ITP Proposal: Semaglutide (GLP-1 Agonist)

Rationale: GLP-1 receptor agonists (GLP-1 RAs) are drugs used to treat type 2 diabetes by increasing insulin release and reducing blood sugar levels. GLP-1 RAs work by activating the GLP-1 receptor, which is found on cells in the pancreas that produce insulin [21]. Since the insulin hormone regulates blood glucose levels, this results in an increase in the production and release of insulin, which lowers blood sugar levels. Semaglutide is a modified form of the naturally occurring hormone GLP-1, synthesized by the addition of a palmitoyl group to the N-terminus of GLP-1, which increases its resistance to degradation by the enzyme dipeptidyl peptidase-4 (DPP-4). This modification allows semaglutide to have a longer half-life and increased potency compared to native GLP-1 [1].

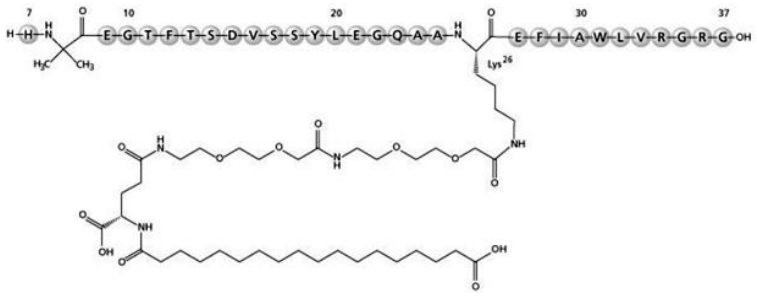

Fig 1. Structural formula of semaglutide, a peptide molecule that is mostly protein bound [32].

Published work shows that semaglutide can benefit health outcomes. In individuals with type 2 diabetes, semaglutide improves glycemic control [1,20,29]. Dysregulation of glucose metabolism is a key contributor to the development of age-related diseases [9] and improving glycemic control may have beneficial effects on lifespan and healthspan by reducing the risk of complications associated with diabetes [4], such as cardiovascular disease [2] and neurodegeneration. In a neurodegenerative mouse model of Parkinson's disease, mice treated with semaglutide performed significantly better than control on cognitive and physical tests, such as the open field test ($p<0.001$) and grip strength test ($p<0.001$) [9]; and in another model of PD, semaglutide was found to protect against 6-OHDA toxicity by enhancing autophagy in SH-SY5Y cells [23]. Semaglutide was found to have a positive effect on brain atrophy and demyelination caused by EAE (experimental autoimmune encephalomyelitis, a common mouse disease model for multiple sclerosis), increasing protein expression of p-CREB, BDNF, and MBP by 3.45, 1.89, and 2.24 times respectively compared to the EAE group ($p < 0.05$) [24]. There was significantly improved locomotor function and lifespan (129 days against 99 days in control) in mice (n=30) with infantile neuroaxonal dystrophy (INAD) that were treated with semaglutide as well [10]. In a mouse model study of ischemic stroke, mice treated with semaglutide had attenuated hippocampal neuron loss ($p<0.001$), improved motor function in tasks such as the grip strength test ($p<0.001$), decreased inflammation and cell death in lieu of decreasing microglia count ($p<0.001$), and increased levels of neurogenesis biomarkers such as DCX ($p<0.001$) compared to untreated MCAO mice [11,13]. Liraglutide, an analog of semaglutide, has been shown to improve memory in mice at an intermediate stage of Alzheimer's disease and in aged APP/PS1 mice [14].

Semaglutide can also cause weight loss and improve cardiometabolic risk factors in obese patients and patients with type II diabetes [2,11,12]. Obesity is a major risk factor for a number of age-related diseases, including cardiovascular disease, type 2 diabetes, and certain cancers [12]. In a

randomized controlled trial (n=957), semaglutide was found to cause significant weight loss compared to a placebo in non diabetic patients with obesity, with the highest dose resulting in a 13.8% reduction in body weight and 37-65% of participants losing at least 10% of their body weight compared to only 10% in control ($p<0.001$) [16]. In mice, semaglutide was found to significantly reduce body weight by 22% at the highest dose of 100 nmol/kg and 10% at the lowest dose 1 nmol/kg [6]. It also caused a 17% to 18% reduction in body weight in mice and a dose-dependent reduction in body weight in rats [6]. Additionally, it suppressed food intake and reduced fat mass with no significant effect on lean mass in mice, and decreased chocolate intake and increased intake of standard chow in rats; in part this is due to semaglutide's ability to upregulate levels of prolactin-releasing hormone (PrLH) in the arcopallium, which was about 22-fold higher than in control [6]. Semaglutide also prevented obesity-related muscle decline in mice fed HFD. Their muscle fibers were organized, and damage was minimal compared to the control HFD group, which showed a reduction in the number of mitochondria in the gastrocnemius muscle, indicating dissolving matrix, loss of cristae and vacuolization. [32].

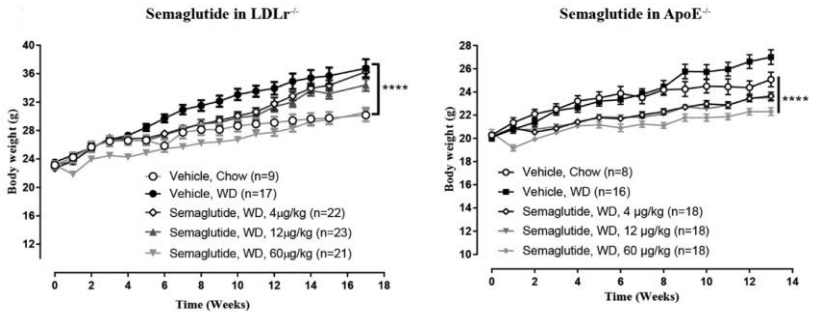

Fig 2. Semaglutide significantly reduced increases in body weight caused by WD in LDLr(-/-) and ApoE(-/-) mice (****$p<0.0001$, vehicle, WD) [2].

Furthermore, semaglutide has been shown to have anti-inflammatory effects. Semaglutide has a significant dose-dependent attenuation to the development of aortic plaque in both LDLr(-/-) and ApoE(-/-) mouse models; it also partially prevented the changes in gene expression induced by a Western diet and reduced inflammation-related genes and collagen gene expression in the liver of LDLr(-/-) mice [2]. In another study in ApoE(-/-) mice, semaglutide administration resulted in a decrease in body weight compared to vehicle treatment, and significantly reduced plasma total cholesterol levels by 34% ($p < 0.0001$) [48]. Moreover, semaglutide significantly decreased the expression of inflammation-related proteins, such as NF-κB, TNF-α, and IL-1β, in the myocardial tissue ($P < 0.001$) of excessively exercised mice [22]**.** In mice fed high fat diets, semaglutide decreases fat pads (-35%) in the eWAT (white adipose tissue) in these mice. These effects were observed through reductions in plasmatic cytokine concentrations like leptin (-30%) and gene expressions related to inflammation and ER stress like Tnf-alpha (-40%) [17]. Semaglutide also significantly reduced PD-induced inflammation by reducing astrocyte count ($p<0.001$) and lipid peroxidation ($p<0.001$) [9] and ischemic stroke induced inflammation in MCAO mice [13]. When applied topically, semaglutide also causes a down-regulation of the protein NF-κB in the retina of diabetic mice, leading to levels similar to those of non-diabetic mice; the upregulation of proinflammatory cytokines, such as Il-1β, Il-6, Il-18, and Icam-1, seen in diabetic mice treated with vehicle is abrogated by treatment with semaglutide ($p < 0.05$) [25]. Inflammation is a key

contributor to the development of age-related diseases [18], and semaglutide's anti-inflammatory properties may be beneficial in reducing disease risk.

Semaglutide, like previously successful ITP drugs, has widely distributed effects in various organs of interest. In the pancreas, semaglutide stimulates the secretion of insulin by activating the GLP-1 receptor on pancreatic beta cells [1]. Upon binding to its receptor, insulin activates intracellular signaling pathways that result in the uptake of glucose into cells, particularly muscle and fat cells. This lowers blood glucose levels and maintains glucose homeostasis. In the brain, semaglutide may act on neurons involved in the regulation of appetite and energy metabolism [5,6]. The GLP-1 receptor is expressed in a number of brain regions, including the hypothalamus, which is involved in the regulation of food intake and energy expenditure. Activation of the GLP-1 receptor in the hypothalamus may lead to the suppression of appetite and the promotion of energy expenditure [26]. In addition, activation of the GLP-1 receptor has been shown to have a number of other effects in the brain, including the promotion of neurogenesis and the improvement of cognitive function in diseased mouse models [13,14]. And finally, in the gut, semaglutide may act on the GLP-1 receptor to slow gastric emptying and increase the secretion of hormones involved in satiety [20]. This may contribute to the weight loss and improved glucose control observed with semaglutide treatment.

Additionally, there are indications semaglutide may have secondary effects similar to other successful ITP drugs, such as rapamycin and acarbose. Semaglutide reduces liver steatosis, enhances insulin signaling, and attenuates the mTOR pathway in the liver by decreasing levels of Pik3c2a, Akt1, and Rps6kb1, and reinforces the AMPK pathway by increasing levels of Prkag2, Sirt1, Nr1h3, and Srebf1 in mice fed a high fat diet [3]. Semaglutide also activates the AMPK pathway, improves autophagy and inhibits the production of ROS in LPS-treated H9C2 cells– the levels of p-AMPK in H9C2 cells were significantly increased in the presence of semaglutide at all tested concentrations (0.3 and 0.9 mmol/L) compared to the LPS alone group ($P < 0.001$) [22]. In addition, orthogonal to semaglutide, a study found that the drug acarbose may increase the production of GLP-1 in the intestine, which can stimulate the production of FGF21 in the liver through the activation of PPARα and Sirt1. This suppression of IGF-I production by acarbose (and likely semaglutide) may contribute to an increase in lifespan in mice [4]. Like acarbose, semaglutide also significantly lowers postprandial glucose–having up to a 29% reduction in AUC in human subjects within 5 hours of eating a breakfast meal [27,28]. In mice, semaglutide also significantly decreases fasting blood and plasma glucose ($p<0.001$) [29]. In a mouse model of Parkinson’s disease, semaglutide reversed PD associated reduction in autophagy in the substantia nigra ($p<0.001$) [9]. Lastly, semaglutide acts as an appetite suppressant through interference with hedonic pathways [6]. Semaglutide reduced food intake in rats by 52% [5] and in lean mice by 40% (34) ($p<0.001$) compared to control and lowered caloric intake by 25% over three meals in obese patients [19]. Semaglutide suppressed eating in mice ($n=6$) fed normal chow during the dark phase but not the light phase, and resulted in a transient decrease in body weight ($p<0.0001$) due to suppressed eating [26]. This suggests that semaglutide could share some of the positive implications that caloric restriction has [7], due to its significant appetite and caloric intake suppression abilities.

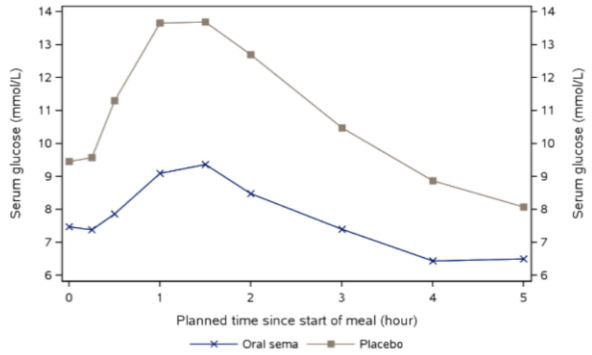

Fig 3. Semaglutide (oral, 14mg) significantly lowered AUC for serum glucose spike in human subjects after consuming a meal [32].

Overall, because semaglutide has been shown to improve glycemic control, have neuroprotective properties, cause weight loss and improve cardiometabolic risk factors, and have anti-inflammatory effects, we propose it as a potential candidate for use in a study of mouse lifespan extension.

Treatment Protocol: With a high binding affinity to HSA relative to other GLP-1 analogues, semaglutide (ED_{50} ≤ 2 nmol/kg) has a mean half life of 168 h in humans [30]. In a db/db mouse dose-response study from 0.3 nmol/kg body weight to 100 nmol/kg body weight per day, a duration of action up to 48 h was observed [31]. Semaglutide is metabolized via proteolytic cleavage of the peptide backbone and sequential β-oxidation of the fatty acid chain, with no single organ acting as the major route of elimination. When measured in plasma, it is estimated to have a retention time of 39.4 to 39.9 min [30]. The peptide is a BCS class 4 molecule, has low solubility at pH range 2 to 6, and is suitable for room temperature storage.

Semaglutide's efficacy is dose dependent–in body weight reduction and corresponding lowering of blood glucose levels [26]. From previous literature, the upper and lower limits of tested doses are 100 nmol/kg body weight per day and 1 nmol/kg body weight per day, for a duration of 3 weeks. Notably, most prior mouse studies on the neuro-, hepato-, nephro- protective and weight loss effects of semaglutide have selected subcutaneous or intravenous injections at 25 or 30 nmol/kg body weight per day [9, 14, 32, 33, 34]. With a molecular weight of 4113.58 g/mol, this corresponds to roughly 0.1 mg/kg body weight per day or 0.6 ppm in food.

However, semaglutide only has an absolute bioavailability of around 1% [35], and its oral bioavailability is often lower (ranging from 0.4% to 1%) given its peptide nature. As such, in FDA-approved forms of semaglutide, the oral dose is 7 to 14 times the subcutaneous dose on a mg basis and given daily instead of weekly. Thus, we propose semaglutide to be administered at the lower dose: 0.7mg/kg body weight per day or 4.2ppm.

Semaglutide tablets are often co-formulated with SNAC (salcaprozate sodium, a permeation enhancer), which facilitates absorption in the stomach across the gastric epithelium via the transcellular route and protects against proteolytic degradation [36]. Although the prescribing information for RYBELSUS® does not suggest to split the pills (possibly to prevent a loss in bioavailability), there is no literature supporting this claim. Additionally, unlike other permeation

enhancers, SNAC does not require a protective enteric coating to maintain efficacy [45]. Thus we propose to purchase and split readily formulated RYBELSUS® tablets.

To assess the physiological effect of semaglutide, we propose body weight and composition to be measured starting at 8 months of age [41]. Composition can be recorded by a NMR imaging instrument, such as the EchoMRI™ 3-in-1 from EchoMRI LLC. Body weight and fat weight should decrease over time, with effects being observable 3 weeks [6]. Optionally, 24 h food intake can be assessed weekly to determine appetite suppression.

Additional physiological data relating to cognitive performance and pathology of specific organs of interest can be used to verify other beneficial properties of semaglutide. For cognitive performance, we propose an open field test using a one-way shuttle box (MED-APAP-D1R; MED Associates, Inc.) with two equal-sized compartments following test conditions outlined by Wang et. al [42]. It was found after 5 training days, the delay for PTZ-treated (chronic epilepsy model) mice to enter the dark cage was shorter than in controls ($P<0.001$) and longer in semaglutide-treated mice than in PTZ mice ($P<0.05$ and $P<0.01$, respectively for low and high dose) [42]. We theorize a difference in time delays will be observed between control and semaglutide-treated mice in more aged mice of at least 12 months of age. For pathology, we propose evaluation of gene expression relative to control of tissue samples from organs of interest such as the liver and kidney [33,34]. Transcriptomics can be performed using RNA sequencing of 15mg fresh tissue samples [46], to elucidate changes in expression of genes relating to metabolism, inflammation, and ECM organization. In prior studies, statistically significant differences in gene expression were observed at 12 weeks and 11 weeks, for the liver and kidney, respectively [39,40].

For an alternative marker, we propose an OGTT (oral glucose tolerance test) to be performed at 4 week [38] or 8 week [39] intervals starting at 8 months of age. Following a 12 hour fast, glucose (2 g/kg body weight) can be administered orally by gavage. Then a 50 μL of tail vein blood is required, to be sampled at 0, 30, 60, 90 and 120 minutes post-glucose load to obtain AUC values [38]. A blood glucose meter can be obtained from LifeScan LLC (OneTouch Ultra®2). In a DIO (dietary induced obesity) model, it was found semaglutide improved glucose tolerance during an OGTT with statistically significant differences in plasma glucose and insulin at 15, 30, 60 and 120 minutes (($P<0.05$ or $P<0.001$, relative to vehicle) [47].

Safety: the FDA has already completed required animal testing on semaglutide several years ago. Most notably, there is a risk of thyroid C-cell tumors in mice and rats; a study was conducted for 2 years on CD-1 mice to examine the effects of RYBELSUS on carcinogenicity. Male mice were given subcutaneous doses of 0.3, 1, and 3 mg/kg/day, which were 9-, 33-, and 113-fold the maximum recommended human dose of RYBELSUS 14 mg. Female mice were given doses of 0.1, 0.3, and 1 mg/kg/day, which were 3-, 9-, and 33-fold the maximum recommended human dose. The results of the study showed that there was a statistically significant increase in thyroid C-cell adenomas and a numerical increase in C-cell carcinomas in both male and female mice at all dose levels, but only at levels that were more than 3 times the human exposure and above. [43]. However, at the relatively lower recommended dose, no acute toxicity was observed in prior studies ranging from 16 weeks on retinal neuroinflammation [25] to 38 weeks on hepatoprotective effects [28]. The highest subcutaneous dose in mice from literature is 100 nmol/kg body weight

per day or 2.4 ppm in food. When applying the same 7 to 14 scaling factor for an oral dose, this would equate to 16.8 to 33.6 ppm, which is much higher than the recommended 4.2 ppm.

Costs: Using bulk rate commercial sources from Canadian Pharmacy World (CIPA certified pharmacy) of readily formulated RYBELSUS® tablets would cost $0.79/mg [44]. Cost estimation assumes a total of 2100 kg of food is consumed for the three sites from age 4 months to death (assumes approximately 3 years), for a total cost of $2323 per year of treatment.

Statement of Understanding

In submitting this proposal, we agree to the following:

- We understand that all information presented in the proposal can be freely shared with members of the ITP Steering Committee and Access Panel during their evaluation of proposals but will otherwise be considered confidential.
- If our proposal, or a modification of it (such as altered dosage or frequency of administration), is accepted for inclusion in a research protocol, we will be asked to help evaluate the data and to prepare the data for written and oral publications, on each of which we will be offered co-authorship. We understand the ITP intends to submit the results of all ITP-supported studies for publication – regardless of whether they produce data showing positive or negative effects on health status in mice.
- We understand that data generated by ITP-supported experiments using the compound/diet proposed will be made publicly available and can be used by anyone in applications for further research support. We also will be free to use ITP-generated data in the context of our applications for research support or for any other purpose.

References

1. Lau, Jesper. “Discovery of the Once-Weekly Glucagon-Like Peptide-1 (GLP-1) Analogue Semaglutide.” ACS Publications, 11 Sept. 2015, https://pubs.acs.org/doi/10.1021/acs.jmedchem.5b00726.
2. Rakipovski G, Rolin B, Nøhr J, et al. The GLP-1 Analogs Liraglutide and Semaglutide Reduce Atherosclerosis in ApoE−/− and LDLr−/− Mice by a Mechanism That Includes Inflammatory Pathways. J Am Coll Cardiol Basic Trans Science. 2018 Dec, 3 (6) 844–857. https://doi.org/10.1016/j.jacbts.2018.09.004
3. Pedro Henrique Reis-Barbosa, Ilitch Aquino Marcondes-de-Castro, Thatiany de Souza Marinho, Marcia Barbosa Aguila, Carlos Alberto Mandarim-de-Lacerda. The mTORC1/AMPK pathway plays a role in the beneficial effects of semaglutide (GLP-1 receptor agonist) on the liver of obese mice. Clinics and Research in Hepatology and Gastroenterology, 2022, https://doi.org/10.1016/j.clinre.2022.101922.
4. McCarty, Mark F., and James J. DiNicolantonio. “Acarbose, Lente Carbohydrate, and Prebiotics Promote Metabolic Health and Longevity by Stimulating Intestinal Production of GLP-1.” Open Heart, vol. 2, no. 1, Jan. 2015, https://doi.org/10.1136/openhrt-2014-000205.
5. Ghidewon, M., et al. “Growth Differentiation Factor 15 (GDF15) and Semaglutide Inhibit Food Intake and Body Weight through Largely Distinct, Additive Mechanisms.” Diabetes, Obesity and Metabolism, vol. 24, no. 6, Mar. 2022, pp. 1010–20, https://doi.org/10.1111/dom.14663.
6. Gabery, Sanaz, et al. “Semaglutide Lowers Body Weight in Rodents via Distributed Neural Pathways.” JCI Insight, vol. 5, no. 6, Mar. 2021, https://doi.org/10.1172/jci.insight.133429.
7. Anderson, Rozalyn M., et al. “Caloric Restriction and Aging: Studies in Mice and Monkeys.” Toxicologic Pathology, vol. 37, no. 1, Jan. 2009, pp. 47–51, https://doi.org/10.1177/0192623308329476.
8. Chia, Chee W., et al. “Age-Related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk.” Circulation Research, vol. 123, no. 7, Sept. 2018, pp. 886–904, https://doi.org/10.1161/circresaha.118.312806.
9. Zhang, Liping, et al. “Semaglutide Is Neuroprotective and Reduces α-Synuclein Levels in the Chronic MPTP Mouse Model of Parkinson’s Disease.” Journal of Parkinson’s Disease, vol. 9, no. 1, Feb. 2019, pp. 157–71, https://doi.org/10.3233/jpd-181503.
10. Poupon-Bejuit, L., Hughes, M.P., Liu, W. et al. A GLP1 receptor agonist diabetes drug ameliorates neurodegeneration in a mouse model of infantile neurometabolic disease. Sci Rep 12, 13825 (2022). https://doi.org/10.1038/s41598-022-17338-1
11. Husain, Mansoor, et al. “Semaglutide (SUSTAIN and PIONEER) Reduces Cardiovascular Events in Type 2 Diabetes across Varying Cardiovascular Risk.” Diabetes, Obesity and Metabolism, vol. 22, no. 3, Feb. 2020, pp. 442–51, https://doi.org/10.1111/dom.13955.
12. O’Neil, Patrick M., et al. “Efficacy and Safety of Semaglutide Compared with Liraglutide and Placebo for Weight Loss in Patients with Obesity: A Randomised, Double-Blind, Placebo and Active Controlled, Dose-Ranging, Phase 2 Trial.” The Lancet, vol. 392, no. 10148, Aug. 2018, pp. 637–49, https://doi.org/10.1016/s0140-6736(18)31773-2.

13. Yang, Xiaoyan, et al. “The Diabetes Drug Semaglutide Reduces Infarct Size, Inflammation, and Apoptosis, and Normalizes Neurogenesis in a Rat Model of Stroke.” Neuropharmacology, vol. 158, 2019, p. 107748., https://doi.org/10.1016/j.neuropharm.2019.107748.
14. McClean, Paula L., and Christian Hölscher. “Liraglutide Can Reverse Memory Impairment, Synaptic Loss and Reduce Plaque Load in Aged App/PS1 Mice, a Model of Alzheimer's Disease.” Neuropharmacology, vol. 76, 2014, pp. 57–67., https://doi.org/10.1016/j.neuropharm.2013.08.005.
15. Zhang, Lingyu, et al. “DA5-CH and Semaglutide Protect against Neurodegeneration and Reduce α-Synuclein Levels in the 6-OHDA Parkinson’s Disease Rat Model.” Parkinson’s Disease, vol. 2022, Nov. 2022, pp. 1–11, https://doi.org/10.1155/2022/1428817.
16. O’Neil, Patrick M., et al. “Efficacy and Safety of Semaglutide Compared with Liraglutide and Placebo for Weight Loss in Patients with Obesity: A Randomised, Double-Blind, Placebo and Active Controlled, Dose-Ranging, Phase 2 Trial.” The Lancet, vol. 392, no. 10148, Aug. 2018, pp. 637–49, https://doi.org/10.1016/s0140-6736(18)31773-2.
17. Martins, Fabiane F., et al. “Semaglutide (GLP-1 Receptor Agonist) Stimulates Browning on Subcutaneous Fat Adipocytes and Mitigates Inflammation and Endoplasmic Reticulum Stress in Visceral Fat Adipocytes of Obese Mice.” Cell Biochemistry and Function, vol. 40, no. 8, Sept. 2022, pp. 903–13, https://doi.org/10.1002/cbf.3751.
18. Claudio Franceschi, Judith Campisi, Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases, The Journals of Gerontology: Series A, Volume 69, Issue Suppl_1, June 2014, Pages S4–S9, https://doi.org/10.1093/gerona/glu057
19. Blundell, John, et al. “Effects of Once-weekly Semaglutide on Appetite, Energy Intake, Control of Eating, Food Preference and Body Weight in Subjects with Obesity.” Diabetes, Obesity and Metabolism, vol. 19, no. 9, May 2017, pp. 1242–51, https://doi.org/10.1111/dom.12932.
20. Davies, Melanie, et al. “Effect of Oral Semaglutide Compared With Placebo and Subcutaneous Semaglutide on Glycemic Control in Patients With Type 2 Diabetes.” JAMA, vol. 318, no. 15, Oct. 2017, p. 1460, https://doi.org/10.1001/jama.2017.14752.
21. Meier, Juris J. “GLP-1 Receptor Agonists for Individualized Treatment of Type 2 Diabetes Mellitus.” Nature Reviews Endocrinology, vol. 8, no. 12, Sept. 2012, pp. 728–42, https://doi.org/10.1038/nrendo.2012.140.
22. Li, Qiang, et al. “Semaglutide Attenuates Excessive Exercise-Induced Myocardial Injury through Inhibiting Oxidative Stress and Inflammation in Rats.” Life Sciences, vol. 250, 2020, p. 117531., https://doi.org/10.1016/j.lfs.2020.117531.
23. Liu, Dong-xing, et al. “SEMAGLUTIDE Protects against 6-OHDA Toxicity by Enhancing Autophagy and Inhibiting Oxidative Stress.” Parkinson's Disease, vol. 2022, 2022, pp. 1–10., https://doi.org/10.1155/2022/6813017.
24. Sadek, Mohamed A, et al. “Semaglutide, a Novel Glucagon-like Peptide-1 Agonist, Amends Experimental Autoimmune Encephalomyelitis-Induced Multiple Sclerosis in Mice: Involvement of the PI3K/AKT/GSK-3B Pathway.” International Immunopharmacology, vol. 115, 2023, p. 109647., https://doi.org/10.1016/j.intimp.2022.109647.

25. Simó, Rafael, et al. “Effects of the Topical Administration of Semaglutide on Retinal Neuroinflammation and Vascular Leakage in Experimental Diabetes.” Biomedicines, vol. 9, no. 8, 2021, p. 926., https://doi.org/10.3390/biomedicines9080926.
26. Brierley, Daniel I., et al. “Central and Peripheral GLP-1 Systems Independently and Additively Suppress Eating.” 2020, https://doi.org/10.1101/2020.08.03.234427.
27. “European Public Assessment Reports: Rybelsus .” European Medicines Agency, 3 Nov. 2020, https://www.ema.europa.eu/en/medicines/what-we-publish-when/european-public-assessment-reports-background-context.
28. DiNicolantonio, James J, et al. “Acarbose: Safe and Effective for Lowering Postprandial Hyperglycaemia and Improving Cardiovascular Outcomes.” Open Heart, vol. 2, no. 1, 2015, https://doi.org/10.1136/openhrt-2015-000327.
29. Pechenov, Sergei, et al. “Development of an Orally Delivered GLP-1 Receptor Agonist through Peptide Engineering and Drug Delivery to Treat Chronic Disease.” Scientific Reports, vol. 11, no. 1, 2021, https://doi.org/10.1038/s41598-021-01750-0.
30. Jensen, Lene, et al. “Absorption, Metabolism and Excretion of the GLP-1 Analogue Semaglutide in Humans and Nonclinical Species.” European Journal of Pharmaceutical Sciences, vol. 104, June 2017, pp. 31–41, https://doi.org/10.1016/j.ejps.2017.03.020.
31. Marty, Vincent N., et al. “Long-Acting Glucagon-Like Peptide-1 Receptor Agonists Suppress Voluntary Alcohol Intake in Male Wistar Rats.” Frontiers in Neuroscience, vol. 14, Dec. 2020, https://doi.org/10.3389/fnins.2020.599646.
32. Ren, Qingjuan, et al. “An Effective Glucagon-Like Peptide-1 Receptor Agonists, Semaglutide, Improves Sarcopenic Obesity in Obese Mice by Modulating Skeletal Muscle Metabolism.” Drug Design, Development and Therapy, vol. Volume 16, Oct. 2022, pp. 3723–35, https://doi.org/10.2147/dddt.s381546.
33. Møllerhøj, Mathias B., et al. “Hepatoprotective Effects of Semaglutide, Lanifibranor and Dietary Intervention in the GAN Diet-induced Obese and Biopsy-confirmed Mouse Model of NASH.” Clinical and Translational Science, vol. 15, no. 5, Feb. 2022, pp. 1167–86, https://doi.org/10.1111/cts.13235.
34. Dalbøge, Louise S., et al. “Nephroprotective Effects of Semaglutide as Mono- and Combination Treatment with Lisinopril in a Mouse Model of Hypertension-Accelerated Diabetic Kidney Disease.” Biomedicines, vol. 10, no. 7, July 2022, p. 1661, https://doi.org/10.3390/biomedicines10071661.
35. FDA. Quality Review Data Sheet. Aug. 2019. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/213051Orig1s000ChemR.pdf
36. Bækdal, Tine A., et al. “Relationship Between Oral Semaglutide Tablet Erosion and Pharmacokinetics: A Pharmacoscintigraphic Study.” Clinical Pharmacology in Drug Development, vol. 10, no. 5, Mar. 2021, pp. 453–62, https://doi.org/10.1002/cpdd.938.
37. FDA. RYBELSUS (Semaglutide) Tablets, Reference ID: 4494169Label. Sept. 2019. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/213051s000lbl.pdf
38. Peterson, Richard G., et al. “Glucose Dysregulation and Response to Common Anti-Diabetic Agents in the FATZO/Pco Mouse.” PLOS ONE, vol. 12, no. 6, June 2017, https://doi.org/10.1371/journal.pone.0179856.
39. Wang, Maorong, et al. “Novel Fatty Chain-Modified GLP-1R G-Protein Biased Agonist Exerts Prolonged Anti-Diabetic Effects through Targeting Receptor Binding Sites.” RSC Advances, vol. 10, no. 14, 2020, pp. 8044–53, https://doi.org/10.1039/c9ra10593j.

40. Jensen, Ditte Marie, et al. “Semaglutide Treatment Attenuates Vessel Remodelling in Apoe-/- Mice Following Vascular Injury and Blood Flow Perturbation.” *SSRN Electronic Journal*, 2022, https://doi.org/10.2139/ssrn.4093532.
41. Harrison, David E., et al. “Acarbose Improves Health and Lifespan in Aging HET3 Mice.” Aging Cell, vol. 18, no. 2, Jan. 2019, p. e12898, https://doi.org/10.1111/acel.12898.
42. Dahiya, Simran, et al. “The Effect of GLP-1 Receptor Agonists in Pre-Clinical Rodent Models of Parkinson’s Disease: A Systematic Review and Meta-Analysis.” Clinical Parkinsonism & Related Disorders, vol. 6, 2022, p. 100133, https://doi.org/10.1016/j.prdoa.2022.100133.
43. Highlights of Prescribing Information Warnings and Precautions These ... https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/213217s000lbl.pdf.
44. “Order Rybelsus 14 Mg Dosage Price.” Canadian Pharmacy World, https://www.canadianpharmacyworld.com/brand/Rybelsus/14+mg. Accessed 11 Jan. 2023.
45. Twarog, Caroline, et al. “Intestinal Permeation Enhancers for Oral Delivery of Macromolecules: A Comparison between Salcaprozate Sodium (Snac) and Sodium Caprate (C10).” *Pharmaceutics*, vol. 11, no. 2, 2019, p. 78., https://doi.org/10.3390/pharmaceutics11020078.
46. Hansen, H.H., Ægidius, H.M., Oró, D. et al. Human translatability of the GAN diet-induced obese mouse model of non-alcoholic steatohepatitis. BMC Gastroenterol 20, 210 (2020). https://doi.org/10.1186/s12876-020-01356-2.
47. “The Dietary-Induced Obese (DIO) Mouse: A Robust Platform for the Development of Anti-Obesity Pharmacotherapies.” Sygnature Discovery, 26 Mar. 2021, https://www.sygnaturediscovery.com/news-and-events/blog/renasci-dio-mouse-model-glp-1-semaglitude/.