UNIVERSITY OF CALIFORNIA SAN DIEGO

The Effect of Glucagon-Like Peptide-1 Receptor Agonist on Mouse Placental Lipid Metabolism

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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The Thesis of Tianyi Zang is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2022

DEDICATION

I dedicate this thesis to my family for their guidance and support during my graduate years.

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ABSTRACT OF THE THESIS

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Glucagon-like peptide-1 (GLP-1) is an incretin that lowers blood glucose level by enhancing glucose-stimulated insulin secretion (GSIS). GLP-1 receptor agonists (GLP-1-RAs) have been approved to treat type 2 diabetes mellitus and obesity. However, little is mentioned about GLP-1-RA's effects during pregnancy. Pregnancy induces maternal metabolic adaptation

due to the increased fetal energy demands. Studying how pregnancy alters metabolism can help understand pregnancy-related disorders. Our lab found that pregnancy increases the pancreatic α-cell-derived GLP-1 level, which leads to enhanced insulin secretion (Qiao et al., 2022). Since insulin is known to directly affect lipid metabolism, the role of GLP-1 in lipid metabolism, especially placental lipid metabolism, is worth investigating. In this thesis, C57BL/6 mice were injected with semaglutide, a long-acting GLP-1-RA, during their late pregnancy. Tissue samples were collected at E18.5 to study the impact of GLP-1R activation on placental lipid metabolism. Using the oil red O staining, our results showed that semaglutide injection significantly increased placental lipid levels. Surprisingly, semaglutide injection did not alter maternal serum insulin and triglycerides concentrations. There was also no significant change in AKT, AMPK, and mTOR pathways in placentas of semaglutide-treated dams. However, there was a trend of increase in lipoprotein lipase (LPL) mRNA in placentas of semaglutide-treated dams. We speculate that increased LPL expression might increase lipid level in placentas since LPL hydrolyzes circulating triglycerides and facilitates fatty acid uptake by trophoblast cells. However, further studies are needed to confirm this mechanism and to fully understand the role of GLP-1 in lipid metabolism during pregnancy.

INTRODUCTION

Obesity is a common metabolic disease which prevails worldwide, and the World Health Organization (WHO) has classified it as a pandemic issue (1). It is highly associated with type 2 diabetes mellitus (T2DM), manifested by elevated blood glucose levels and decreased insulin sensitivity and function (2). 5% to 8% of adults suffer from T2DM, mainly due to are high caloric intake and lack of exercise (3). While obesity and T2DM have been well-studied, developing these disorders during pregnancy is more complicated since they pose long-term adverse health risks for both mother and offspring. Obesity can lead to miscarriage and congenital abnormalities in early pregnancy and increased risk of Cesarean section delivery, which may result in wound infection and rupture (1, 4). In addition, postpartum obese women have a higher risk of venous thromboembolism, depression, and breastfeeding difficulties (4), and their newborns will have increased body fat and a higher risk of childhood obesity (1, 4, 5).

Most importantly, maternal obesity is highly correlated with gestational diabetes mellitus (GDM), which becomes more prevalent due to the obesity epidemic (5-8). GDM is a common metabolic disorder occurring in 6% of pregnancies (6), yet its etiology is very complex, involving both genetic and environmental factors (7). Failure to expand pancreatic β-cell mass and insufficiency in insulin secretion during pregnancy can lead to GDM (8). It also increases the risk of preeclampsia and causes about 50% of women to develop T2DM later in life (4, 5). Treatment of GDM begins with lifestyle changes, including healthy diets and physical activities, to regulate blood glucose level and gestational weight gain (6-8). If not enough, treatment with metformin, glyburide, or insulin should be initiated (6-8). Medications for GDM are similar to those for T2DM, focusing on insulin secretion and sensitization but still having unwanted side effects to patients (2). Insulin injection is a safer treatment for pregnant women since insulin is a large molecule that

does not cross the placenta (8). On the other hand, oral drugs such as metformin and glyburide can be transported via placenta to the fetus and can be detected in umbilical cord blood (8). Currently, long-term effects of anti-diabetic drugs on offspring via in utero exposure is still uncertain (7). This thesis will focus on the effects of glucagon-like peptide-1 receptor agonist, one of the available treatments for obesity and T2DM, during pregnancy.

Glucagon-like peptide-1 (GLP-1) is a 30-amino-acid peptide hormone that functions primarily as an incretin hormone which lowers the blood glucose level by increasing glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells and inhibiting glucagon secretion in pancreatic α-cells (9-12). GLP-1 is cleaved from proglucagon, the product of glucagon gene, via prohormone convertase 1 (PC1) in the intestinal L-cells (13). The secretion of GLP-1 in the gut is triggered by food intake, and GLP-1 is rapidly broken down into GLP-1(9-36)-amide by dipeptidyl peptidase IV (DPPIV) (9, 10). Recent studies proved that, besides the intestinal L-cells, high glucose levels and destruction of β-cells stimulate GLP-1 production in the α-cells (13, 14). α-cell to β-cell communication is essential in regulating insulin secretion and glucose homeostasis (15). GLP-1 increases the β-cell mass by stimulating β-cell proliferation and differentiation, and inhibiting β-cell apoptosis (16). In addition, studies also showed that GLP-1 is secreted in the brain, which functions to regulate neural activities (13, 14). It can enhance the proliferation of neuron cells and improve memory and learning (17). Most importantly, long-term animal studies demonstrated that, at pharmacological levels, GLP-1 reduces food intake and body weight (11, 12).

The receptor of GLP-1 (GLP-1R) is a 463-amino-acid transmembrane protein belonging to the family B G protein-coupling receptors (GPCRs) (10, 17). Besides GLP-1 peptide, GLP-1R is also the receptor for oxyntomodulin and exendin-4 (17). The activation of GLP-1R stimulates G_s protein to increase the level of cyclic adenosine monophosphate (cAMP), which activates

protein kinase A and promotes proinsulin gene transcription (10, 17). Furthermore, GLP-1R activation causes depolarization of the pancreatic β-cell membrane, which facilitates calcium influx via voltage-dependent calcium channels and results in exocytosis of insulin from the β-cells (17). Besides, GLP-1R is also found in many other tissues, and GLP-1R signaling results in a wide variety of effects including increased peripheral glucose disposal, delayed gastric emptying, improved blood lipid metabolism, and reduced fat deposition (18).

Due to GLP-1's incretin effects, GLP-1R agonists (GLP-1-RAs) are approved for the treatment of T2DM. Currently, the available GLP-1-RAs consist of GLP-1 analogs and mimetics that are specially designed to prolong the short half-life of GLP-1. Despite being only 53% identical to GLP-1, exenatide, an exendin-4 analog, is an effective anti-diabetic treatment since it can resist the cleavage of DPPIV (10). Liraglutide, a once-daily treatment for T2DM, is an acylated GLP-1 analog that can bind to serum albumin to reduce degradation (19). Semaglutide, a new GLP-1 analog and a potential once-weekly treatment for T2DM, was designed to have even lower GLP-1 receptor affinity and higher albumin affinity than liraglutide (19, 20). In fact, the public assessment report for semaglutide showed that its terminal half-life reached 8 hours in mice and 148 hours in humans (21). Therefore, this study chose the long-acting semaglutide as the experimental drug. GLP-1-RA has several advantages over other anti-diabetic treatments. First, since GLP-1-RA specifically enhances GSIS, insulin secretion is increased only when the blood glucose level is high. This reduces the risk of hypoglycemia, occurring often at the wrong timing of insulin injections. Moreover, GLP-1-RA can reduce body weight while other anti-diabetic treatments usually lead to increased body weight. This allows GLP-1-RA to target T2DM and obesity simultaneously. Finally, GLP-1-RA, because of its appetite-reducing and gastric-emptying

effects, is an approved and effective weight loss therapy in obese patients, and lower body weight greatly reduces their risk of developing T2DM.

This thesis aims to investigate the impact of GLP-1-RA on mouse placental lipid metabolism. Lipid metabolism is a delicate balance of lipid synthesis and breakdown, in which the liver plays a crucial role. However, obesity may cause the accumulation of hepatic triglycerides (TG) and lead to nonalcoholic fatty liver disease (NAFLD) (22). Lipids, mainly consisting of TG and cholesterol, are digested and absorbed by the intestinal cells, transported in blood by binding to lipoproteins, such as chylomicron and very low-density lipoprotein (VLDL), and hydrolyzed by lipoprotein lipases (LPL) into fatty acids (23). Fatty acids are either stored in the form of nonesterified or free fatty acids, bound by serum albumin, or reesterified into lipids (23). Lipids are accumulated by fatty acid uptake from the blood and de novo lipogenesis and cleared by tissue lipolysis and its secretion into the blood (22, 24). Dyslipidemia occurs when there are high concentrations of TG and low-density lipoprotein (LDL) carried cholesterol yet low concentration of high-density lipoprotein (HDL) carried cholesterol (25).

Lipid metabolism during pregnancy is altered by the maternal metabolic adaptation in response to the increased fetal energy demands. Complications before and during pregnancy, such as obesity and excessive gestational weight gain, increase the risk of childhood obesity and impaired glucose metabolism (26). The distribution of lipids among the mother, the placenta, and the fetus depends on the different gestational stages. The accumulation of lipid and fatty acid in maternal adipose tissue occurs in the first and second trimesters, while the transfer of fatty acid to fetus increases in the third trimester (23, 27, 28). Increased fat deposition in early pregnancy provides sufficient fatty acid supply to the fetus in late pregnancy, which is essential for fetal cell structure and energy metabolism (23). The placenta preferentially transfers fatty acid from mother

to fetus via lipoprotein receptor, lipase, and fatty acid-binding protein, and the contact surface area between maternal blood and syncytiotrophoblasts and the amount of fatty acid transport protein (FATP) are two factors that limit the rate of fatty acid uptake by the trophoblasts (23).

Lipid metabolism is a complex physiological process involving various hormonal regulations. The level of insulin, fluctuating between fasting and eating, determine whether lipids are synthesized or degraded (29). Insulin activates de novo lipogenesis in adipose tissue and liver, increases lipid uptake from blood, yet inhibits lipolysis in tissues (22, 30). In addition, a study showed that intravenous infusion of insulin reduces the blood TG level (31). Studies also verified that T2DM and insulin resistance are associated with dyslipidemia, and diabetic dyslipidemia greatly increases the risk of cardiovascular diseases (25, 32, 33). Insulin resistance causes overproduction of VLDL and chylomicron in liver and intestine and accumulation of free fatty acids in blood (25, 33). Moreover, obesity-induced insulin resistance leads to increased fatty acid secretion from the adipose tissue and increased fatty acid uptake by tissues (22). Pregnancy also modulates lipid metabolism by altering the insulin signaling. Insulin needs increase during pregnancy due to higher maternal caloric intake, gestational weight gain, and presence of placental hormones (8). The increased insulin sensitivity in early pregnancy contributes to the maternal fat deposition in adipose tissue (23, 28). On the other hand, placental hormones impair insulin receptor signaling while stimulating pancreatic β-cell proliferation and GSIS (34, 35). Therefore, despite having high insulin levels in late pregnancy, the mother is still in an insulin-resistant state, which greatly suppresses the effects of insulin on lipid metabolism to ensure adequate delivery of fatty acid to the fetus.

Besides insulin, lipid metabolism is also regulated by many other signaling pathways. Phosphatidylinositol 3’-kinase (PI3K) and Akt, or protein kinase B, activate sterol-regulatory

element-binding protein (SREBP) to increase fatty acid and cholesterol accumulation (36). AMP-activated protein kinase (AMPK) reduces hepatic TG level by inhibiting acetyl CoA carboxylase (ACC), which prevents de novo lipogenesis and promotes fatty acid oxidation (37). Moreover, the activation of the mechanistic target of rapamycin (mTOR) increases TG accumulation through stimulating lipogenesis and inhibiting lipolysis and β-oxidation (29). In addition, the regulation of adipose TG lipase (ATGL) and hormone-sensitive lipase (HSL) can improve obesity-induced insulin resistance (38, 39). Besides, pregnancy-induced changes in hormone levels also regulates lipid metabolism. Early pregnancy has high estrogen, progesterone, and cortisol levels which promote lipogenesis (28), and estrogen also suppresses maternal liver LPL level, resulting in higher hepatic lipid level (23). Late pregnancy has high HSL level, which enhance lipolysis in maternal tissue, and low expression of peroxisome proliferator-activated receptor gamma (PPARγ), which accelerates lipid metabolism (28).

As a peptide hormone, GLP-1 can also regulate lipid metabolism via various pathways. GLP-1 is effective in improving the obesity-induced insulin resistance. A study showed that overweight/obese children and adolescents have higher fasting GLP-1 levels, which increase insulin secretion and expand pancreatic β-cell mass, to compensate for the insulin resistance (40). Moreover, GLP-1 can improve lipid metabolism to potentially treat atherosclerosis and NAFLD (41). GLP-1-RA lowers the risk of major adverse cardiac events (MACE) by impacting insulin resistance, body weight, blood pressure, lipid profile, and heart and vascular endothelium (42). It also prevents hypertriglyceridemia and non-alcoholic steatohepatitis (NASH) by reducing the hepatic TG level, the hepatic and intestinal production of TG-rich lipoprotein, and the intestinal absorption of dietary lipids, while increasing the peripheral utilization of TG (25, 33, 41, 43). Liraglutide, one of the GLP-1-RAs, may potentially improve NASH by reducing insulin resistance

in liver and adipose tissue to inhibit lipolysis and hepatic lipid production (44). In addition, liraglutide can restore autophagy by enhancing the lysosomal functions (45), inhibit fatty acid synthase (FASN) in adipocytes via PKA and MAPK signaling pathways (46), and promote the browning of subcutaneous white adipose tissue (WAT) (47). On the other hand, a recent phase II clinical trial showed that the once-daily treatment of semaglutide, a long-acting GLP-1 analog, has a stronger weight-loss effect than once-daily liraglutide treatment and qualifies as a future anti-obesity drug (20). Compared with liraglutide, semaglutide also has a stronger effect of reducing the blood TG level (48). Overall, most studies showed positive impacts of GLP-1-RAs on lipid metabolism.

Although many studies have proved that GLP-1-RAs are effective as anti-diabetic and anti-obesity treatments, little has been mentioned about how GLP-1 pathway functions in pregnancy. Our lab found that pregnancy increases the pancreatic-α-cell-derived GLP-1 level, which results in more insulin secretion (49). Our results matched the study from Moffett et al. which proved that an increase in intra-islet GLP-1 level activates β-cell expansion during pregnancy (50). However, the effects of activating GLP-1R on mother and fetus remain uncertain (51). This thesis aims to investigate the effects of GLP-1-RA during pregnancy. Since insulin is known to directly impact lipid metabolism, the role of GLP-1 in lipid metabolism, especially placental lipid metabolism, is worth researching. In this study, C57BL/6 mice were injected with semaglutide during their late pregnancy. Tissue samples were collected at E18.5 to study the effects of GLP-1R activation on placental lipid metabolism.

RESULTS

Experimental Setup

In order to study the impact of glucagon-like peptide-1 receptor agonist (GLP-1-RA) on mouse placental lipid metabolism, semaglutide was subcutaneously injected into mice during their late pregnancy. Figure 1 showed a schematic timeline of saline/semaglutide injections and tissue collection during mouse pregnancy. Pregnancy was determined by the presence of a vaginal plug, and the day pregnancy was detected was assigned the embryonic day (E) 0.5. Semaglutide was injected at E13.5, E15.5, and E17.5, and saline was used as controls. Then, blood and placentas were collected at E18.5 from the saline/semaglutide-treated dam and processed as described in the Material and Methods section.

Semaglutide injection increased placental lipid levels

Placentas were collected from the saline/semaglutide-injected mice at E18.5 and were stained with oil red O for visualization of placental lipid accumulation, consisting primarily of triglycerides (Figure 2). Structurally, the placenta is divided into three sections – decidua, junctional zone, and labyrinth. Decidua is the part connecting the placenta to the maternal uterine lining. Labyrinth is the region that mixes the maternal and fetal blood vessels. Junctional zone is the gap between decidua and labyrinth.

Most of the oil red O staining accumulated in the decidua, while small oil red O dots appeared in the labyrinth. From the images, the semaglutide-treated placenta had more oil red O staining in the decidua region than the saline-treated placenta (Figure 2A). Moreover, there were more oil red O dots in the semaglutide group, showing in the magnified placenta labyrinth images (Figure 2B). To verify this result more accurately, oil red O staining of the placenta labyrinth was

quantified using ImageJ software, and the data showed that the semaglutide-injected group had significantly increased oil-red-O-stained area compared to the saline-treated group (Figure 2C). Overall, these results proved that semaglutide injection in mice during late pregnancy significantly increased the placental lipid level.

Semaglutide injection did not alter the maternal serum triglyceride (TG) and insulin concentrations

Since semaglutide injection increased the placental lipid level, serum TG level was also measured to confirm whether the semaglutide's effect was restricted to the placenta. Serum was collected from the saline/semaglutide-injected mice at E18.5. The result from the TG assay kit showed that the serum TG concentrations were not significantly different between the saline and semaglutide groups (Figure 3A). Thus, we speculate that the effect of semaglutide might be limited to the placenta.

On the other hand, since semaglutide increases the insulin level by promoting the glucose-stimulated insulin secretion (GSIS), the serum insulin concentration was measured to confirm its incretin effect. Surprisingly, the result from the mouse insulin ELISA kit showed that serum insulin concentrations were not significantly different between the saline and semaglutide groups (Figure 3B). Therefore, the incretin effect of semaglutide during pregnancy might not be apparent, yet more studies are needed to confirm this. In sum, semaglutide injection in mice during late pregnancy did not change the serum concentrations of TG or insulin.

No significant change was found in AKT, AMPK, and mTOR pathways in placentas of semaglutide-treated dams

To find out the mechanism by which semaglutide injection increased placental lipid levels, its effect on different signaling pathways in placenta was investigated. AKT, AMPK, and mTOR proteins were all involved in the signaling pathways that regulate lipid metabolism, and their expression levels were measured by western blot. Unfortunately, the results showed that semaglutide injection did not change the expression levels of phospho-AKT, phospho-AMPK, and phospho-mTOR in placentas of E18.5 dams (Table 1, Figure 4). Hence, more experiments are needed to discover any possible mechanism of its lipid-elevating effect.

Semaglutide injection resulted in a trend of increase in lipoprotein lipase (LPL) mRNA in placentas of dams

Attempted to continue searching for a potential mechanistic link between semaglutide and elevated placental lipid levels, we performed reverse transcription quantitative PCR for multiple genes involved in the regulation lipid metabolism. Experimental results showed that the LPL mRNA expression level trended upward in placentas of semaglutide-injected dams (Table 2, Figure 5). Since LPL can hydrolyze circulating TG, it is possible that placental LPL level is correlated with the placental lipid level.

FIGURES

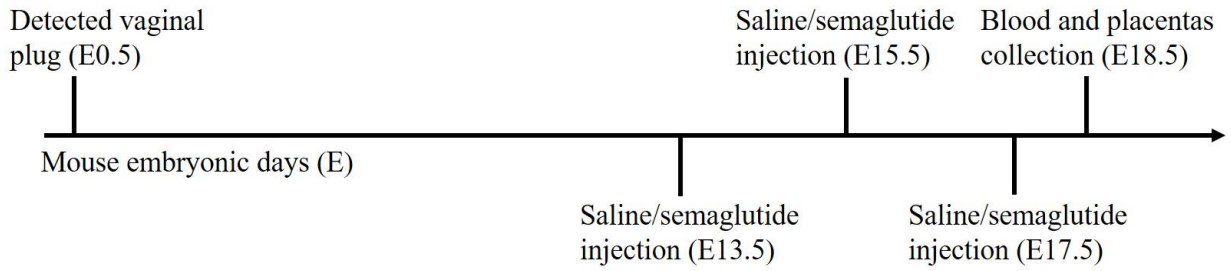
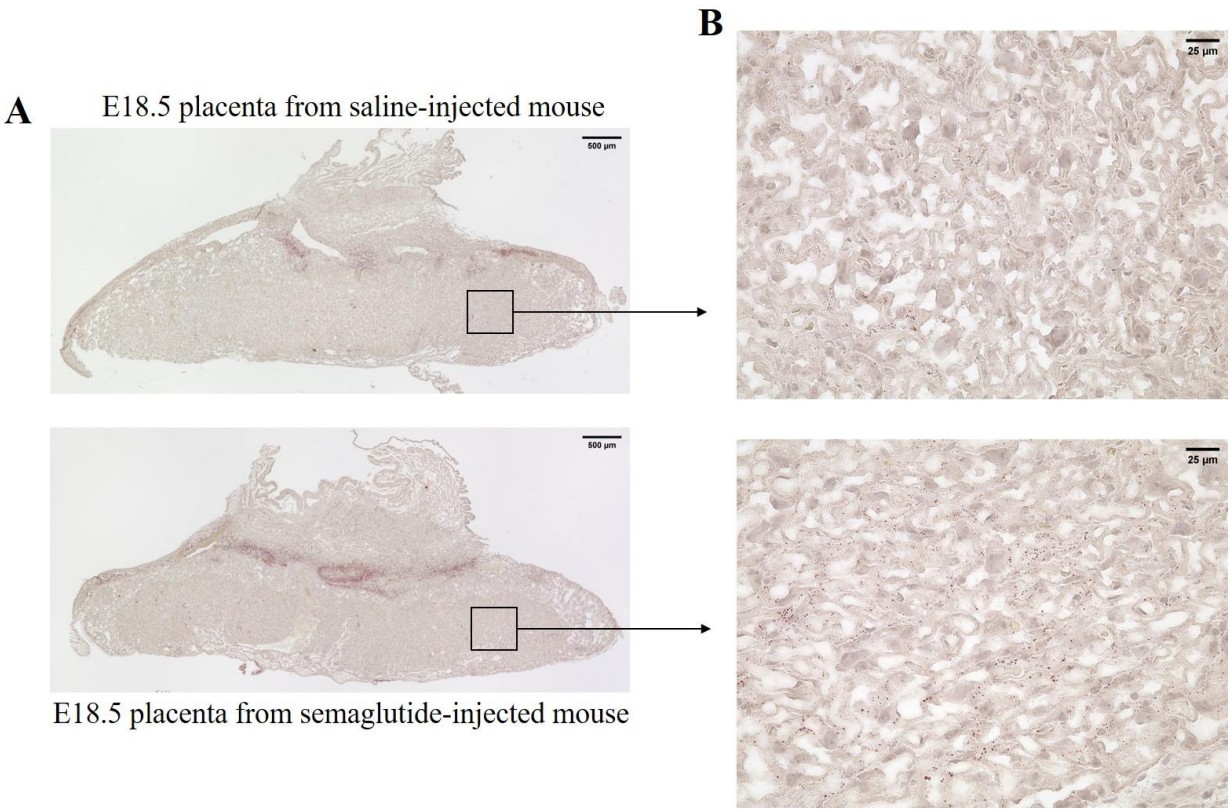


Figure 1. Schematic timeline of saline/semaglutide injections and tissue collection during mouse pregnancy. Pregnancy was determined by the presence of a vaginal plug, and the day pregnancy was detected was assigned the embryonic day (E) 0.5. Saline/semaglutide was injected subcutaneously at E13.5, E15.5, and E17.5, and the blood and placentas were collected at E18.5.



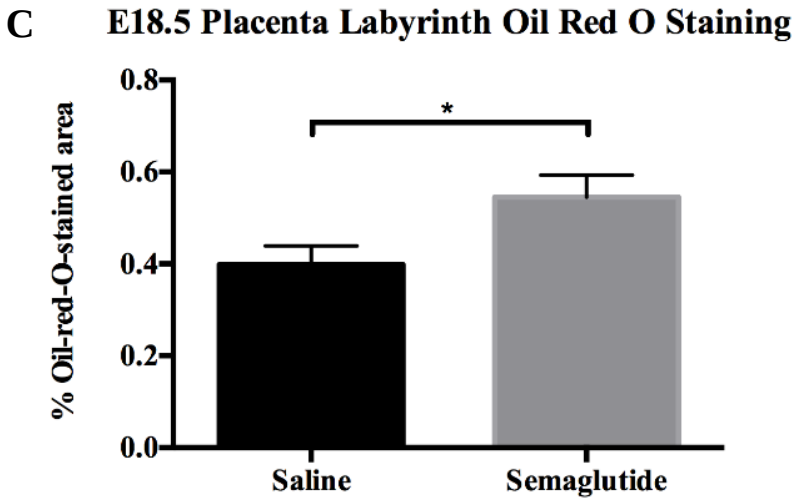


Figure 2. Semaglutide injection increased the placental lipid levels. Placentas were collected from the saline/semaglutide-injected mice at E18.5 and were stained with oil red O for visualization of placental lipid accumulation (A) Representative images of the overall placenta structure, scale bar is 500μm. (B) Representative images of the magnified placenta labyrinth, scale bar is 25μm. Saline group is at the top and semaglutide group is at the bottom. (C) Quantification of placenta labyrinth oil red O staining, detailed calculation is shown in the Method section, saline n=12, semaglutide n=10. (* represents $p<0.05$)

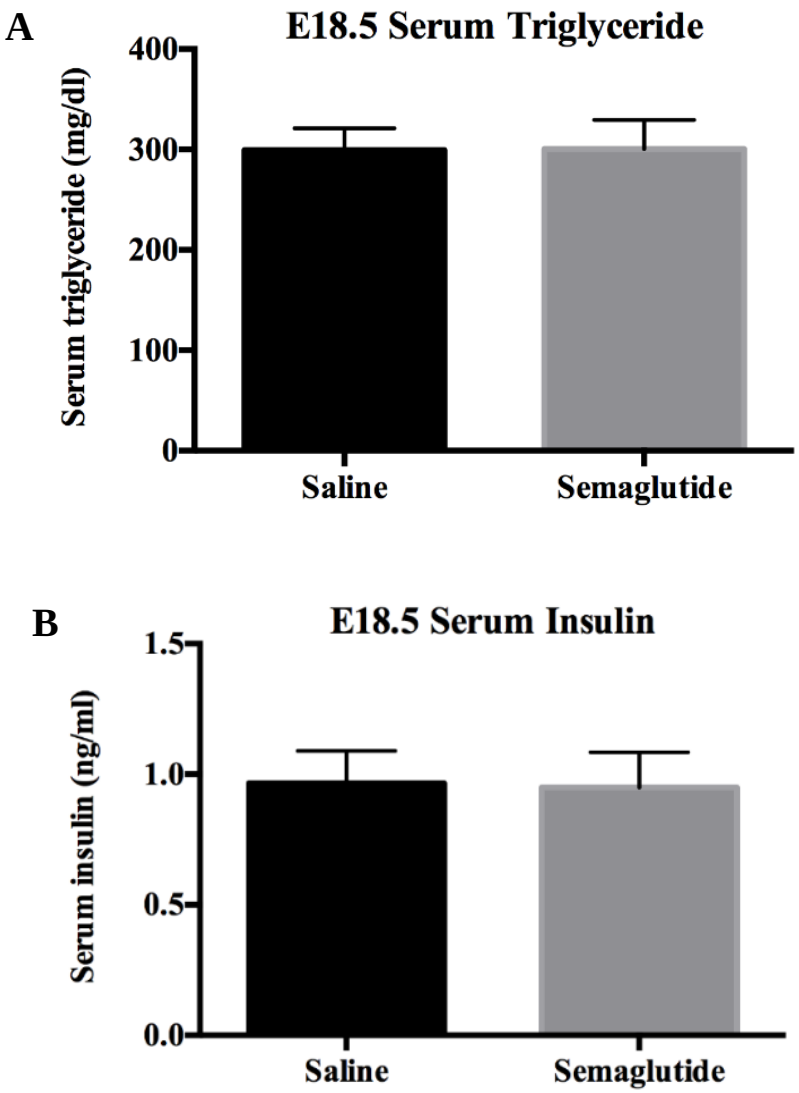


Figure 3. Semaglutide injection did not alter the maternal serum triglyceride (TG) and insulin concentrations. Serum was collected from the saline/semaglutide-injected mice at E18.5 for TG assay and insulin ELISA. (A) Serum TG concentration (mg/dL), saline n=15, semaglutide n=12. (B) Serum insulin concentration (ng/ml), saline n=12, semaglutide n=10.

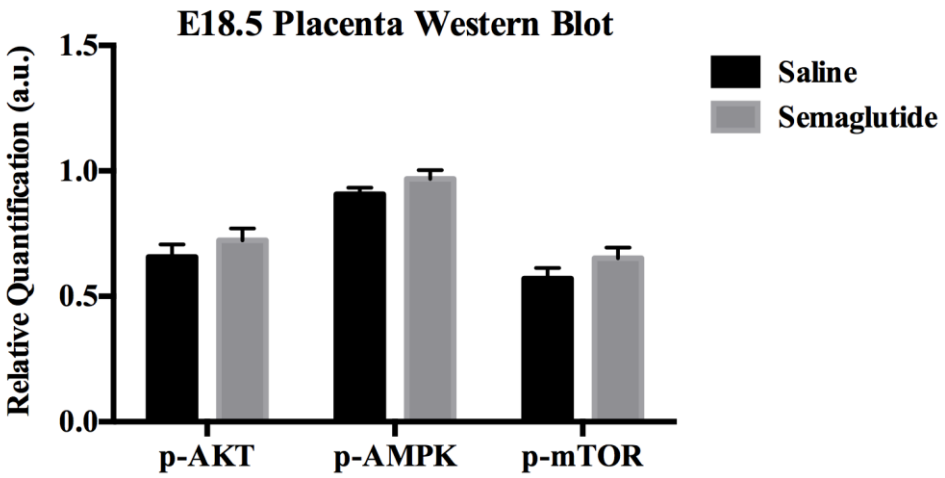


Figure 4. No significant change was found in AKT, AMPK, and mTOR pathways in placentas of semaglutide-treated dams. Placentas were collected from the saline/semaglutide-injected mice at E18.5 for western blot. Phospho-AKT, phospho-AMPK, and phospho-mTOR protein expression levels were normalized to GAPDH, saline n=12, semaglutide n=12. Protein bands were shown in Table 1.

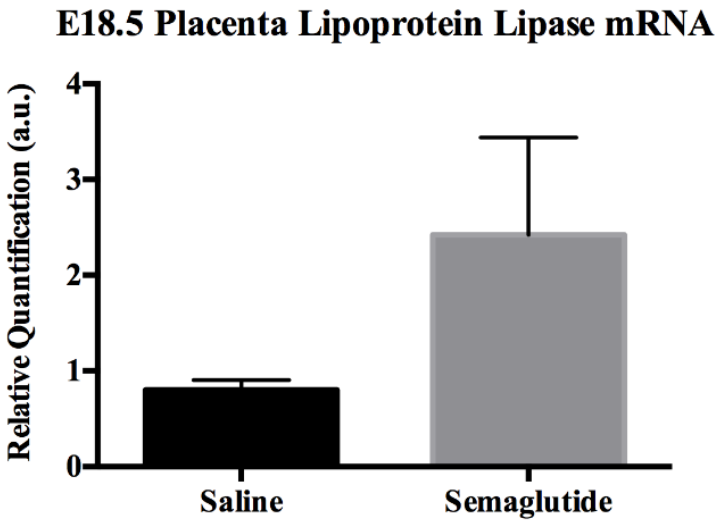


Figure 5. Semaglutide injection resulted in a trend of increase in lipoprotein lipase (LPL) mRNA in placentas of dams. Placentas were collected from the saline/semaglutide-injected mice at E18.5 for reverse transcription quantitative PCR. LPL mRNA expression level is normalized to 18S rRNA, saline n=12, semaglutide n=12.

TABLES

Table 1. Protein bands for E18.5 placenta western blot. Protein bands for phospho-AKT, phospho-AMPK, phospho-mTOR, and GAPDH. Saline n=6 and semaglutide n=6 for each membrane. Quantification of the protein bands are shown in Figure 4.

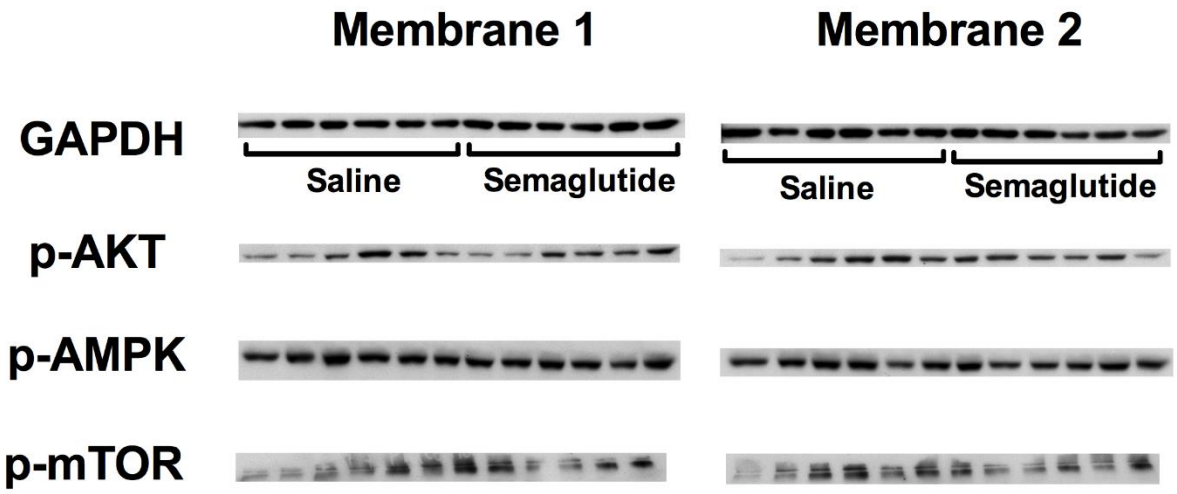


Table 2. Primer sequences for E18.5 placenta real-time quantitative PCR. Primer sequences for lipoprotein lipase (LPL) gene and 18S rRNA gene.

Gene	Forward (5' to 3')	Reverse (5' to 3')
LPL	GAAGTCTGACCAATAAGAAGGTCAA	TGTGTGTAAGACATCTACAAAATCAGC
18S rRNA	CGAAAGCATTTGCCAAGAAT	AGTCGGCATCGTTTATGGTC

DISCUSSION

This thesis discussed the impact of glucagon-like peptide-1 receptor agonist (GLP-1-RA) on mouse placental lipid metabolism. In this study, C57BL/6 mice were injected with semaglutide during late pregnancy, and tissue samples were collected at E18.5. Our main finding was that semaglutide injection increased the placental lipid levels (Figure 2). The lipid-raising effect of GLP-1-RA appears to contradict most current studies, which propose a lipid-lowering effect of GLP-1-RA. However, there was very limited research on GLP-1-RA's impact during pregnancy, and we could not find any research discussing its impact on placental lipid metabolism specifically. Therefore, more experiments are needed to verify and explain this outcome.

We also found that semaglutide injection did not change the serum insulin concentration (Figure 3A). It is known that semaglutide, as an incretin hormone, can increase the insulin level by stimulating glucose-stimulated insulin secretion (GSIS), yet surprisingly, our experiments proved that the insulin level was unchanged. Some may argue that for semaglutide to be effective, the frequency and cycle of injections need to be increased. However, we speculate that it is the unique nature of pregnancy that leads to this result. During late pregnancy, the increased fetal energy demands and the needs for sufficient nutrient transport to the fetus lead to maternal insulin resistance. Semaglutide's incretin effect on pregnant mice might not be significant due to the reduced insulin sensitivity in dams. Not only does the mother no longer need more insulin, but insulin resistance also inhibits the binding of insulin to its receptors, causing unbound insulin to degrade. Hence, the serum insulin concentration might not be altered by any incretins. Meanwhile, the effectiveness of semaglutide injection could be verified by the increased pancreatic β-cell mass in E18.5 pancreas (unpublished data from our lab's graduate student Cindy Lu), which suggested that semaglutide injection promoted proliferation of cells that secrete insulin.

Since semaglutide injection did not change the serum triglycerides (TG) concentration (Figure 3B), semaglutide's lipid-increasing effect and its mechanism might be limited to the placenta. In search of any potential mechanism, we investigated how semaglutide injection affected various lipid metabolism pathways in the placenta. Western blot results showed that there was no significant change in AKT, AMPK, and mTOR pathway in placentas of semaglutide-injected dams (Figure 4), meaning that the action of semaglutide might not involve AKT, AMPK, and mTOR pathways. On the other hand, the result from the reverse transcription quantitative PCR showed that semaglutide injection resulted in a trend of increase in lipoprotein lipase (LPL) mRNA in placentas of dams (Figure 5). This surprising result provides direction for a mechanistic link between GLP-1 and its lipid-elevating effect during pregnancy.

LPL is an enzyme that hydrolyzes the circulating lipoproteins such as TG-rich lipoprotein (TRL), chylomicron, and very low-density lipoprotein (VLDL), and the resulting fatty acids and monoacylglycerol are absorbed by the tissue locally (52). During pregnancy, the circulating maternal fatty acids released by LPL are absorbed by the placenta and bind to the cytosolic fatty acid-binding proteins (FABPs) (53). Then, they are either transported to the fetal blood, oxidized by the trophoblast cells, or re-esterified and stored as lipid droplets in the placenta (53). Pregnancy-induced insulin resistance promotes lipolysis of the maternal tissues and inhibits fatty acid uptake by the maternal tissues, leading to increased lipid accumulation in the maternal blood (54). The increased blood lipid level activates placental LPL (pLPL) (54). Therefore, it is possible that semaglutide injection increased pLPL levels, and pLPL hydrolyzes the circulating lipids and facilitates fatty acid uptake by the trophoblasts.

However, it is still unclear whether the injection directly or indirectly impacted pLPL levels. The study from Egholm et al. showed that GLP-1Rs are expressed in the endothelial cells, and the

activation of G_s protein promotes the proliferation of endothelial cells (55). Since lipoproteins bind to pLPL at the vascular endothelium (56), the activation of endothelial GLP-1R by semaglutide may directly increase the number of endothelial cells and the pLPL levels. On the other hand, since semaglutide injection increased the pancreatic β-cell mass (unpublished result from Cindy Lu), cells that secrete insulin, the mother might respond by further increasing maternal insulin resistance to block insulin's effect and ensure adequate nutrient transport to the fetus. Thus, semaglutide injection indirectly resulted in more insulin resistance that led to higher pLPL levels. However, the detailed mechanisms of how GLP-1 alters placental lipid metabolism via pLPL are still unknown. More future studies are needed to verify this potential mechanism and to fully understand the role of GLP-1 in lipid metabolism during pregnancy.

MATERIAL AND METHODS

Material

ECL Western Blotting Substrate was from Abcam (Cambridge, United Kingdom). Protein Assay was from Bio-Rad Laboratories, Inc. (Hercules, CA). Antibodies against phospho-AKT, phospho-AMPK, and phospho-mTOR were from Cell Signaling Technology, Inc. (Danvers, MA). Mouse Insulin ELISA Kit was from Mercodia (Uppsala, Sweden). Semaglutide was from Novo Nordisk Inc. (Plainsboro Township, NJ). The antibody against GAPDH was from Santa Cruz Biotechnology (Dallas, TX). Oil red O and primers against 18S rRNA gene and lipoprotein lipase gene were from Sigma-Aldrich (St. Louis, MO). NuPAGE gels, Alexa flour-conjugated goat anti-rabbit antibody, TRIzol Reagent, SuperScript III reverse transcriptase, oligo$(dT)_{12\text{-}18}$ primer, and PowerUp SYBR Green Master Mix were from Thermo Fisher Scientific (Waltham, MA). LabAssay Triglyceride was from Wako Chemicals (Richmond, VA).

Mice

C57BL/6 mice from Jackson Laboratory (Bar Harbor, ME) were used to design the mouse model of this study. Ten to twelve-week-old nulliparous female mice were randomly selected for mating. For this thesis, a mouse was determined pregnant by the presence of a vaginal plug and was assigned the embryonic day (E) 0.5.

Experiments using mice were carried out under the Association for Assessment and Accreditation of Laboratory Animal Care guidelines with approval from the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego.

Saline/semaglutide injections

Pregnant mice received either saline or semaglutide injections (6μg/kg) subcutaneously at E13.5, E15.5, and E17.5.

Blood and placenta collection/processing

Blood and placentas were collected in the fed state at E18.5. Serum was obtained from the blood after centrifuging under 5,000rpm for 20min at 4^{o}C at and was then preserved at -20^{o}C. Placentas were either stored at -80^{o}C or fixed with 4% paraformaldehyde (PFA) in PBS overnight at 4^{o}C for tissue embedding. After that, 4% PFA was changed to 10% sucrose overnight at 4^{o}C, then to 30% sucrose overnight at 4^{o}C. Finally, placentas were embedded in OCT compound (Tissue-Tek), snap-frozen with liquid nitrogen, and cryosectioned into 4-μm-thick sections on the slides which were preserved at -20^{o}C.

Insulin ELISA

The insulin concentration of E18.5 serum was measured using the mouse insulin ELISA kit. The enzyme conjugate solution and the wash buffer solution were diluted to 1X. 10μL of Calibrators, controls, and sample serum were pipetted into each well of the microplate. 100μL of 1X enzyme conjugate solution was added into each well, and the microplate was incubated on a shaker at room temperature for 2hrs. After that, solution in the wells was discarded. Each well was then washed 6 times with 1X wash buffer, and wash buffer was discarded after every wash. 200μL of Substrate TMB was added into each well. After incubating at room temperature for 15min, 50μL of Stop Solution was added into each well, and the solution was mixed by briefly shaking the

microplate. Finally, the optical density at 450nm was measured by a microplate reader (BioTek) within 30min, and the results were converted to insulin concentration (ng/mL).

Triglyceride (TG) assay

The TG concentration of E18.5 serum was measured using LabAssay Triglyceride. First, the Chromogen reagent was prepared by dissolving Chromogen Substrate to Buffer Solution, and the standard solution is diluted according to the protocol. In each well of the microplate, 300μL of Chromogen reagent was mixed with 2μL of serum, standard solution, or blank solution, and the microplate was incubated at 37^{o}C for 5min. The absorbance at 600nm was measured using the microplate reader (BioTek), and the results were converted to TG concentration (mg/dL).

Oil red O staining

Oil red O staining can visualize the lipid accumulation in tissue. Oil red O solution was prepared by dissolving oil red O in 60% isopropanol. Slides with the frozen placenta sections were labeled, air-dried, and soaked in water for 3min. The slides were incubated in 60% isopropanol for 1min and stained with oil red O solution for 15min. After that, the slides were incubated in 60% isopropanol for 30sec and soaked in water until clear. Then, they were stained with hematoxylin for 1min, soaked in water until clear, and mounted with aqueous mounting medium.

Images of the stained sections were recorded within 24hrs using the microscope software (BZ-X800E; Keyence, Laguna Hills, CA). For calculations, the oil-red-O-stained area was divided by the tissue area, and the average percentage from images of one placenta was used as one data point. ImageJ software (NIH) was used for the calculations of areas in the images.

Reverse transcription quantitative PCR (RT-qPCR) assay

Messenger RNA (mRNA) was extracted from the placenta tissue using TRIzol Reagent. The placenta tissue was mixed with 1mL of TRIzol Reagent per 50-100mg of tissue and homogenized using a homogenizer (Bertin Technologies) for 5min. Then, 400μL of chloroform and 100μL of 8M potassium acetate were added, followed by 5min of centrifugation at 10,000rpm. The supernatant from the top layer was transferred to new tubes and mixed with 400μL of isopropanol. After centrifuging for 5min, the mRNA pellet was formed, and the liquid around it was discarded. Then, 500μL of 70% ethanol was added, followed by another centrifugation. Finally, the liquid around the pellet was discarded again, and the tubes were air-dried overnight.

Water was mixed with the mRNA pellet depending on the pellet size, and the tubes were incubated at 60^{o}C for 10min. The concentration of each mRNA sample was measured by BioPhotometer (Eppendorf), and the amount of water needed to dilute each mRNA sample was calculated. Random oligo(dT)$_{12-18}$ primers and the diluted mRNA samples were mixed in tubes and incubated at room temperature for 10min. Then, the tubes were heated at 65^{o}C for 5min, spined down, and incubated at room temperature for 10min. 4μL of 5X First-Strand Buffer, 1μL of 0.1M DTT, 1μL of dNTP, and 1μL of SuperScript III Reverse Transcriptase were added to the tubes. After spinning down, the tubes were put into the PCR machine (Bio-Rad) with the protocol of 5min at 65^{o}C, 1hr at 42^{o}C, and 5min at 90^{o}C. The resulting cDNA was diluted with 20μL of water. After that, 5 μL of PowerUp SYBR Green Master Mix, 0.5μL of specific forward and reverse primers (Table 1), 1μL of cDNA samples, and water were mixed and pipetted into wells of a microplate, which was covered and put into the QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). All mRNA expression levels were normalized to 18S rRNA.

Western blot assay

Total protein was extracted from the placenta tissue by homogenizing the tissue with lysis buffer in a homogenizer (Bertin Technologies), and the supernatant was transferred to new tubes after centrifuging at 5,000rpm for 20min. Then, the tubes were centrifuged again at 15,000rpm for 20min, and the supernatant was collected. The concentration of each protein sample was measured using Protein Assay (Bio-Rad) and the microplate reader (BioTek). After that, the protein samples were mixed with dye and incubated at 105^{o}C for 10min. Different volumes of the protein samples, depending on the concentration, were pipetted into the NuPAGE gels along with the marker. Electrophoresis started at 100V for 10min and continued at 140V for 1hr until the dye reached the bottom. The polyvinylidene fluoride (PVDF) membranes were labeled and prewetted with 100% methanol. After that, filter papers, NuPAGE gels, and PVDF membranes were soaked in the transfer buffer, made of tris, glycine, and methanol, and stacked in the sandwich method. The roller was used to prevent bubbles between layers. The protein samples were transferred from gel to membrane at 100V for 2hrs in transfer boxes with ice.

After the transfer, the PVDF membranes were blocked with 5% milk in tris-buffered saline with Tween (TBST) for 1hr, washed with TBST for 5min, and blotted with primary antibodies (phospho-AKT, phospho-AMPK, phospho-mTOR, and GAPDH antibodies) overnight at 4^{o}C. Then, the membranes were washed with TBST for 5min twice and blotted with secondary antibodies (goat anti-rabbit antibody) for 1hr. After that, the membranes were washed with TBST for 5min 3 times and incubated with the ECL Western Blotting Substrate for 5min before exposure in dark room. The exposure time varied for different antibodies, and the protein bands on the film were quantified using Quantity One software (Bio-Rad). All protein expression levels were normalized to GAPDH.

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using Student t-test or ANOVA, followed by Bonferroni post-tests using Prism software. Differences were considered significant at $P < 0.05$.

REFERENCES

1. Satpathy, H. K., Fleming, A., Frey, D., Barsoom, M., Satpathy, C., & Khandalavala, J. (2008). Maternal obesity and pregnancy. *Postgraduate medicine*, *120*(3), E01–E9. https://doi.org/10.3810/pgm.2008.09.1920

2. Tan, S. Y., Mei Wong, J. L., Sim, Y. J., Wong, S. S., Mohamed Elhassan, S. A., Tan, S. H., Ling Lim, G. P., Rong Tay, N. W., Annan, N. C., Bhattamisra, S. K., & Candasamy, M. (2019). Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. *Diabetes & metabolic syndrome*, *13*(1), 364–372. https://doi.org/10.1016/j.dsx.2018.10.008

3. Pfeiffer, A. F., & Klein, H. H. (2014). The treatment of type 2 diabetes. *Deutsches Arzteblatt international*, *111*(5), 69–82. https://doi.org/10.3238/arztebl.2014.0069

4. Catalano, P. M., & Shankar, K. (2017). Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child. *BMJ (Clinical research ed.)*, *356*, j1. https://doi.org/10.1136/bmj.j1

5. Poblete, J. A., & Olmos, P. (2021). Obesity and Gestational Diabetes in Pregnant Care and Clinical Practice. *Current vascular pharmacology*, *19*(2), 154–164. https://doi.org/10.2174/1570161118666200628142353

6. Mack, L. R., & Tomich, P. G. (2017). Gestational Diabetes: Diagnosis, Classification, and Clinical Care. *Obstetrics and gynecology clinics of North America*, *44*(2), 207–217. https://doi.org/10.1016/j.ogc.2017.02.002

7. Johns, E. C., Denison, F. C., Norman, J. E., & Reynolds, R. M. (2018). Gestational Diabetes Mellitus: Mechanisms, Treatment, and Complications. *Trends in endocrinology and metabolism: TEM*, *29*(11), 743–754. https://doi.org/10.1016/j.tem.2018.09.004

8. Lende, M., & Rijhsinghani, A. (2020). Gestational Diabetes: Overview with Emphasis on Medical Management. *International journal of environmental research and public health*, *17*(24), 9573. https://doi.org/10.3390/ijerph17249573

9. Holst J. J. (2007). The physiology of glucagon-like peptide 1. *Physiological reviews*, *87*(4), 1409–1439. https://doi.org/10.1152/physrev.00034.2006

10. Donnelly D. (2012). The structure and function of the glucagon-like peptide-1 receptor and its ligands. *British journal of pharmacology*, *166*(1), 27–41. https://doi.org/10.1111/j.1476-5381.2011.01687.x

11. Sandoval, D. A., & D'Alessio, D. A. (2015). Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. *Physiological reviews*, *95*(2), 513–548. https://doi.org/10.1152/physrev.00013.2014

12. Nauck, M. A., Quast, D. R., Wefers, J., & Pfeiffer, A. (2021). The evolving story of incretins (GIP and GLP-1) in metabolic and cardiovascular disease: A pathophysiological update. *Diabetes, obesity & metabolism*, *23 Suppl 3*, 5–29. https://doi.org/10.1111/dom.14496

13. Whalley, N. M., Pritchard, L. E., Smith, D. M., & White, A. (2011). Processing of proglucagon to GLP-1 in pancreatic α-cells: is this a paracrine mechanism enabling GLP-1 to act on β-cells?. *The Journal of endocrinology*, *211*(1), 99–106. https://doi.org/10.1530/JOE-11-0094

14. McLean, B. A., Wong, C. K., Campbell, J. E., Hodson, D. J., Trapp, S., & Drucker, D. J. (2021). Revisiting the Complexity of GLP-1 Action from Sites of Synthesis to Receptor Activation. *Endocrine reviews*, *42*(2), 101–132. https://doi.org/10.1210/endrev/bnaa032

15. Bethea, M., Bozadjieva-Kramer, N., & Sandoval, D. A. (2021). Preproglucagon Products and Their Respective Roles Regulating Insulin Secretion. *Endocrinology*, *162*(10), bqab150. https://doi.org/10.1210/endocr/bqab150

16. Karaca, M., Magnan, C., & Kargar, C. (2009). Functional pancreatic beta-cell mass: involvement in type 2 diabetes and therapeutic intervention. *Diabetes & metabolism*, *35*(2), 77–84. https://doi.org/10.1016/j.diabet.2008.09.007

17. Koole, C., Pabreja, K., Savage, E. E., Wootten, D., Furness, S. G., Miller, L. J., Christopoulos, A., & Sexton, P. M. (2013). Recent advances in understanding GLP-1R (glucagon-like peptide-1 receptor) function. *Biochemical Society transactions*, *41*(1), 172–179. https://doi.org/10.1042/BST20120236

18. Zhao, X., Wang, M., Wen, Z., Lu, Z., Cui, L., Fu, C., Xue, H., Liu, Y., & Zhang, Y. (2021). GLP-1 Receptor Agonists: Beyond Their Pancreatic Effects. *Frontiers in endocrinology*, *12*, 721135. https://doi.org/10.3389/fendo.2021.721135

19. Lau, J., Bloch, P., Schäffer, L., Pettersson, I., Spetzler, J., Kofoed, J., Madsen, K., Knudsen, L. B., McGuire, J., Steensgaard, D. B., Strauss, H. M., Gram, D. X., Knudsen, S. M., Nielsen, F. S., Thygesen, P., Reedtz-Runge, S., & Kruse, T. (2015). Discovery of the Once-Weekly Glucagon-Like Peptide-1 (GLP-1) Analogue Semaglutide. *Journal of medicinal chemistry*, *58*(18), 7370–7380. https://doi.org/10.1021/acs.jmedchem.5b00726

20. Christou, G. A., Katsiki, N., Blundell, J., Fruhbeck, G., & Kiortsis, D. N. (2019). Semaglutide as a promising antiobesity drug. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, *20*(6), 805–815. https://doi.org/10.1111/obr.12839

21. European Medicines Agency: EMEA/H/C/004174/0000 – Ozempic European Public Assessment Report, https://www.ema.europa.eu/en/documents/assessment-report/ozempic-epar-public-assessment-report_en.pdf, June, 2022.

22. Kawano, Y., & Cohen, D. E. (2013). Mechanisms of hepatic triglyceride accumulation in nonalcoholic fatty liver disease. *Journal of gastroenterology*, *48*(4), 434–441. https://doi.org/10.1007/s00535-013-0758-5

23. Chavan-Gautam, P., Rani, A., & Freeman, D. J. (2018). Distribution of Fatty Acids and Lipids During Pregnancy. *Advances in clinical chemistry*, *84*, 209–239. https://doi.org/10.1016/bs.acc.2017.12.006

24. Alves-Bezerra, M., & Cohen, D. E. (2017). Triglyceride Metabolism in the Liver. *Comprehensive Physiology*, *8*(1), 1–8. https://doi.org/10.1002/cphy.c170012

25. Patel, V. J., Joharapurkar, A. A., Shah, G. B., & Jain, M. R. (2014). Effect of GLP-1 based therapies on diabetic dyslipidemia. *Current diabetes reviews*, *10*(4), 238–250. https://doi.org/10.2174/1573399810666140707092506

26. Parrettini, S., Caroli, A., & Torlone, E. (2020). Nutrition and Metabolic Adaptations in Physiological and Complicated Pregnancy: Focus on Obesity and Gestational Diabetes. *Frontiers in endocrinology*, *11*, 611929. https://doi.org/10.3389/fendo.2020.611929

27. Furse, S., Fernandez-Twinn, D. S., Chiarugi, D., Koulman, A., & Ozanne, S. E. (2021). Lipid Metabolism Is Dysregulated before, during and after Pregnancy in a Mouse Model of Gestational Diabetes. *International journal of molecular sciences*, *22*(14), 7452. https://doi.org/10.3390/ijms22147452

28. Huda, S. S., Sattar, N., & Freeman, D. J. (2009). Lipoprotein metabolism and vascular complications in pregnancy. *Clinical Lipidology*, *4*(1), 91-102.

29. Caron, A., Richard, D., & Laplante, M. (2015). The Roles of mTOR Complexes in Lipid Metabolism. *Annual review of nutrition*, *35*, 321–348. https://doi.org/10.1146/annurev-nutr-071714-034355

30. Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E., & Raptis, S. A. (2011). Insulin effects in muscle and adipose tissue. *Diabetes research and clinical practice*, *93 Suppl 1*, S52–S59. https://doi.org/10.1016/S0168-8227(11)70014-6

31. Hoff, A., & Piechowski, K. (2021). Treatment of Hypertriglyceridemia with Aggressive Continuous Intravenous Insulin. *Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques*, *24*, 336–342. https://doi.org/10.18433/jpps32116

32. Furse S. (2022). Lipid metabolism is dysregulated in a mouse model of diabetes. *Metabolomics : Official journal of the Metabolomic Society*, *18*(6), 36. https://doi.org/10.1007/s11306-022-01884-w

33. Farr, S., Taher, J., & Adeli, K. (2014). Glucagon-like peptide-1 as a key regulator of lipid and lipoprotein metabolism in fasting and postprandial states. *Cardiovascular & hematological disorders drug targets*, *14*(2), 126–136. https://doi.org/10.2174/1871529x14666140505125300

34. Yeo, E., Brubaker, P. L., & Sloboda, D. M. (2022). The intestine and the microbiota in maternal glucose homeostasis during pregnancy. *The Journal of endocrinology*, *253*(1), R1–R19. https://doi.org/10.1530/JOE-21-0354

35. Hill D. J. (2018). Placental control of metabolic adaptations in the mother for an optimal pregnancy outcome. What goes wrong in gestational diabetes?. *Placenta*, *69*, 162–168. https://doi.org/10.1016/j.placenta.2018.01.002

36. Krycer, J. R., Sharpe, L. J., Luu, W., & Brown, A. J. (2010). The Akt-SREBP nexus: cell signaling meets lipid metabolism. *Trends in endocrinology and metabolism: TEM*, *21*(5), 268–276. https://doi.org/10.1016/j.tem.2010.01.001

37. Foretz, M., Even, P. C., & Viollet, B. (2018). AMPK Activation Reduces Hepatic Lipid Content by Increasing Fat Oxidation In Vivo. *International journal of molecular sciences*, *19*(9), 2826. https://doi.org/10.3390/ijms19092826

38. Grabner, G. F., Xie, H., Schweiger, M., & Zechner, R. (2021). Lipolysis: cellular mechanisms for lipid mobilization from fat stores. *Nature metabolism*, *3*(11), 1445–1465. https://doi.org/10.1038/s42255-021-00493-6

39. Watt M. J. (2009). Storing up trouble: does accumulation of intramyocellular triglyceride protect skeletal muscle from insulin resistance?. *Clinical and experimental pharmacology & physiology*, *36*(1), 5–11. https://doi.org/10.1111/j.1440-1681.2008.05075.x

40. Kubota, S., & Yabe, D. (2021). Elevation of Fasting GLP-1 Levels in Child and Adolescent Obesity: Friend or Foe?. *The Journal of clinical endocrinology and metabolism*, *106*(9), e3778–e3780. https://doi.org/10.1210/clinem/dgab301

41. Tsimihodimos, V., & Elisaf, M. (2018). Incretins and Lipid Metabolism. *Current medicinal chemistry*, *25*(18), 2133–2139. https://doi.org/10.2174/0929867324666170414164244

42. Andrikou, E., Tsioufis, C., Andrikou, I., Leontsinis, I., Tousoulis, D., & Papanas, N. (2019). GLP-1 receptor agonists and cardiovascular outcome trials: An update. *Hellenic journal of cardiology : HJC = Hellenike kardiologike epitheorese*, *60*(6), 347–351. https://doi.org/10.1016/j.hjc.2018.11.008

43. Mulvihill, E. E. (2018). Regulation of intestinal lipid and lipoprotein metabolism by the proglucagon-derived peptides glucagon like peptide 1 and glucagon like peptide 2. *Current opinion in lipidology*, *29*(2), 95.

44. Armstrong, M. J., Hull, D., Guo, K., Barton, D., Hazlehurst, J. M., Gathercole, L. L., Nasiri, M., Yu, J., Gough, S. C., Newsome, P. N., & Tomlinson, J. W. (2016). Glucagon-like peptide 1 decreases lipotoxicity in non-alcoholic steatohepatitis. *Journal of hepatology*, *64*(2), 399–408. https://doi.org/10.1016/j.jhep.2015.08.038

45. Fang, Y., Ji, L., Zhu, C., Xiao, Y., Zhang, J., Lu, J., Yin, J., & Wei, L. (2020). Liraglutide Alleviates Hepatic Steatosis by Activating the TFEB-Regulated Autophagy-Lysosomal Pathway. *Frontiers in cell and developmental biology*, *8*, 602574. https://doi.org/10.3389/fcell.2020.602574

46. Chen, J., Zhao, H., Ma, X., Zhang, Y., Lu, S., Wang, Y., Zong, C., Qin, D., Wang, Y., Yingfeng Yang, Y., Wang, X., & Liu, Y. (2017). GLP-1/GLP-1R Signaling in Regulation of Adipocyte Differentiation and Lipogenesis. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology*, *42*(3), 1165–1176. https://doi.org/10.1159/000478872

47. Zhao, L., Zhu, C., Lu, M., Chen, C., Nie, X., Abudukerimu, B., ... & Lu, Y. (2019). The key role of a glucagon-like peptide-1 receptor agonist in body fat redistribution. *Journal of Endocrinology*, *240*(2), 271-286.

48. Iqbal, J., Wu, H. X., Hu, N., Zhou, Y. H., Li, L., Xiao, F., Wang, T., Jiang, H. L., Xu, S. N., Huang, B. L., & Zhou, H. D. (2022). Effect of glucagon-like peptide-1 receptor agonists on body weight in adults with obesity without diabetes mellitus-a systematic review and meta-analysis of randomized control trials. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, *23*(6), e13435. https://doi.org/10.1111/obr.13435

49. Qiao, L., Saget, S., Lu, C., Zang, T., Dzyuba, B., Hay, W. W., & Shao, J. (2022). The Essential Role of Pancreatic α-cells in Maternal Metabolic Adaptation to Pregnancy. *Diabetes*, db210923. Advance online publication. https://doi.org/10.2337/db21-0923

50. Moffett, R. C., Vasu, S., Thorens, B., Drucker, D. J., & Flatt, P. R. (2014). Incretin receptor null mice reveal key role of GLP-1 but not GIP in pancreatic beta cell adaptation to pregnancy. *PloS one*, *9*(6), e96863. https://doi.org/10.1371/journal.pone.0096863

51. Graham, D. L., Madkour, H. S., Noble, B. L., Schatschneider, C., & Stanwood, G. D. (2021). Long-term functional alterations following prenatal GLP-1R activation. *Neurotoxicology and teratology*, *87*, 106984. https://doi.org/10.1016/j.ntt.2021.106984

52. Wang, H., & Eckel, R. H. (2009). Lipoprotein lipase: from gene to obesity. *American journal of physiology. Endocrinology and metabolism*, *297*(2), E271–E288. https://doi.org/10.1152/ajpendo.90920.2008

53. Gil-Sánchez, A., Demmelmair, H., Parrilla, J. J., Koletzko, B., & Larqué, E. (2011). Mechanisms involved in the selective transfer of long chain polyunsaturated Fatty acids to the fetus. *Frontiers in genetics*, *2*, 57. https://doi.org/10.3389/fgene.2011.00057

54. Hernandez, T. L., Friedman, J. E., & Barbour, L. A. (2020). Insulin resistance in pregnancy: implications for mother and offspring. In *Insulin Resistance* (pp. 67-94). Humana, Cham.

55. Egholm, C., Khammy, M. M., Dalsgaard, T., Mazur, A., Tritsaris, K., Hansen, A. J., Aalkjaer, C., & Dissing, S. (2016). GLP-1 inhibits VEGFA-mediated signaling in isolated human endothelial cells and VEGFA-induced dilation of rat mesenteric arteries. *American journal of physiology. Heart and circulatory physiology*, *311*(5), H1214–H1224. https://doi.org/10.1152/ajpheart.00316.2016

56. Olivecrona G. (2016). Role of lipoprotein lipase in lipid metabolism. *Current opinion in lipidology*, *27*(3), 233–241. https://doi.org/10.1097/MOL.0000000000000297