## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Protocol for a randomized controlled trial of the combined effects of the GLP-1 receptor agonist liraglutide and exercise on maintenance of weight loss and health after a very low-calorie diet |
| **AUTHORS** | Jensen, Simon; Lundgren, Julie; Janus, Charlotte; Juhl, Christian; Olsen, Lisa; Rosenkilde, Mads; Holst, Jens; Stallknecht, Bente; Madsbad, Sten; Torekov, Signe |

## VERSION 1 – REVIEW

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| **REVIEWER** | Edoardo Mannucci<br>University of Florence, Italy<br>I have received consultancy fees from Novo Nordisk |
| **REVIEW RETURNED** | 27-May-2019 |

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| **GENERAL COMMENTS** | The manuscript appropriately describes a nicely designed protocol for a clinical trial. I have only three minor points:<br>1) Body composition is indicated as one of the primary outcomes, but the sample size is determined on the basis of a power calculation performed for body weight only. Unless a further power calculation for lean/fat mass ratio is performed, body composition should be classified as a secondary endpoint<br>2) I do not understand the allocation procedure with respect to physical exercise: how can you ensure that the study personnel is unaware of that allocation at the moment of enrollment?<br>3) I would avoid to use the brand name of liraglutide in the "Ethical Considerations" section. |

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| **REVIEWER** | Katarina Kos<br>University of Exeter, UK |
| **REVIEW RETURNED** | 05-Aug-2019 |

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| **GENERAL COMMENTS** | The study protocol is well described and just requires a few minor modifications.<br>Abstract: be more specific of primary endpoint: body composition: lean/fat mass ratio<br>Exclusion criteria: be clearer about the exclusion of diabetes in the wording, why is diabetic gastroparesis an issue if diabetes were excluded?<br>Introduction: the quotation of reference on long term weight loss is unclear: it should specifically say that if when 10% of weight is lost (6, Wing ), as in what weight loss is expected, however as the study is aiming at 5% weight loss it will be more interesting what to expect in regards of weight maintenance. Please provide respective references and discuss rational in why you aim at 5% weight loss. When discussing other effects of GLP-1 differentiate |

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| | human from rodent work, in vitro to in vivo work and mention controversial findings.<br>Please clarify when in relation to assessments the study drug is taken as it is likely to affect some of the assessments (e.g. blood tests, meal tests etc.). |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer(s)' Comments to Author:

Reviewer: 1
The manuscript appropriately describes a nicely designed protocol for a clinical trial.

Answer: Thank you.

I have only three minor points:

1) Body composition is indicated as one of the primary outcomes, but the sample size is determined on the basis of a power calculation performed for body weight only. Unless a further power calculation for lean/fat mass ratio is performed, body composition should be classified as a secondary endpoint

Answer: Thank you for this important comment. We have now classified body composition as a secondary endpoint:
Page 10, line 209: “The secondary endpoints are changes in body composition (lean/fat mass ratio)… from V1 to V3.”

2) I do not understand the allocation procedure with respect to physical exercise: how can you ensure that the study personnel is unaware of that allocation at the moment of enrollment?

Answer: At enrollment in the study (V0/screening) the later study arm allocation is unknown for all, incl. study personal and study participants. Allocation to treatment will be done after completion of the test day after the 8-week very low-calorie diet phase (V1/baseline), and participants randomized to a treatment arm with physical exercise will initiate the ramp-up phase to exercise. The allocation will be performed by an un-blinded study nurse not otherwise associated with the trial according to the subject randomization list provided by Novo Nordisk. After the randomization allocation procedure, the study personnel will not be blinded with regards to physical exercise, whereas study medication is blinded with the use of placebo. In order to clarify, we have added that the allocation procedure is done after the initial very low-calorie diet phase and that exercise intervention is not blinded to study personnel:
Page 7, line 122: “The trial is double-blinded with regards to study medication but not exercise intervention.”
Page 11, line 235: “After the initial eight-week VLCD phase, participants will be randomized after the test day (V1/baseline) to one of the four study groups in a 1:1:1:1 ratio in accordance with a subject randomization list (SRL) provided by Novo Nordisk (NN).”

3) I would avoid to use the brand name of liraglutide in the "Ethical Considerations" section.

Answer: We agree, and have changed “Saxenda” to ”liraglutide 3.0 mg” throughout the ”Ethical Considerations” section, page 20.

Reviewer: 2
The study protocol is well described and just requires a few minor modifications.

Answer: Thank you.

Abstract: be more specific of primary endpoint: body composition: lean/fat mass ratio

Answer: We have now classified body composition as a secondary endpoint. Further, we have specified it as lean/fat mass ratio.
Page 10, line 209: “The secondary endpoints are changes in body composition (lean/fat mass ratio) … from V1 to V3.”

Exclusion criteria: be clearer about the exclusion of diabetes in the wording, why is diabetic gastroparesis an issue if diabetes were excluded?

Answer: We have changed the wording from ”diabetic gastroparesis” to ”gastroparesis” as liraglutide is not recommended to individuals with this condition, as stated in the Summary of Product Characteristics for Saxenda (https://www.ema.europa.eu/en/documents/product-information/saxenda-epar-product-information_en.pdf): ”This medicine is not recommended if you have a severe stomach or gut problem which results in delayed stomach emptying (called gastroparesis)…”
Page 32, table 1: Exclusion criteria: “Gastroparesis”

Introduction: the quotation of reference on long term weight loss is unclear: it should specifically say that if when 10% of weight is lost (6, Wing ), as in what weight loss is expected, however as the study is aiming at 5% weight loss it will be more interesting what to expect in regards of weight maintenance.
Please provide respective references and discuss rational in why you aim at 5% weight loss.

Answer: Thank you for this very relevant comment. We have elaborated on the rational for at least 5 % weight loss before randomization. For recommended weight loss of more than 5 %, we have referred to: 1) the AHA/ACC 2013 Guidelines for the Management of Overweight and Obesity, recommending that the objective with overweight and obese patients is to produce weight loss that is clinically meaningful which is generally considered to be approximately a 5-10 percent loss of initial bodyweight, which is associated with reductions in key cardiometabolic risk factors (e.g., blood pressure, dyslipidemia, risk of diabetes)[1]; and 2) the AACE/ACE comprehensive clinical practice guidelines for medical care of patients with obesity[2], recommending patients with overweight or obesity and one comorbidity (e.g. T2DM, dyslipidemia, hypertension) to lose 5 to15 % or more and patients with overweight or obesity and metabolic syndrome or prediabetes to lose 10 %[2]. Furthermore, we have provided references of expected benefits from a 5 to 10 % weight loss.
In addition, we have added in the Methods section, interventions, that > 5 % was chosen, as this is generally considered a clinical meaningful weight loss due to improvements in CVD risk factors and T2D prevention.
In order to clarify what to expect with regard to weight regain following weight loss, we have changed the phrase about weight maintenance and cited 3 systematic reviews describing expected magnitude of weight regain post weight loss interventions. As indicated by Barte et al.[3], this magnitude of weight regain can be expected across different degrees of initial weight loss and thus we find these references appropriate to illustrate the challenge of weight loss maintenance in the context of the present study design with >5 % initial weight loss.
Page 4, line 52: “Obesity management guidelines recommends weight loss of more than 5 % of initial body weight to improve cardiometabolic risk factors, with greater weight loss producing greater benefits [1,2]. A 5 to 10 % weight loss improves lipid profile (~20% reduction in triglycerides, ~15 %

reduction in LDL-cholesterol, ~8 % increase in HDL-cholesterol levels) [2,4,5], reduces systolic and diastolic blood pressure (~5 and ~4 mmHg, respectively) [1,6], reduces HbA1c [1,2], and improves insulin sensitivity [7–9]. However, weight regain reverse these health benefits [10,11]. Furthermore, intentional weight loss is typically followed by a 30 to 50 % regain of lost weight within the first year [3,12,13]."
Page 8, line 153: "Although some benefits may be evident already at modest weight loss of 2-3 % (e.g. triglycerides and HbA1c)[1], >5 % is chosen in the present study because it is associated with improved cardiovascular disease risk factors [1,2,14] and type 2 diabetes prevention [15] and thus generally considered a clinically meaningful weight loss [1,16,17]."

When discussing other effects of GLP-1 differentiate human from rodent work, in vitro to in vivo work and mention controversial findings.

Answer: We agree that a distinction should be made between findings in vivo versus in vitro and humans versus rodents. We have added the following in the introduction:
Page 5, line 91: "GLP-1 has also emerged as an immunomodulatory agent [18,19]. In mice, GLP-1 RA administration reduces macrophage accumulation and inflammatory markers in the arterial wall [20], adipose tissue [21], and heart [22]. Similarly, GLP-1 RAs have shown antiinflammatory effects in human coronary artery endothelial cells and aortic endothelial cells [23]. In humans with T2D, short term GLP-1 RA treatment exert antiinflammatory actions, reflected in reduced levels of the macrophage activation molecule sCD163 [24] and reduced production of several proinflammatory markers, such as TNF-α, IL1β, and IL-6 in peripheral blood mononuclear cells [24,25]. Another study showed no improvement of obesity-associated adipose tissue dysfunction in T2D patients after GLP-1RA treatment [26]. One year treatment with GLP-1 RAs reduce the inflammation marker, high-sensitivity C-reactive protein, in overweight and obese individuals [27] and T2D patients [28]. Notably, in patients with T2D and high cardiovascular risk, GLP-1 RAs reduced the rate of occurrence of first major cardiovascular event [29,30]."

Please clarify when in relation to assessments the study drug is taken as it is likely to affect some of the assessments (e.g. blood tests, meal tests etc.).

Answer: This is very relevant, and we have described that study medication should preferably be taken in the morning on test days.
Page 13, line 276: "Study medication should preferably be taken on the morning of the tests."

References

1 Jensen MD, Ryan DH, Apovian CM, et al. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults. Circulation 2014;129:S102–38. doi:10.1161/01.cir.0000437739.71477.ee
2 Garvey WT, Mechanick JI, Brett EM, et al. American association of clinical endocrinologists and American college of endocrinology comprehensive clinical practice guidelines for medical care of patients with obesity. Endocr Pract 2016;22:1–203. doi:10.4158/EP161365.GL
3 Barte JCM, Ter Bogt NCW, Bogers RP, et al. Maintenance of weight loss after lifestyle interventions for overweight and obesity, a systematic review. Obes Rev. 2010;11:899–906. doi:10.1111/j.1467-789X.2010.00740.x
4 Akram D, Astrup A, Atinmo T, et al. Obesity: Preventing and managing the global epidemic. World Health Organization. Tech Rep Ser 2000.
5 Nordmann AJ, Nordmann A, Briel M, et al. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: A meta-analysis of randomized controlled trials. Arch Intern Med. 2006;166:285–93. doi:10.1001/archinte.166.3.285

6 Weiss EP, Albert SG, Reeds DN, et al. Effects of matched weight loss from calorie restriction, exercise, or both on cardiovascular disease risk factors: a randomized intervention trial. Am J Clin Nutr 2016;104:576–86. doi:10.3945/ajcn.116.131391
7 Weiss EP, Reeds DN, Ezekiel UR, et al. Circulating cytokines as determinants of weight loss-induced improvements in insulin sensitivity. Endocrine 2017;55:153–64. doi:10.1007/s12020-016-1093-4
8 Magkos F, Fraterrigo G, Yoshino J, et al. Effects of Moderate and Subsequent Progressive Weight Loss on Metabolic Function and Adipose Tissue Biology in Humans with Obesity. Cell Metab 2016;23:591–601. doi:10.1016/j.cmet.2016.02.005
9 Fayh APT, Lopes AL, Fernandes PR, et al. Impact of weight loss with or without exercise on abdominal fat and insulin resistance in obese individuals: A randomised clinical trial. Br J Nutr 2013;110:486–92. doi:10.1017/S0007114512005442
10 Kroeger CM, Hoddy KK, Varady KA. Impact of weight regain on metabolic disease risk: A review of human trials. J Obes. 2014;2014:614519. doi:10.1155/2014/614519
11 Thomas TR, Warner SO, Dellsperger KC, et al. Exercise and the metabolic syndrome with weight regain. J Appl Physiol 2010;109:3–10. doi:10.1152/japplphysiol.01361.2009
12 Anderson JW, Konz EC, Frederich RC, et al. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr 2001;74:579–84. doi:10.1093/ajcn/74.5.579
13 Curioni CC, Lourenc -O PM. Long-term weight loss after diet and exercise: a systematic review. Int J Obes 2005;29:1168–74. doi:10.1038/sj.ijo.0803015
14 Wing RR, Lang W, Wadden RA, et al. Benefits of Modest Weight Loss in Improving Cardiovascular Risk Factors in Overweight and Obese Individuals With Type 2 Diabetes. Diabetes Care 2011;34:1481–6. doi:10.2337/dc10-2415.
15 Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403. doi:10.1056/NEJMoa012512
16 Donnelly JE, Blair SN, Jakicic JM, et al. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 2009;41:459–71. doi:10.1249/MSS.0b013e3181949333
17 Williamson DA, Bray GA, Ryan DH. Is 5% weight loss a satisfactory criterion to define clinically significant weight loss? Obesity 2015;23:2319–20. doi:10.1002/oby.21358
18 Torekov SS. Glucagon-like peptide-1 receptor agonists and cardiovascular disease: from LEADER to EXSCEL. Cardiovasc Res 2018;114:e70–1. doi:10.1093/cvr/cvy124
19 Insuela DBR, Carvalho VF. Glucagon and glucagon-like peptide-1 as novel anti-infl ammatory and immunomodulatory compounds. Eur J Pharmacol 2017;812:64–72. doi:10.1016/j.ejphar.2017.07.015
20 Arakawa M, Mita T, Azuma K, et al. Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. Diabetes 2010;59:1030–7. doi:10.2337/db09-1694
21 Lee YS, Park MS, Choung JS, et al. Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. Diabetologia 2012;55:2456–68. doi:10.1007/s00125-012-2592-3
22 Noyan-Ashraf MH, Shikatani EA, Schuiki I, et al. A glucagon-like peptide-1 analog reverses the molecular pathology and cardiac dysfunction of a mouse model of obesity. Circulation 2013;127:74–85. doi:10.1161/CIRCULATIONAHA.112.091215
23 Garczorz W, Gallego-Colon E, Kosowska A, et al. Exenatide exhibits anti-inflammatory properties and modulates endothelial response to tumor necrosis factor α-mediated activation. Cardiovasc Ther 2018;36. doi:10.1111/1755-5922.12317
24 Hogan AE, Gaoatswe G, Lynch L, et al. Glucagon-like peptide 1 analogue therapy directly modulates innate immune-mediated inflammation in individuals with type 2 diabetes mellitus. Diabetologia 2014;:781–4. doi:10.1007/s00125-013-3145-0
25 Chaudhuri A, Ghanim H, Vora M, et al. Exenatide exerts a potent antiinflammatory effect. J Clin Endocrinol Metab 2012;97:198–207. doi:10.1210/jc.2011-1508

26 Pastel E, McCulloch LJ, Ward R, et al. GLP-1 analogue-induced weight loss does not improve obesity-induced AT dysfunction. Clin Sci 2017;131:343–53. doi:10.1042/CS20160803
27 Pi-Sunyer X, Astrup A, Fujioka K, et al. A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. N Engl J Med 2015;373:11–22. doi:10.1056/NEJMoa1411892
28 Bunck MC, Diamant M, Eliasson B, et al. Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition. Diabetes Care 2010;33:1734–7. doi:10.2337/dc09-2361
29 Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2016;375:311–22. doi:10.1056/NEJMoa1603827
30 Marso SP, Bain SC, Consoli A, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. N Engl J Med 2016;375:1834–44. doi:10.1056/NEJMoa1607141

**VERSION 2 – REVIEW**

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| **REVIEWER** | Katarina Kos<br>University of Exeter, United Kingdom |
| **REVIEW RETURNED** | 16-Sep-2019 |

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| **GENERAL COMMENTS** | My comments were satisfactorily addressed and I have no further concerns apart from a few minor typos which will be obvious with further proof reading. |