 1 month.

12.5. Drug accountability and destruction: The trial products will be dispensed to each patient as required according to treatment group. A total DUN list provided by Novo Nordisk to the primary investigator will be used to link randomisation codes to treatments. A blinded randomisation list will be created by our own statisticians. Different parts of the code list will be allocated to the other four participant trial sites, and when a patient will be randomised, a randomisation code will be assigned from this list. The correct DUN must be dispensed to the patient.

The investigator from each centre is responsible for ensuring that:

. trial product is not dispensed to any person not included in the trial,
. drug accountability,
. patients are instructed to return all used, partly used and unused trial product including empty packaging material at each dispensing visit and at End of Treatment visit,
. all returned trial products is kept and storage separately from non-allocated trial products.

Destruction of trial products will be done according to local law after accountability is finalised at site and reconciled by monitor. Destruction of trial products must be documented.

13. RANDOMISATION PROCEDURE AND BREAKING OF BLINDED CODES

The trial is a double-blind trial. A randomization session will be carried out for all patients by using the blinded randomization list. At the randomization visit patients meeting all inclusion/exclusion criteria will be randomized to one of two parallel treatments groups:

Group A: (i) 7-week subcutaneous liraglutide treatment once daily, (ii) a 4-week wash-out period, and (iii) 7-week subcutaneous placebo once daily.

Group B: (i) 7-week subcutaneous placebo once daily, (ii) a 4-week wash-out period, and (iii) 7-week subcutaneous liraglutide treatment once daily.

13.1 Breaking of blinded codes: If the site needs to break the treatment code, the sponsor should, if possible, be contacted before the code is broken.

The code for a particular patient may be broken in a medical emergency if knowing the actual treatment would influence the treatment of the patient. The reason for code break should be documented in the medical record. Whenever a code is broken the person breaking the code must print the Code Break Confirmation Notification, and sign and date the document.

If the code has been broken, the patient should be discontinued from the trial product but be asked to continue in the trial.

14. ASSESSMENT OF SAFETY

14.1. Definitions

An Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation participants administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An Adverse Reaction (AR) is all untoward and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

A Serious Adverse Event (SAE) is an experience that at any dose results in any of the following: (1) Deah; (2) Life-threatening experience; (3) Inpatient hospitalization or prolongation of existing hospitalization; (4) A persistent or significant disability/incapacity; or (5) A congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgement, they may jeopardise the patient or require medical or surgical intervention to prevent one of the outcomes listed in this definition. Suspicion of transmission of infectious agents must be always be considered an SAE. Note: the term “life-threatening” in the previous definition of SAE refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.

A Serious Adverse Reaction (SAR) is an adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting investigator, believed with reasonable probability to be due to one of the study treatments, based on the information provided.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is:

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or summary of product characteristics for an approved product).

14.2 Causality: The relationship of each adverse event to the trial medication must be determined by a medically qualified individual according to the following definitions:
Related: The adverse event follows a reasonable temporal sequence from trial medication administration. It cannot reasonably be attributed to any other cause.
Not Related: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

14.3. Procedures for recording adverse events: All AEs occurring during the study observed by the investigator or reported by the participant, whether or not attributed to study medication, will be recorded on the CRF. The following information will be recorded: study name, patient identification (e.g., patient number, sex, and age), trial drug, description of the event and diagnosis when possible, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device, action taken, and reporter. Follow-up information should be provided as necessary. AEs considered related to the study medication as judged by a medically qualified investigator or the sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable. The relationship of AEs to the study medication will be assessed by a medically qualified investigator. Any pregnancy occurring during the clinical study and the outcome of the pregnancy, should be recorded and followed up for congenital abnormality or birth defect.

14.4. Reporting procedures for serious adverse events: All SAEs must be reported to the Sponsor or designated organization within one working day of discovery or notification of the event. The Sponsor or designated organization will perform an initial check of the report, request any additional information. All SAE information must be recorded on an SAE forms and faxed to the Sponsor or designated organization. Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form and faxed to the Sponsor or designated organization.

As a minimum, the investigator should copy Novo Nordisk when expediting SARs or SUSARs, and should report all SARs related to Novo Nordisk product to the local Novo Nordisk affiliate safety

department. The submission to Novo Nordisk must be within day 15 from the investigator's first knowledge about a valid case.

14.5. Pregnancies: Female patients must be instructed to notify the investigator immediately if they become pregnant during the trial. The invstigator must report any pregnancy in patients who received Novo Nordisk provided trial product.

The investigator must follow the pregnancy until the pregnancy outcome and the newborn infant(s) until the age of 1 month. The investigator must report all information on pregnancy, including AEs in the patient, the foetus, and newborn infant. All this information must be forwarded to Novo Nordisk.

When an abnormality is reported in the foetus or newborn infant, information is needed from the male partner. Informed consent must be obtained prior to this.

14.6 Indemnity insurance: A written assurance indemnity or clinical trial insurance to protect the institutions from claims of non-negligent harm resulting from the clinical research and to coverage patients for untoward events will be subscribed by the principal investigator. The amount of compensations paid should be appropriate to the nature, severity, and persistence of the injury. This insurance indemnity will be in accordance with Royal decree 223/2004, of 6th February 2004, establishing the requisites regarding clinical trials.

15. PUBLICATION STRATEGY

The diffusion of the results will follow the procedures that we are currently using. Therefore, the results will first be presented at national and international congresses and oral presentation will be prioritized. Afterwards, we will submit the papers to top journals (first quartile journals or with impact factor > 5). If a late break result is obtained this will be sent as a "Rapid Communication" to a relevant journal that allows this type of publication or it will be sent to a prestigious on-line journal (i.e. PLoS One). Simultaneously, a parallel diffusion through informative channels (newspapers, radio or TV interviews) will be conducted

Novo Nordisk will be informed of any intended publication related to the clinical trial results, and a period of 4 weeks will be stablished in order to receive Novo Nordisk comments.

The Novo Nordisk involvement in this clinical trial will always be acknowledged in any diffusion action related with its results.

16. MAIN REFERENCES

Ahrén B. GLP-1 and extra-islet effects. Horm Metab Res 2004; 36: 842-5.

Barrett-Connor E, Frette C. NIDDM, impaired glucose tolerance, and pulmonary function in older adults. The Rancho Bernardo Study. Diabetes Care 1996; 19(12) : 1441-4.

Benito E, Blazquez E, Bosch MA. Glucagon-Like Peptide-1-(7-36)amide increases pulmonary surfactant secretion through a cyclic adenosine 3',5'-monophosphate-dependent protein kinase mechanism in rat type II pneumocytes. Endocrinology 1998; 139: 2363-8.

Davis TM, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: the Fremantle Diabetes Study. Diabetes Res Clin Pract 2000; 50(2): 153-9.

Davis WA, Knuiman M, Kendall P, Grange V, Davis TME. Glycemic exposure is associated with reduced pulmonary function in type 2 diabetes. The Fremantle Diabetes Study. Diabetes Care 2004; 27:752-7.

Fernández-Real JM, Chico B, Shiratori M, Nara Y, Takahashi H, Ricart W. Circulating surfactant protein A (SP-A), a marker of lung injury, is associated with insulin resistance. Diabetes Care 2008; 31: 958-63.

Lecube A, Sampol G, Lloberes P et al (2009) Diabetes is an independent risk factor for severe nocturnal hypoxemia in obese patients. A case-control study. PloS One 4:e4692

Lecube A, Sampol G, Muñoz X, Hernández C, Mesa J, Simó R. Type 2 diabetes impairs pulmonary function in morbidly obese women. A case-control study. Diabetologia 2010; 53: 1210-6.

Lecube A, Sampol G, Muñoz X, Lloberes P, Hernández C, Simó R. Insulin resistance is related to impaired lung function in morbidly obese women. A case-control study. Diabetes Metab Res Rev 2010; 26(8): 639-45.

Mannino DM, Thorn D, Swensen A, Holguin F. Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD. Eur Respir J 2008;32(4):962-9.

Nicolaie T, Zavoianu C, Nuta P. Pulmonary involvement in diabetes mellitus. Rom J Intern Med 2003; 41(4) : 365-74.

Vara E, Arias-Díaz J, García C, Balibrea JL, Blázquez E. Glucagon-like peptide-1(7-36) amide stimulates surfactant secretion in human type 2 pneumocytes. Am J Respir Crit Care Med 2001; 163: 840-6.

Yeh HC, Punjabi NM, Wang NY, Pankow JS, Duncan BB, Cox CE, Selvin E, Brancati FL. Cross-sectional and prospective study of lung function in adults with type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. Diabetes Care 2008; 31(4): 741-6.

Yeh HC, Punjabi NM, Wang NY et al. Cross-sectional and prospective study of lung function in adults with type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. Diabetes Care 2008; 31:741-6.

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