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The role of glucagon-like peptide-1 on food intake, glucokinase expression and fatty acid metabolism

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THE ROLE OF GLUCAGON-LIKE PEPTIDE-1 ON FOOD INTAKE, GLUCOKINASE EXPRESSION AND FATTY ACID METABOLISM

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By

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DEDICATION

I would like to dedicate this to my major professor Dr. Martin and my family.

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ABBREVIATIONS

AGRP	Agouti-related peptide
AOX1	Aldehyde oxidase 1
ARC	Arcuate nucleus
CPT1	Carnitine palmitoyltransferase 1
FAS	Fatty acid synthase
GK	Glucokinase
GLP-1	Glucagon-like peptide-1
PPARγ	Peroxisome proliferator-activated receptor-gamma
NPY	Neuropeptide Y
SCD1	Stearoyl-CoA desaturase 1
SREBP1c	Sterol regulatory element-binding protein-1c

ABSTRACT

The increase in blood levels of GLP-1 with dietary resistant starch is thought to be associated with the activities of hypothalamic NPY/AgRP neurons and the gene expression of glucokinase in the ARC of the hypothalamus. Exendin-4 has been shown to be associated with fatty acid synthesis and oxidation. In this project, it was proposed that 1) food intake will be decreased in GLP-1R KO mice, the glucokinase (GK) mRNA expression in the liver and in the hypothalamus, the hypothalamic NPY mRNA expression will be up-regulated by exendin-4 treatment; and 2) exendin-4 will decrease fatty acid synthesis and increase fatty acid oxidation.

In order to investigate these hypotheses, animal experiments were performed. Exendin-4 treated WT and GLP-1R KO mice were injected with exendin-4 at 100μg/Kg body weight or vehicle for 7 days. The exendin-4 treated mice and the vehicle mice were subdivided into fed and fasted groups. There were 8 groups of mice (n=8). Food intake after refeeding at 1h, 2h and 4h was measured. At end of the study, the blood glucose level, the GK, CPT1, AOX1, SCD1, FAS, SREBP1c and PPARγ mRNA expressions in liver, and the GK and NPY mRNA expressions in hypothalamus were measured.

In this study, the food intake of GLP-1R KO mice at 1h, 2h and 4h was significantly less than in WT mice, and the FAS expression in liver was significantly higher in GLP-1R KO mice than WT mice. Exendin-4 treatment decreased blood glucose, and increased the GK gene expression in liver, but not in the hypothalamus. The food intake and the NPY gene expression were not altered by exendin-4. Only the AOX mRNA expression was increased by exendin-4. CPT1, SCD1, FAS, SREBP1c, and PPARγ mRNA were not changed. GLP-1 might have beneficial effects on improving the impairment of nutrient sensing system induced by aging. The up-regulated FAS gene expression might be one possible mechanism for higher body fat in

GLP1R KO mice. The lowering body fat storage effect of exendin-4 may be due in part to the increase of the fatty acid oxidation in liver.

CHAPTER 1

INTRODUCTION

Resistant starch, a non-digestible fermentable dietary fiber, has been shown in human studies to have many health benefits. These include increasing satiety, reducing the blood cholesterol and triglyceride levels, decreasing body fat, improving the insulin sensitivity, and promoting anti-cancer effects (Robertson, Currie et al. 2003; Brown 2004; Higgins 2004). Resistant starch also lowers fat storage in animals and significantly increases the expression and release of glucagon-like peptide -1 (GLP-1) in the large intestine (Keenan, Zhou et al. 2006; Zhou, Hegsted et al. 2006). GLP-1, one kind of satiety peptide, may inhibit food intake, stimulates insulin release, decelerates the gastric emptying, reduces plasma glucose level, lowers body weight and improves insulin sensitivity (Turton, O'Shea et al. 1996; Young, Gedulin et al. 1999; Meier, Gallwitz et al. 2003; Holst 2007). GLP-1 was thought to affect the activities of hypothalamic NPY/AgRP neurons and it was demonstrated that the increase of NPY mRNA induced by a 48h fast could be significantly attenuated by intracerebroventricular (icv) injection of GLP-1 in rats (Seo, Ju et al. 2008).

Elderly persons are known to have impaired ability to adjust food intake following over-eating or under-eating. Such age-related impairment of nutrient sensing is called anorexia of aging, and is the reason that older people have an impaired ability to defend their body weight (Roberts, Fuss et al. 1994; Hays and Roberts 2006). It has been found that aged rats had decreased NPY responsiveness to fasting, so an impaired ability to defend body weight is thought to be associated with the impaired activation of the hypothalamic NPY pathway (Gruenewald, Marck et al. 1996; Kaneda, Makino et al. 2001). Obesity and aging have also been shown to impair the nutrient sensing system in peripheral tissues such as muscle, liver, adipose

and pancreas (Hajnoczky and Hoek 2007). Calorie restriction (CR) has been shown to reverse such impairment of the nutrient sensing system in peripheral tissue (Dilova, Easlon et al. 2007).

It has been found that age-related impairment of glucose tolerance could be improved by GLP-1 treatment (Wang, Perfetti et al. 1997). In our lab's preliminary data, resistant starch was found to increase gene expression of glucokinase in the ARC in the hypothalamus. Glucokinase was thought to function as a glucose sensor to facilitate the adaptation in response to the fluctuations in blood glucose levels (Roncero, Alvarez et al. 2000; Li, Xi et al. 2003; Roncero, Alvarez et al. 2004). In our studies, we have observed increased (mice) or similar (rats) energy intake for rodents fed resistant starch compared to rodents fed a diet with equal energy density. Consistently high levels of GLP-1 and PYY as a result of fermentation of resistant starch may overcome meal spikes of these hormones that would otherwise lead to satiety. Tolerance to PYY may be the mechanism by which RS induces food intake (Zhou, Martin et al. 2008). It was recently reported that repeated i.p. infusions of PYY increased food intake (Reidelberger, Haver et al. 2008). Our observations are consistent with a report that dietary resistant starch did not suppress appetite(Scribner, Pawlak et al. 2008). We hypothesize that resistant starch will mimic the effects of calorie restriction. We expect the increasing of GLP-1 by resistant starch could improve the impairment of nutrient sensing system induced by obesity and aging.

Exendin-4, a long-acting GLP-1 receptor agonist, has a much longer circulating and biological half-life and is thought to be more potent than GLP-1 (Deacon, Pridal et al. 1996; Szayna, Doyle et al. 2000). Exendin-4, just as GLP-1, inhibits the food intake, stimulates insulin release, decreases plasma glucose level, reduces body weight and improves insulin sensitivity (Young, Gedulin et al. 1999; Szayna, Doyle et al. 2000; Parkes, Pittner et al. 2001). Exendin-4 was also demonstrated to be associated with fatty acid metabolism. The key regulators of *de*

novo hepatic lipogenesis, SREBP-1c, SCD1, ACC, PPAR-γ1, 2, and FAS were down-regulated, and the key elements in β-oxidation of free fatty acids, PPARα and AOX were up-regulated by exendin-4 treatment (Ding, Saxena et al. 2006; Samson, Gonzalez et al. 2008).

In this study, we investigated the effects of exendin-4 and GLP-1 receptor knock out on the nutrient sensing system and fatty acid metabolism. The hypothesis for this work is that 1) the food intake will be decreased in GLP-1R KO mice, the glucokinase (GK) mRNA expression in the liver and in the hypothalamus, the hypothalamic NPY mRNA expression will be up-regulated by exendin-4 treatment; and 2) exendin-4 can decrease fatty acid synthesis and increase fatty acid oxidation. To test this hypothesis, we measured food intake, the expressions of GK, CPT1, AOX1, SCD1, FAS, SREBP1c and PPARγ mRNA in liver, and the GK and NPY mRNA expressions in hypothalamus in WT mice and GLP-1R KO mice with vehicle or exendin-4 treatment.

CHAPTER 2

REVIEW OF LITERATURE

Glucagon-Like Peptide-1(GLP-1)

GLP-1, a gut peptide, comes from the preproglucagon gene product in the L cells of the intestine. It is released in response to nutrients, such as FFA and carbohydrate (Badman and Flier 2005). This response occurs within minutes of food ingestion, even though the L cells are in the distal intestine (Roberge and Brubaker 1993). The circulating half-life of GLP-1 is only 2 min as GLP-1 can be quickly inactivated by the enzyme dipeptidyl peptidase IV (DPP-IV) (Kieffer, McIntosh et al. 1995). This peptide has several functions which includ inhibiting food intake, lowering body weight, decelerating the gastric emptying, stimulating insulin release, reducing plasma glucose level and improving insulin sensitivity.

GLP-1 and Food intake: Intracerebroventricular (ICV) GLP-1 potently inhibits food intake in fasted rats (Turton, O'Shea et al. 1996). Food intake was decreased and satiety was enhanced by intravenously infused GLP-1 in both healthy subjects and patients with type 2 diabetes (Gutzwiller, Drewe et al. 1999).

One study showed that when long-acting DPP-IV–resistant GLP-1 agonists were given peripherally to rats, food intake and body weight both decreased (Larsen, Fledelius et al. 2001). Exendin-4, a long-acting GLP-1 receptor agonist, results in immediate reduction in food intake after 5 days of once daily injections and also produces a sustained reduction in food intake and weight-gain when given twice daily injections for a total of 56 days in Zucker fatty rats (Szayna, Doyle et al. 2000).

Exendin (9-39), a specific GLP-1-receptor antagonist, blocks the effects of ICV injected GLP-1 on food intake. However, it only had effects on satiated rats, not on fasted rats. It

increased the food intake more than double in satiated rats (Turton, O'Shea et al. 1996). Another study also showed that exendin (9-39) could augment 1-h food intake by 300% after three repeated injections every 20 min in satiated animals (Schick, Zimmermann et al. 2003).

The vagal-brainstem-hypothalamic pathway is necessary for the GLP-1 inhibitory effect on food intake as this effect is abolished by ablation of this pathway (Abbott, Monteiro et al. 2005). GLP-1 also functions at lateral and medial hypothalamic sites to reduce food intake (Schick, Zimmermann et al. 2003).

The GLP-1 inhibitory effect on food intake is thought to be through the activities of hypothalamic NPY/AgRP neurons. NPY and AgRP are both potent orexigenic neuropeptides, and involved in control of food intake. Food intake, and the increased gene expression of NPY and AgRP are induced by 48h fasting and are significantly attenuated by the intracerebroventricular (icv) injection of GLP-1 in rats (Seo, Ju et al. 2008). Exendin (9-39) has also been found to increase the feeding response to NPY (Turton, O'Shea et al. 1996).

But in our lab's preliminary data, resistant starch which could significantly increases the expression and release of glucagon-like peptide -1 (GLP-1) in the large intestine, did not inhibit the food intake. Actually, resistant starch could significantly increase the cumulative food intake. One reason might be the time and dosage differences. The food intake inhibitory effect of GLP-1 on 48-h fasted rats could be significant by one time single icv administration of GLP-1 (10μg) (Seo, Ju et al. 2008). Food intake was decreased and satiety was enhanced by intravenously infused GLP-1 (1.5 pmol. kg-1. min-1) for 2 days in both healthy subjects and patients with type 2 diabetes (Gutzwiller, Drewe et al. 1999). But in our study, rats were fed by resistant starch for long-term and the GLP-1 levels were consistently higher over a 24-h period. Consistently high levels of GLP-1 and PYY as a result of fermentation of resistant starch may overcome meal

spikes of these hormones that would otherwise lead to satiety. Tolerance to PYY may be the mechanism by which RS induces food intake (Zhou, Martin et al. 2008). It was recently reported that repeated i.p. infusions of PYY increased food intake (Reidelberger, Haver et al. 2008). Our observations are consistent with a report that dietary resistant starch did not suppress appetite (Scribner, Pawlak et al. 2008). Another reason might be that resistant starch had increased energy expenditures. The resistant starch fed mice might compensate the increased energy expenditure by increasing food intake. These results strongly support our hypothesis that resistant starch fed animals might have better adjustment for fasting, thus could help to attenuate anorexia of aging. This study is focused on the age-associated anorexia.

GLP-1 and Obesity: GLP-1 reduces body weight 6 days after ICV injection, and exendin-(9-39) increases body weight after 3 days ICV injection. Interestingly, the increase in body weight when exendin-(9-39) and NPY were given together was significantly higher than when given NPY alone (Meeran, O'Shea et al. 1999). Exendin (9-39) was thought to increase the feeding response to NPY (Turton, O'Shea et al. 1996).

It has been found that a novel long-acting injectable GLP-1 derivative, NN2211, could reduce body weight in both normal rats and obese rats with obesity generated by neonatal monosodium glutamate treatment (MSG) (Abbott, Monteiro et al. 2005). Five days prandial subcutaneous injections of recombinant glucagon-like peptide-1 (7-36) reduces body weight in healthy obese subjects (Naslund, King et al. 2004). Peripheral infusions of glucagon-like peptide-1 inhibit food intake, decrease carbohydrate oxidation and gastric emptying rate in healthy obese subjects (Flint, Raben et al. 2001). However, GLP-1 receptor -/- mice, have normal body weight and normal feeding behavior, even for 11- and 16-month-old GLP-1R-/-

mice fed with high fat diet (Scrocchi and Drucker 1998). However, GLP-R-/- in our lab had more body fat (unpublished data).

GLP-1 and Diabetes: The binding of GLP-1 to its receptors increases the levels of β cell intracellular cAMP and insulin gene transcription. GLP-1 also functions synergistically with glucose to stimulate insulin release in a glucose dependent manner (Drucker, Philippe et al. 1987; Holz, Kuhtreiber et al. 1993; Holst 2007).

GLP-1 enhances cell proliferation of the endocrine and exocrine components of the pancreas, decreases apoptotic cells within the islet as well in the exocrine pancreas, reduces the caspase-3 expression, increases insulin content, and also decreases the fragmented nuclei in the islet cells in Zucker diabetic rats (Farilla, Hui et al. 2002).

Exogenous GLP-1 decelerates gastric emptying after solid and liquid meals and increases the meal retention time in the distal stomach in healthy subjects (Little, Pilichiewicz et al. 2006). Exogenous GLP-1 also slows gastric emptying after solid meals and normalizes glucose concentrations in patients with type 2 diabetes (Meier, Gallwitz et al. 2003).

Exendin-4, which has 53% sequence similarity to glucagon-like peptide (GLP)-1, decreases blood glucose levels, reduces body weight and improves insulin sensitivity in obese diabetic (ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (Macaca mulatta) (Young, Gedulin et al. 1999). Continuous subcutaneous infusion of GLP-1 for 6 weeks decreases fasting and 8 h mean plasma glucose and free fatty acids, reduces body weight, and improves insulin sensitivity and beta-cell function in patients with type 2 diabetes (Zander, Madsbad et al. 2002).

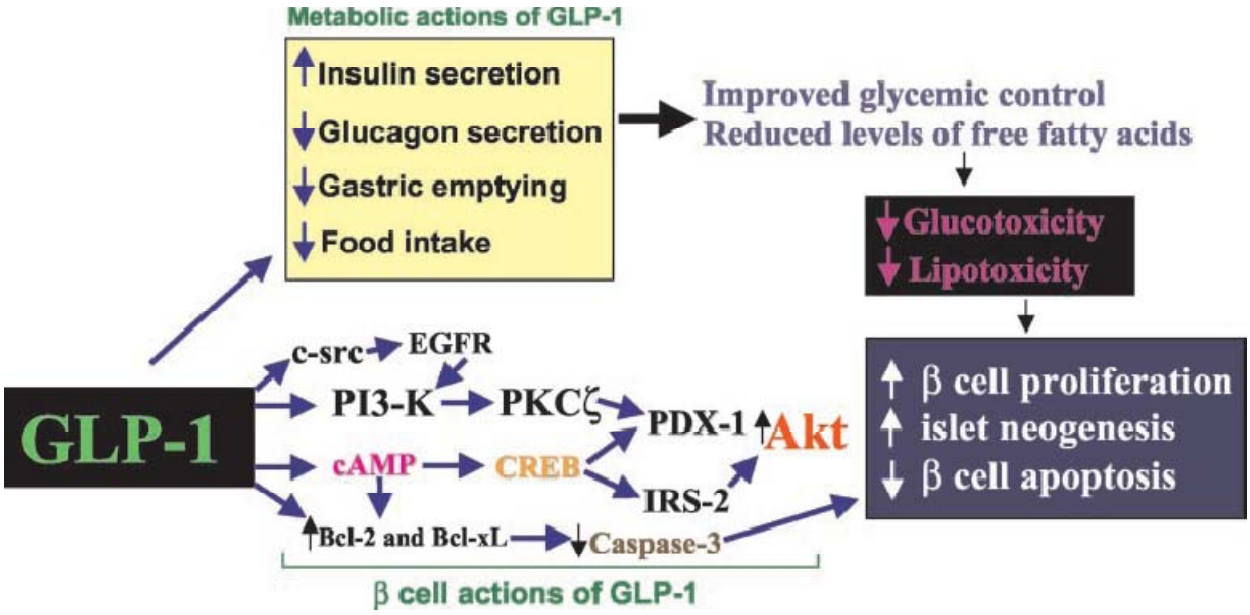

Figure 1. The effects of GLP-1

The overall effects and metabolic actions, β cell function, and CNS function of GLP-1 are illustrated in Figure 1 (Drucker 2003).

Glucagon-Like Peptide-1 Receptor -/- Mice

Glucagon-like peptide-1 receptor -/- mice were first generated in the Drucker lab in 1995 in order to investigate the physiological importance of GLP-1. Although GLP-1 was thought to inhibit food intake, there were no significant differences in 2-hour and 6-hour food intake after a 20-hour fasting in either female or male GLP-1R-/- mice compared to WT mice. In addition there were also no significant differences in body weight for both female and male GLP-1R-/- mice from 4 to 24 weeks of age compared to WT mice (Scrocchi, Brown et al. 1996). There was also no evidence of abnormal body weight in 11- and 16-month-old aging GLP-1R-/- mice, or obesity in GLP-1R-/- mice after 18 weeks of high fat feeding (Scrocchi and Drucker 1998). So, *in vivo* the body weight and feeding behavior were not connected to the disruption of GLP1/GLP1R signaling in the central nervous system.

But in our lab's preliminary data, GLP-1R KO male mice had significantly higher body weight and higher body fat compared with WT male mice after 12 weeks EC or RS diet feeding. But before the EC or RS diet feeding, these GLP-1R KO male mice which were fed on chow diet had the similar body weight and body fat compared with WT male mice. And GLP-1R KO female mice which were fed consistently chow diet had similar body weight compared with WT female mice. These reflects that the diet type might influence the body weight gain of GLP-1R KO mice.

Following an oral glucose challenge or intraperitoneal glucose challenge, GLP1R-/- mice have abnormal blood glucose tolerance and decreased levels of circulating insulin (Scrocchi, Brown et al. 1996). There was no evidence for deterioration of glucose tolerance in 11- and 16-month-old aging GLP-1R-/- mice, but it did result in increased insulin secretion during testing for glucose tolerance, indicating that there may be a change in insulin sensitivity. In addition no evidence for deterioration in glucose tolerance was detected in GLP-1R-/- mice after 18 weeks of high fat feeding (Scrocchi and Drucker 1998). Therefore, GLP1/GLP1R signaling was related to a modest glucose intolerance.

Compared with the WT mice, the GLP-1R -/- mice had an elevated glucose-dependent insulinotropic polypeptide (GIP) secretion and enhanced GIP action after an oral glucose tolerance test. Compared with the WT mice, the GLP-1R -/- mice had higher insulin response to GIP perfusion of the pancreas and isolated islets when the glucose level was higher. The GLP-1R -/- mice still had normal glucagon secretion. Such compensatory changes in the GIP secretion and action in GLP-1R -/- mice, appears to account for the modification of the anticipated phenotype resulting from interruption of the GLP1/GLP1R signaling pathway (Pederson, Satkunarajah et al. 1998).

Exendin-4

Exendin-4, which is isolated from the Gila monster salivary gland, is a 39-amino acid peptide, and is a long-acting GLP-1 receptor agonist (Goke, Fehmann et al. 1993). Compared with GLP-1, exendin-4 has a much longer circulating and biological half-life and is thought to be more potent (Deacon, Pridal et al. 1996; Szayna, Doyle et al. 2000).

Compared with untreated animals, exendin-4 had an immediate reduction in food intake after 5 days of once daily injections in Zucker fatty rats. When Zucker fatty rats were treated with twice daily injections for a total 56 days, exendin-4 produced a sustained reduction in food intake and weight-gain. After 4 weeks of exendin-4 treatment, Zucker fatty rats began to have reduced fat deposition in the subcutaneous region of the abdominal region. Then after 8 weeks of exendin-4 treatment, the visceral fat deposition began decreasing (Szayna, Doyle et al. 2000).

Exendin-4 increases the basal and glucose-stimulated insulin release in static incubation of isolated islets, in dynamically perfused isolated islets and also in anesthetized rats. The insulinotropic effects of exendin-4 occurs both in vitro and in vivo (Parkes, Pittner et al. 2001). Exendin-4 increased the differentiation and proliferation of beta-cells in normal rats. The regeneration of the pancreas and expansion of beta-cell mass were also enhanced with exendin-4 treatment in the partial pancreatectomy rat model of type 2 diabetes (Xu, Stoffers et al. 1999). Treatment of Wistar diabetic rats with exendin-4 from postnatal day 2 to day 6 increases body weight, beta-cell mass and pancreatic insulin content, reduces basal plasma glucose on day 7. Treatment with exendin-4 from postnatal day 2 to day 6 resulted in the basal plasma glucose level and plasma glucose clearance rates which were much better even at 2 months of age. This suggested that exendin-4 had both short- and long-term beneficial effects on beta-cell mass

recovery and glucose homeostasis when given during the neonatal diabetic period (Tourrel, Bailbe et al. 2001).

The antidiabetic effect of exendin-4 has been tested in several animal models of type 2 diabetes. Exendin-4 reduces body weight, blood glucose levels and improves insulin sensitivity in hyperglycemic db/db and ob/ob mice, diabetic rhesus monkeys, and diabetic fatty Zucker rats (Young, Gedulin et al. 1999).

The antidiabetic effect of exendin-4 has also been tested in healthy volunteers, and patients with type 1 and type 2 diabetes. Exendin-4 delays gastric emptying, decreases food intake, decreases fasting blood glucose levels and decrease the peak change of postprandial glucose level in healthy volunteers (Edwards, Stanley et al. 2001). In patients with type 1 diabetes, exendin-4 treatment 15 min before breakfast delays gastric emptying, reduces blood glucose levels, blood pancreatic polypeptide, and glucagon levels after breakfast (Dupre, Behme et al. 2004). Exendin-4 also delays gastric emptying, reduces postprandial blood glucose levels, and decreases insulin and glucagon levels following twice daily subcutaneous injections with meals for 5 d in patients with type 2 diabetes (Kolterman, Buse et al. 2003). Additionally, hyperglycemic patients with type 2 diabetes who were unable to control blood glucose level even with maximally effective doses of combined metformin-sulfonylurea therapy, exendin-4 significantly decreased A1C and body weight in a 30-week, double-blind, placebo-controlled study (Kendall, Riddle et al. 2005).

Exendin-4 alters fatty acid metabolism. Exendin-4 reduced body weight, decreased fat content in liver tissue, reduced hepatic oxidative stress, and reversed hepatic steatosis in ob/ob mice with 60 days of treatment. The key regulators of *de novo* hepatic lipogenesis, SREBP-1c and SCD1, was down-regulated, and the key element in β-oxidation of free fatty acids, PPARα,

was up-regulated in the liver tissue of the ob/ob mice treated with exendin-4. Besides these three genes, ACC, which is associated with *de novo* hepatic lipogenesis was down-regulated, and AOX, which is associated with β-oxidation of free fatty acids, was up-regulated in rat hepatocytes with exendin-4 treatment (Ding, Saxena et al. 2006). One group constructed a mouse model with elevated steady-state levels of Ex4 by a single injection of a helper-dependent adenoviral (HDAd) vector which expresses exendin-4 long-term in vivo. When such mice are fed a high-fat-diet, they had lower body weight, improved glucose homeostasis, decreased hepatic fat, lower liver total lipid and triglyceride levels and an improved adipokine profile compared with mice without injected with a control vector. The ACC1, ACC2, SCD1, FAS, and PPAR-γ1, 2, which are associated with *de novo* hepatic lipogenesis were down-regulated in HDAd-Ex4 mice (Samson, Gonzalez et al. 2008).

Glucokinase (GK)

Glucokinase, also called hexokinase IV, is a glucose phosphorylating enzyme. It can facilitate the following reaction: Glucose + ATP → glucose-6-phosphate + ADP (Iynedjian 1993). Compared with the other hexokinases, glucokinase has a lower affinity for glucose. Its half-saturated point for glucose is about 8 mmol/L. Another important characteristic of glucokinase is that it is not inhibited by its product, glucose-6-phosphate. These two characteristics of glucokinase enable continued glucose phosphorylation and result in glucose transport not being rate limiting in GK - containing cells. Also the type of GLUT in these cells is not insulin dependent. Thus, the glucose phosphorylating reaction is a "supply-driven" reaction, with the flow being driven by the glucose supply, not by the demand for glucose-6-phosphate. These two characteristics also enable the glucose sensor function of glucokinase in GK - containing cells (Matschinsky 1996).

At this time there are about 150 mutations of the glucokinase gene that have been found in humans. Such mutations can lead to at least three kinds of syndromes. First, persistent hyperinsulinemic hypoglycemia, it is thought to be associated with the activation of glucokinase. Second, permanent neonatal diabetes, which happens when the newborns have two of the same alleles are thought to be associated with the inactivation of glucokinase. Third, maturity-onset diabetes of the young, occurs when the patient has one defective allele which is thought to be associated with the inactivation of glucokinase. All these functional consequences which come from GK gene mutations provide potent evidence for the glucokinase glucose-sensor concept (Glaser, Kesavan et al. 1998; Davis, Cuesta-Munoz et al. 1999; Njolstad, Sovik et al. 2001; Christesen, Jacobsen et al. 2002).

Mice with the global, or pancreatic beta-cell or hepatocyte-specific knockouts of GK had been already developed. Mice with the global, or pancreatic beta-cell -specific knockouts of GK die within a few days after birth from severe diabetes. Mice with hepatocyte-specific of GK knockouts are viable, and only mildly hyperglycemic (Postic, Shiota et al. 1999).

In hepatocytes, the catalytic activity of glucokinase is regulated by glucokinase regulatory protein. When the glucose concentration is low, glucokinase is bound to glucokinase regulatory protein and stays in the nucleus. When the glucose concentration is high or fructose is low, glucokinase will translocate to a free state in the cytoplasm. The effect of high glucose concentrations is less potent and slower than the effect of fructose (Toyoda, Miwa et al. 1994; Brown, Kalinowski et al. 1997). In hepatocytes, the primary inducer of glucokinase is insulin (Bedoya, Matschinsky et al. 1986).

The primary inducer of glucokinase in pancreatic β cells is glucose (Liang, Najafi et al. 1992). The glucokinase expression that is driven by glucose can be regulated by many factors. It

can be up-regulated by cAMP, biotin, retinoic acid and insulin, and down-regulated by Ca^{2+} channel blockers (Sorenson and Brelje 1997; Cabrera-Valladares, German et al. 1999; Romero-Navarro, Cabrera-Valladares et al. 1999; Fernandez-Mejia, Vega-Allende et al. 2001).

Glucokinase mRNA is also found in several rat brain regions, including the ventromedial and arcuate nuclei of the hypothalamus and cerebral cortex. Such glucokinase may be similar to pancreatic glucokinase, as it has an RNA splicing pattern of the glucokinase gene product in pancreatic islets. Also, glucokinase regulatory protein mRNA is found in the rat brain. All these findings suggest that the glucokinase in rat brain functions as the glucose sensor to facilitate the adaptation of the brain in response to thefluctuations in blood glucose levels (Roncero, Alvarez et al. 2000; Li, Xi et al. 2003; Roncero, Alvarez et al. 2004).

Neuropeptide Y (NPY)

NPY is a potent orexigenic neuropeptide. It has been reported that chronic central NPY administration could lead to persistent hyperphagia, body weight gain, and a significant increase in body fat accumulation (Jeanrenaud and Rohner-Jeanrenaud 2001). Up-regulation of NPY in the arcuate nucleus of the hypothalamus can be found during fasting (Brady, Smith et al. 1990). NPY can increase the net energy gain by many mechanisms. It can increase the food intake, decrease energy expenditure, and can stimulate glucocorticoid and insulin secretion to increase the deposition of triglycerides in white adipose tissue (Jeanrenaud and Rohner-Jeanrenaud 2001). NPY-knock-out mice have normal body weight and adiposity (Erickson, Clegg et al. 1996). However, the response to fasting for the NPY KO mice is a decreased food intake compared with the WT mice (Bannon, Seda et al. 2000).

AgRP is another potent orexigenic neuropeptide. It can increase food intake and decrease energy expenditure by antagonism of the melanocortin-3 receptor and melanocortin-4 receptor

(Morton and Schwartz 2001). The NPY/AgRP system is very important in both short- and long-term control of energy balance through the regulation of many kinds of hormones and nutrients. It has been demonstrated that if there is defective inhibition of NPY/AgRP neurons, there may be hyperphagia, decreased energy expenditure of glucose and lipid metabolism, and obesity (Kalra, Dube et al. 1999). It has also been reported that if NPY Y1 feeding receptors are blocked or immunoneutralized AgRP, the ghrelin-induced feeding would be impaired (Asakawa, Inui et al. 2001).

The NPY receptors are G-protein coupled receptors. At this time, six receptors have been isolated, NPY Y1–Y6, and for these six NPY receptors, the Y6 receptor is found only active in mice, but inactive in primates and absent in rats (Inui 1999; Kalra, Dube et al. 1999). The NPY Y1 and Y5 receptors are thought to be associated with the energy balance effects of NPY (Stanley, Wynne et al. 2005).

The food intake inhibitory effect of GLP-1 was thought to be through the activities of hypothalamic NPY/AgRP neurons. Food intake and the increased gene expression of NPY and AgRP induced by 48h fasting were significantly attenuated by the ICV injection of GLP-1 in rats (Seo, Ju et al. 2008). Exendin (9-39) has also been found to increase the feeding response to NPY (Turton, O'Shea et al. 1996).

Carnitine Palmitoyltransferase 1 (CPT1)

The carnitine palmitoyltransferase 1 is located in the mitochondrial outer membrane. The function of the carnitine palmitoyltransferase 1 is to transfer long-chain fatty acids from the cytosol to the mitochondrial matrix, where they are used for energy production through beta-oxidation (Murthy and Pande 1987; Bieber 1988). The up-regulation of the carnitine palmitoyltransferase 1 in the liver by a peroxisome proliferator-activated receptor-α (PPARα)

agonist leads to the stimulation of the mitochondrial beta-oxidation and the triglyceride (TG) - lowering effect (Minnich, Tian et al. 2001).

The absence of the carnitine palmitoyl transferase 1 will lead to the decreased long-chain fatty acid oxidation and ketogenesis, and increased urinary carnitine concentrations. And absence of this enzyme activity in the liver will lead to severe hypoglycemia and even death although just a few patients have been reported with carnitine palmitoyl transferase 1 deficiency. However, this syndrome can be reversed by administration of medium-chain triglycerides (Bougneres, Saudubray et al. 1981; Angelini, Trevisan et al. 1987; Vianey-Saban, Mousson et al. 1993).

In order to decrease the high risk of carnitine palmitoyl transferase 1 deficiency and prevent potentially life-threatening complications, pre-symptomatic diagnosis is needed. The ratio of free carnitine to the sum of palmitoylcarnitine and stearoylcarnitine [C0/(C16 + C18)] which is obtained by electrospray ionization-tandem mass spectrometry (ESI-MS/MS), has been shown to be very highly specific for carnitine palmitoyl transferase 1 deficiency and can be used for pre-symptomatic diagnosis (Fingerhut, Roschinger et al. 2001).

The rat model with decreased hypothalamic CPT1 activity has been developed by injection of a ribozyme-containing plasmid which was used for down-regulation of the carnitine palmitoyl transferase 1 gene or by injection of biochemical inhibitors of CPT1 into the third cerebral ventricle. This model with decreased long-chain fatty acid oxidation and ketogenesis in selective hypothalamic neurons can lead to substantially decreased food intake and endogenous glucose production (Obici, Feng et al. 2003).

Aldehyde Oxidase 1 (AOX1)

Aldehyde oxidase 1, a xenobiotic metabolizing protein, produces hydrogen peroxide superoxide under certain conditions (Wright, Vaitaitis et al. 1993). Alkane production, a

lipoperoxidation products, was determined in isolated rat hepatocytes during ethanol metabolism in order to study the role of aldehyde oxidase 1 in the pathogenesis of alcohol-induced hepatotoxicity. The alkane production was decreased by the inhibition of aldehyde oxidase 1. Thus it has been thought that aldehyde oxidase 1 may play an important role in the pathogenesis of alcohol-induced liver injury as it produces free radicals during ethanol metabolism (Shaw and Jayatilleke 1990). Reactive oxygen species (ROS) generated by aldehyde oxidase 1 also increase cell damage and fibrogenesis (Pessayre, Mansouri et al. 2002).

Aldehyde oxidase 1 level is higher in rats fed a high fat diet when compared with rats fed standard chow diet. Adiponectin suppresses aldehyde oxidase 1 protein through activating peroxisome proliferator-activated receptor-alpha (PPAR-á), as such suppression is blocked by the PPAR-á antagonist. Adiponectin protects the liver from obesity or alcohol induced steatosis because it reduces the reactive oxygen species (ROS) through the inhibition of aldehyde oxidase 1 (Neumeier, Weigert et al. 2006). Aldehyde oxidase 1 is also very important for adipogenesis, as there is impaired lipid storage and adiponectin release in the differentiated adipocytes when aldehyde oxidase 1 is knocked-down in the preadipocytes (Weigert, Neumeier et al. 2008).

Stearoyl-CoA Desaturase 1 (SCD1)

Stearoyl-CoA desaturase 1, a key regulator of fatty acid composition, regulates a rate-limiting step in the synthesis of unsaturated fatty acids. Stearoyl-CoA desaturase 1 can catalyze the formation of the monounsaturated fatty acids palmitoleyl- and oleyl-CoA from the saturated fatty acids palmitoyl-CoA and stearoyl-CoA. The ratio of monounsaturated fatty acids to saturated fatty acids plays a key role on the cell membrane fluidity and signal transduction. This ratio is important for the regulation of cell growth and differentiation (Enoch, Catala et al. 1976;

Wang, Yu et al. 2005). Dietary cholesterol, fatty acids, several hormones, and growth factors all can influence the expression and activity of stearoyl-CoA desaturase 1 (Ntambi 1999).

There is a reduction in monounsaturated fatty acids, cholesterol, and phospholipid synthesis, and an increase in saturated free fatty acids and triacylglycerol in stearoyl-CoA desaturase 1 knocked-down human lung fibroblasts. A change in the ratio of the monounsaturated fatty acids to saturated fatty acids leads to a reduction of proliferation rate, abolition of anchorage-independent growth, and increased sensitivity to palmitic acid-induced apoptosis in a ceramide-independent manner. This suggests that stearoyl-CoA desaturase 1 plays an important role in maintaining cellular lipid homeostasis (Scaglia and Igal 2005). Stearoyl-CoA desaturase 1 is also essential for cellular cholesterol homeostasis because it is important in esterification of cholesterol to cholesterol esters (Miyazaki, Kim et al. 2000).

Compared with the normal ob/ob mice, the stearoyl-CoA desaturase 1 deficient ob/ob mice have lower body weight, increased energy expenditure, decreased liver triglyceride storage, and decreased VLDL (very low density lipoprotein) production (Cohen, Miyazaki et al. 2002). Compared with wild-type mice, stearoyl-CoA desaturase 1 knockout mice also have lower body adiposity, increased insulin sensitivity, enhanced energy expenditure and increased oxygen consumption. These suggests that stearoyl-CoA desaturase 1 deficiency was associated with decreased lipid synthesis and increased lipid oxidation (Ntambi, Miyazaki et al. 2002).

High fat diet-induced hepatic insulin resistance in rats and mice is reversed by specific knockdown of hepatic stearoyl-CoA desaturase 1 through reducing gluconeogenesis and glycogenolysis. This suggests that stearoyl-CoA desaturase 1 is also essential for high fat diet-induced hepatic insulin resistance (Gutierrez-Juarez, Pocai et al. 2006).

Fatty Acid Synthase (FAS)

The main function of fatty acid synthase is to catalyze the synthesis of long-chain saturated fatty acids from acetyl-CoA and malonyl-CoA, in the presence of NADPH (Wakil 1989).

The systemic or intracerebroventricular inhibition of fatty acid synthase induces a reduction of food intake and body weight through the inhibition of the expression of neuropeptide Y in the hypothalamus. Fatty acid synthase is thought to be associated with feeding regulation and may be a new target for treating obesity (Loftus, Jaworsky et al. 2000).

Fatty acid synthase is also thought to be associated with cancer development, as there is increased expression of fatty acid synthase in many common human cancers. The inhibition of fatty acid synthase may be potentially useful for cancer therapy (Camassei, Cozza et al. 2003; Menendez, Vellon et al. 2004).

There is lower fatty acid synthase mRNA level and activity in heterozygous fatty acid synthase +/- mice. These mice cannot survive. This suggests that fatty acid synthase is necessary for embryonic development (Chirala, Chang et al. 2003).

The expression of fatty acid synthase is regulated by feeding of carbohydrates and insulin level in animals. Upstream stimulatory factors (USF1 and USF2) interact with the E box motif on fatty acid synthase promoter to up-regulate fatty acid synthase gene expression in liver during carbohydrates feeding. This up-regulated fatty acid synthase gene expression induced by dietary carbohydrates is dependent on USF1 and USF2, thus the response is severely delayed in USF1 and USF2 knock-out mice. Sterol response element binding protein 1 (SREBP1) also can transcriptionally regulate the fatty acid synthase gene expression (Paulauskis and Sul 1989; Casado, Vallet et al. 1999).

Sterol Regulatory Element-Binding Protein-1c (SREBP1c)

Sterol regulatory element-binding protein-1c, a transcription factor mainly expressed in liver, activates genes involved in glucose utilization and fatty acid synthesis (Kim, Kim et al. 2004). The expression of SREBP-1c is regulated by nutritional status, being down-regulated during fasting and up-regulated after refeeding (Kim, Sarraf et al. 1998).

The induction of the expression of a family of genes involved in fatty acid synthesis by high-carbohydrate diet is dependent on SREBP-1c, as such induction cannot be observed in SREBP-1 knock-out mice (Shimano, Yahagi et al. 1999). In streptozotocin-induced diabetic mice, the up-regulated expression of SREBP-1c by adenovirus in the liver can induce the up-regulated expression of a family of genes involved in fatty acid synthesis (Becard, Hainault et al. 2001).

In streptozotocin-induced diabetic rats, the gene expression of SREBP-1c is very low in the liver, but this low expression can be reversed significantly by insulin (Shimomura, Bashmakov et al. 1999). SREBP-1c is thought to be associated with the insulin dependent hepatic glucokinase expression. The expressions of SREBP-1c and hepatic glucokinase can both be up-regulated by insulin. The up-regulated expression of SREBP-1c by adenovirus in the liver increases the expression of hepatic glucokinase. The induction of the expression of hepatic glucokinase by insulin and SREBP-1c can be blocked by the over-expression of the dominant negative mutant of SREBP-1c. This suggests that SREBP-1c is essential for insulin-dependent hepatic glucokinase expression (Becard, Hainault et al. 2001; Kim, Kim et al. 2004).

Transgenic mice with adipocyte-specific over-expression of SREBP-1c results in abnormal adipose tissue differentiation, insulin resistance, diabetes mellitus, fatty liver and high blood triglyceride level (Shimomura, Hammer et al. 1998). In many insulin resistance animal models,

such as lipodystrophic and ob/ob mice, insulin receptor substrate-2 (IRS)-2(-/-) mice, and Zucker obese fa/fa rats, the gene expression of SREBP-1c is up-regulated in liver (Kakuma, Lee et al. 2000; Shimomura, Matsuda et al. 2000; Tobe, Suzuki et al. 2001). SREBP-1c is thought to be involved in insulin resistance, diabetes and hepatic steatosis.

Peroxisome Proliferator-Activated Receptor-Gamma (PPARγ)

PPARγ, a transcription factor which is highly expressed in adipocytes, functions as a key regulator of adiopocyte differentiation (Chawla, Schwarz et al. 1994; Tontonoz, Hu et al. 1994). PPARγ can activate several genes to increase lipid uptake and adipogenesis by adipocytes. The knockout of the PPARγ in adipose tissue of mice makes the mice unable to generate adipose tissue and protects the mice from obesity and insulin resistance when fed a high fat diet (Jones, Barrick et al. 2005).

PPARγ was found to be the receptor for the glitazones in 1995 (Lehmann, Moore et al. 1995) and the glucose lowering effect of glitazones was thought to be associated with the activation of PPARγ (Willson, Cobb et al. 1996). The PPARγ agonists have been used clinically to improve the glycemic control and enhance insulin sensitivity in type 2 diabetic patients. Now, PPARγ serves as a target of multimodal insulin sensitizers, PPARγ agonists are now a promising research area even when considering the side-effects on weight gain and fluid retention (Rangwala and Lazar 2004; Pegorier 2005; Cho and Momose 2008).

There are several mechanisms for the insulin sensitizer effect of the PPARγ agonists. First, the insulin sensitizer effect of PPARγ agonists may be due to the effect of PPARγ agonists on lipid. PPARγ agonists stimulate several genes to increase fatty acids uptake, storage, and oxidation in both white and brown adipose tissue, thus decreasing the circulating concentrations of both esterified and nonesterified fatty acids and reducing lipid accumulation in non-adipose

tissue (Shimkets ef al., 1999). These two factors both are involved in the development of insulin resistance.

Second, PPARγ agonists can down-regulate the gene expression of tumor necrosis factor α (TNFα) and leptin which are secreted by adipose tissue and are involved in glucose utilization and/or production in other tissues (Hofmann, Lorenz et al. 1994; De Vos, Lefebvre et al. 1996; Kallen and Lazar 1996).

Finally, PPARγ agonists can reduce the gene expression of pyruvate dehydrogenase kinase 4 (PDK4) in muscle, thus enhancing glucose oxidation in muscle. PPARγ agonists can decrease the hepatic gluconeogenesis through down-regulating the expression of phosphoenolpyruvate carboxykinase in liver. PPARγ agonists also can reduce fatty acid oxidation in muscle and liver (Shimkets ef al., 1999). The effects of PPARγ agonists on fatty acids and glucose is shown in Figure 2 (Kliewer, Xu et al. 2001).

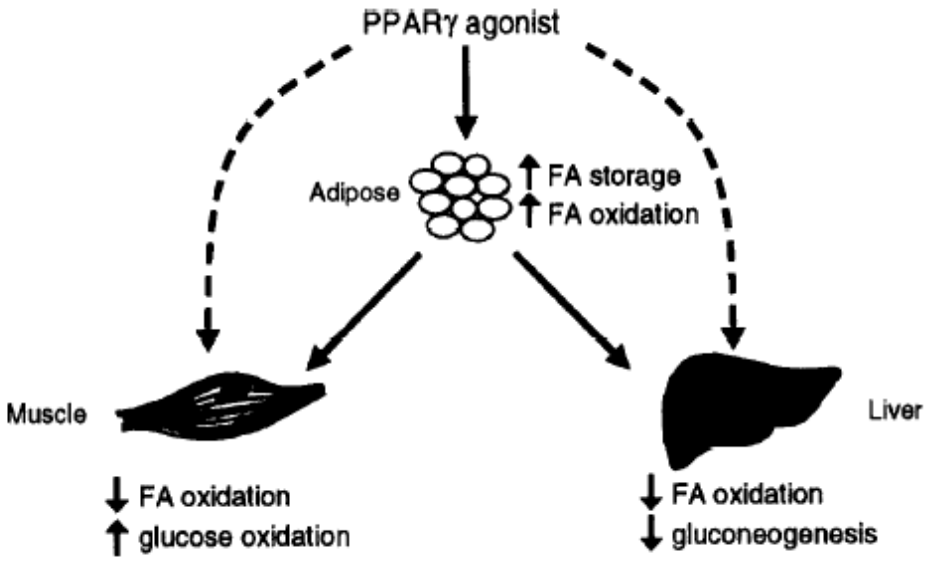

Figure 2. The effects of PPARγ agonists

CHAPTER 3

THE ROLE OF GLUCAGON-LIKE PEPTIDE-1 ON FOOD INTAKE, GLUCOKINASE EXPRESSION AND FATTY ACID METABOLISM

Introduction

Resistant starch has been shown to lower fat storage in animals and significantly increase gene expression of glucagon-like peptide -1 (GLP-1) (Keenan, Zhou et al. 2006; Zhou, Hegsted et al. 2006). GLP-1 affects the activities of hypothalamic NPY/AgRP neurons, inhibits food intake and lowers body weight (Turton, O'Shea et al. 1996; Young, Gedulin et al. 1999; Meier, Gallwitz et al. 2003; Holst 2007). Elderly persons have impaired ability to control food intake following over-eating or under-eating and such impaired ability is thought to be associated with the impaired activation of the hypothalamic NPY pathway (Gruenewald, Marck et al. 1996; Kaneda, Makino et al. 2001). Obesity and aging have been shown to impair the nutrient sensing system in peripheral tissues such as muscle, liver, adipose and pancreas (Hajnoczky and Hoek 2007). In our lab's preliminary data, resistant starch was found to increase gene expression of glucokinase in the ARC in the hypothalamus, which was thought to function as the glucose sensor to facilitate the adaptation in response to fluctuations of the blood glucose levels. We expect that increased GLP-1 by resistant starch could improve the impairment of nutrient sensing system induced by obesity and aging. Exendin-4, a long-acting GLP-1 receptor agonist, has also been shown to be associated with fatty acid synthesis and oxidation (Ding, Saxena et al. 2006; Samson, Gonzalez et al. 2008). We hypothesize that 1) the food intake will be decreased in GLP-1R KO mice, the glucokinase (GK) mRNA expression in the liver and in the hypothalamus, and the hypothalamic NPY mRNA expression will be up-regulated by exendin-4 treatment; and 2) exendin-4 will decrease the fatty acid synthesis and increase the fatty acid oxidation. Therefore, in this study we measured food intake, the GK, CPT1, AOX1, SCD1, FAS, SREBP1c and

PPARγ mRNA expressions in liver, and the GK and NPY mRNA expressions in hypothalamus in WT mice and GLP-1R KO mice with vehicle or exendin-4 treatment.

Materials and Methods

Animals

The original GLP-1R KO mice were provided by the Dr. Daniel J. Drucker lab in the Samuel Lunenfeld Research Institute (SLRI). The mice used in this study were bred by Anne Raggio. They were maintained in a temperature-controlled room (21-23 ^{o}C) on a 12 hour/12 hour light/dark cycle. The light was on from 7AM-7PM. In the experiment, water was available ad libitum. Food was also available ad libitum except for the fasting time.

All mice were housed, four mice per cage, in 6" x 10" x 5" standard plastic cages with bedding to contain their urine and feces, and with wire mesh covers. Pelleted food was placed on wire mesh covers by the researcher. A water bottle was placed on the wire mesh cover with a mouthpiece inserted through a mesh hole. A plastic cover was put over the cage. The cages were changed twice a week. The researchers could observe the mice in the transparent cages. Animal protocols were approved by the Pennington Biomedical Research Institutional Animal Care and Use Committee.

Exendin-4 Treatment

The exendin-4 groups were injected ip with exendin-4 at 100µg/Kg mouse body weight. The exendin-4 was purchased from Amylin Pharmaceuticals, Inc., San Diego, CA. 5mg Exendin-4 was dissolved in 500 ml sterile 0.9% NaCl. The final injection dosage was exendin-4 at 100µl/10g of mouse body weight. The saline groups were injected daily with saline at 100µl/10g mouse body weight.

Experimental Design

Acclimation Period: day 1-7. The female C57BL/6J mice and the female GLP-1R KO mice were both separated into two groups at day 1. The ages and the average body weight of each group were not statistically significantly different. They were fed chow diet for one week to get accustomed to the new environment after regrouping.

Treatment Period: day 8-15. The weights of the mice were measured at the beginning of day 8. Then the body weights were measured every two days. The female C57BL/6J mice and the female GLP-1R KO mice were both divided into two groups, the saline group and the exendin-4 group. This meant there were two independent variables in a 2 X 2 factorial: type of mouse and type of injection. There were a total of 4 groups (n=16). Injection was done at 3:00 pm at day 8, 9, 10, 11, 12. At 9:00 pm of day 12, all mice were fasted for 13 hours. Injection was done at 9:00 am on day 13 and re-feeding was started at 10:00 am. The food intakes of each group were measured at 1h, 2h and 4h after re-feeding. Injection was done at 3:00 pm at day 14. At 9:00 pm of day 14, half of the total 4 groups of mice were fasted for a minimum of 12 hours. At this point the study experimental design became a 2 x 2 x 2 factorial with 8 groups of mice (n=8) and three independent variables. The experimental design is shown in figure 3. Injection was done at 9:00am at day 15 and blood glucose levels were measured using the Freestyle Blood Glucose Meter following injection. The mice were then sacrificed via decapitation and the brain and liver were removed and stored at -80 ^{o}C.

Measurements of GK, CPT1, AOX1, SCD1, FAS, SREBP1c, PPARγ mRNA Expressions in liver

RNA was extracted from liver tissue using TRIzol reagent. The liver tissue was cut into small pieces and homogenized using a Polytron PT 1200E Tissue Homogenizer for 30- 60 seconds in 0.5ml TRIzol reagent. Then 0.5ml TRIzol reagent was added, and the samples

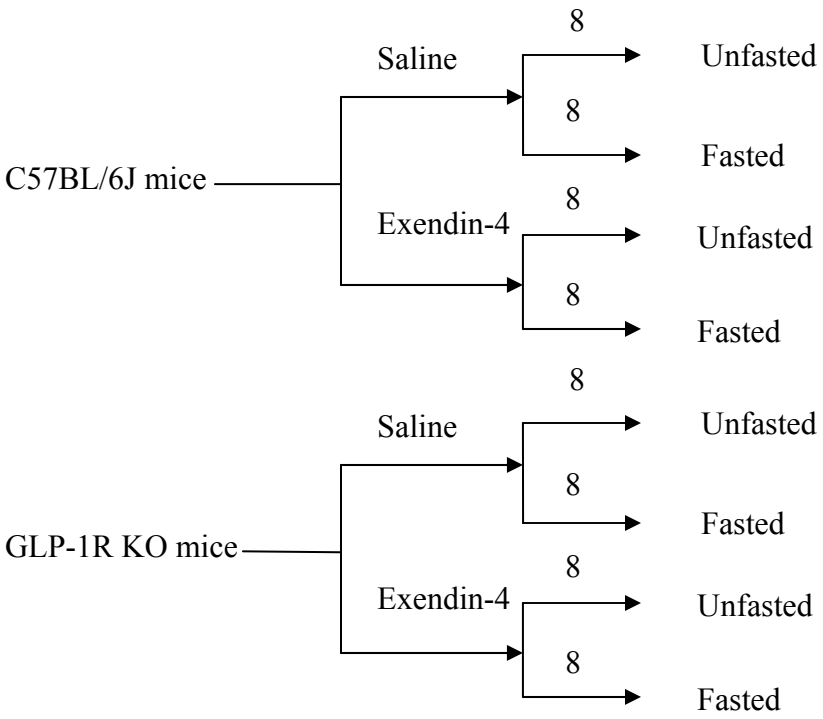

Figure 3 Experimental design

vortexed and incubated at Room Temperature for 5-10 minutes. The homogenized sample was then centrifuged at 12,000 g for 10 minutes at 4^{o}C and then transferred the supernatant (TRIzol reagent layer) which contained RNA to a fresh tube. The next step was the addition of 0.2 ml Chloroform, then the sample was vortexed and incubated at Room Temperature for 2-15 minutes. This was followed by centrifugation at 12,000 g for 15 minutes at 4^{o}C and the supernatant (aqueous phase) which contained RNA was transfered to a fresh tube. The addition of 0.5 ml isopropyl alcohol followed, and then the sample was vortexed and incubated at room temperature for 5-10 minutes. Centrifugation followed at 12,000 g for 10 min at 4^{o}C, and then the supernatant was removed and the gel-like pellet at the bottom of the tube was kept. To the pellet 1 ml of 75% ethanol was added to wash the pellet. Then the sample was centrifuged at 7500 g for 5 min at 4^{o}C, and the 75% ethanol was removed and the gel-like pellet was again kept. The pellet was air-

dried at 4°C for 5-10 min (the RNA pellet was not allowed to dry completely) and the RNA pellet was dissolved in 200 µl RNase-free water. The RNA was then stored at -80 °C.

Two µl of sample RNA was used for RNA quantification using the nanodrop (Nanodrop Technologies, Spectrophotometer ND-1000 UV/Vis). The RNA samples were diluted to 500 ng /µl. Then 2 µl of these samples RNA was used for RNA quantification using the nanodrop again to check the RNA concentration. Then all these RNA samples were diluted to 50 ng/ µl.

The quality of the RNA was measured by electrophoresis using an agarose gel. The gel contains 1 g agarose in 100 ml 0.5 X TBE buffer **(**45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0) and 10 ul 50 mg/ml ethidium bromide**,** was cooled to 60°C. Sample were electrophoresed in 6 X native agarose gel loading buffer (10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol, 60 mM EDTA at 160V for 10min. The 28S and 18S RNA bands were visualized using the Bioaid gel documentation.

Real-time reverse transcriptase polymerase chain reaction was performed to measure the mRNA levels of GK, CPT1, AOX1, SCD1, FAS, SREBP1c, and PPARγ in the liver samples. Results were expressed as the ratio of the GK, CPT1, AOX1, SCD1, FAS, SREBP1c, PPARγ to the constitutive gene cyclophilin. Taqman RT-PCR primers and probes were used. The primers and probes used were CPT1, Mm00550438_m1; AOX1, Mm 00437475_m1; SCD1, Mm 00772290_m1; FAS, Mm 00662319_m1; SREBP1, Mm 00550338_m1; PPARγ, Mm 00440945_m1. The real time RT-PCR reagents were purchased from Applied Biosystems (Foster City, CA). The real time RT-PCR reaction mixture contained 1ul of 10 X Tagman buffer, 5.5 mM MgCl2, 0.3 mM dATP, 0.3 mM dCTP, 0.3 mM dUTP, 0.3 mM dGTP, 500 nM forward primers, 500 nM reverse primers, 200 nMTaqman probes, 0.3 U AmpliTaq Gold DNA polymerase, 5 U MuLV reverse transcriptase, 7.5 U RNase inhibitor, and RNase-free water. For

each gene, 3 µl 5 ng/ul liver template sample was used, total 15 ng. Each sample (total volume 10 µl) was performed in duplicate. RT-PCR conditions were 48 °C for 30 min, 95°C for 10 min for one cycle, 95 °C for 15 sec and 60 °C for 1 min for 40 cycles.

Microdissection of Arcuate Nucleus (ARC) in Hypothalamus

The brains were rapidly obtained from the decapitated mice, then put on dry ice and stored at -80 °C. The first step of the microdissection of the ARC was to dissect the middle brain using a cryostat. Four continuous coronal sections from Bregman -1.22mm to -2.42mm were collected for the ARC micropunch. Each coronal section was 300 um. The tubes were prepared with 100 µl of 2- mecaptoethanol-lysis buffer (ratio is 0.7:100) before the micropunch. The samples were then put on ice. A needle (Stoelting, Chicago, IL), which had an inner diameter of 0.52mm was used to perform the micropunch under a microscope. The tissue was transfered in the needle immediately to the tube after the micropunch and vortexed to make the sample homogenized. 100 µl cold 70% ethanol was added to the lysate and votexed about 10 seconds to mix the sample thoroughly. The sample was put on the dry ice immediately and then stored at -80 °C until the RNA extraction was performed.

Measurements of GK, NPY mRNA Expression in Arcuate Nucleus (ARC) in the Hypothalamus

The Absolutely RNA microprep kit (Stratagene, La Jolla, CA) was used to extract the brain mRNA. First, the thawed sample was vortexed for about 10 sec, transfered to a seated RNA-binding spin cup in a 2-ml collection tube, and centrifuged for 1 min at maximum speed at 4 °C. The filtrate was discarded, 600 µl of 1*low-salt wash buffer was added to the spin cup, which was centrifuged for 1 min at maximum speed at 4 °C. Again, the filtrate was discarded,

and centrifuged for 2 more min at maximum speed at 4 °C to make the fiber matrix dry. The reconstituted RNase-free DNase I and DNase digestion buffer (ratio is 1:5) were mixed together and 30 µl of the mixture was added directly onto the fiber matrix. The spin cup was incubated in a 2-ml collection tube in 37 °C water bath for 15 min. 500 µl of 1*high-salt wash buffer was added to the spin cup, and centrifuged for 1 min at maximum speed at 4 °C. The filtrate was discarded, 600 µl of 1*low-salt wash buffer was added to the spin cup, and centrifuged for 1 min at maximum speed at 4 °C. Again, the filtrate was discarded, 300 µl of 1*low-salt wash buffer was added to the spin cup, and centrifuged for 2 min at maximum speed at 4 °C to make the fiber matrix dry. The 2-ml collection tube was discarded, the spin cup was put in a new 1.5-ml collection tube, and 20 µl of elution buffer was added directly onto the fiber matrix. This was then centrifuged for 3 min at maximum speed at 4 °C after a 2-min incubation at room temperature. The filtrate was collected and stored at -80 °C. 2 µl of brain mRNA was used for mRNA quantification using the Nanodrop. The mRNA samples were diluted to 2 ng/µl.

Real-time reverse transcriptase polymerase chain reaction was performed to measure the mRNA levels of GK and NPY in the brain samples. The ratio of the GK and NPY to the constitutive gene cyclophilin was used to express the results. Taqman RT-PCR primers and probes were used and the sequences of the primers and probes for mice cyclophilin and GK were listed in Table 1. The mice NPY probe Ref. number was SS18395-01, the forward Ref. number is SS18398-01, the reverse Ref. number is SS18398-02. The real time RT-PCR reagents were purchased from Applied Biosystems (Foster City, CA). The real time RT-PCR reaction mixture

contained 1ul of 10 X Tagman buffer, 5.5 mM MgCl2, 0.3 mM dATP, 0.3 mM dCTP, 0.3 mM dUTP, 0.3 mM dGTP, 500 nM forward primers, 500 nM reverse primers, 200 nMTaqman probes, 0.3 U AmpliTaq Gold DNA polymerase, 5 U MuLV reverse transcriptase, 7.5 U RNase inhibitor, and RNase-free water. For cyclophilin, 4 µl 2 ng/µl brain template sample, total 8 ng was used. For the GK and NPY, 2 µl 2 ng/µl brain template sample, total 4ng was used. Each sample (total volume 10 ul) was performed in duplicate. The real time RT-PCR condition was 48 °C for 30 min, 95°C for 10 min for one cycle, 95 °C for 15 sec and 60 °C for 1 min for 40 cycles.

Table 1. The sequences of primers and probes for real time RT-PCR.

F: forward primer, R: reverse primer, P: Taqman probe, CYC: cyclophilin, GK: glucose kinase.

Gene	Sequence
Mice CYC	F: 5'GGCCGATGACGAGCCC3' R: 5'TGTCTTGGAACTTTGTCTGCAA3' P: 5'TGGGGCCGCGTCTCCTTCGA 3'
Mice GK	F: 5'CCACAATGATCTCCTGCTACTATGA3' R: 5'AGCCGGTGCCCACAATC3' P: 5'ACCGCCAATGTGAGGTCGGCA3'

Statistical Analysis

Data is presented as mean ± SEM. The significance of the differences between means was analyzed by two-way ANOVA using the Statistical Analysis System (SAS 9.1). The significant level was set at 0.05.

Results

Food intake

The 1h, 2h and 4h food intakes were not affected by the exendin-4 treatment for either WT mice or GLP-1R KO mice ($P>0.05$). But there was a significant reduction in fasting-refeeding food intake ($P<0.01$) for KO mice at 1h, 2h and 4h compared to wild type by two way ANOVA. (Figure 1)

FAS mRNA Expressions in liver

The FAS expression in liver was significantly higher in GLP-1R KO mice than WT mice in unfasted condition ($P<0.01$). (Figure 2)

Blood glucose level

The blood glucose level was significantly reduced by exendin-4 treatment in the unfasted condition for WT mice ($P<0.0001$). There were no effects of exendin-4 treatment for the fasted condition and GLP-1R KO mice ($P>0.05$). Fasting significantly reduced the blood glucose level of WT mice with vehicle treatment ($P<0.0001$). (Figure 3)

GK mRNA expression in liver

GK mRNA expression in liver was significantly up-regulated by exendin-4 treatment in both unfasted and fasted condition for WT mice ($P<0.001$). As expected, there were no effects of exendin-4 treatment for GLP-1R KO mice ($P>0.05$). (Figure 4)

CPT1, AOX1, SCD1, FAS, SREBP1c, PPARγ mRNA Expressions in liver

Exendin-4 had no effect on CPT1 mRNA expression in the liver samples, but increased the AOX mRNA expression in the fasted condition in WT mice ($P<0.05$). mRNA expressions of SCD1, FAS and SREBP1c, key regulators of *denovo* hepatic lipogenesis, were not changed by exendin-4 treatment for WT mice ($P>0.05$). Exendin-4 also had no effect in PPARγ mRNA

expression in the liver (P>0.05). Fasting significantly up-regulated fatty acid oxidation related gene CPT1 and AOX1 expressions, down-regulated the fatty acid synthesis related genes SCD1, FAS and SREBP1c expression, and up-regulated PPARγ mRNA expressions in the liver (P<0.05). (Figure 5-10)

GK and NPY mRNA Expression in Arcuate Nucleus (ARC) in Hypothalamus

GK and NPY mRNA expressions in Arcuate Nucleus (ARC) in Hypothalamus were not changed by exendin-4 treatment for WT mice and GLP-1R KO mice (P>0.05). (Figure 11-12)

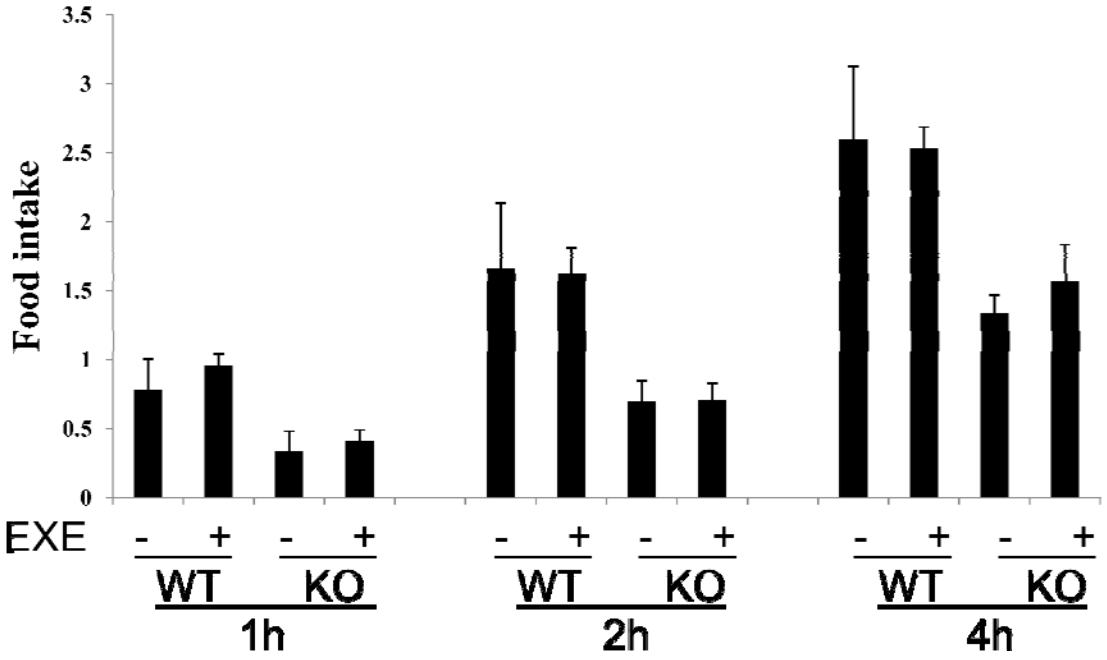

Figure 1. Exendin-4 has no effect on the 1h, 2h and 4h food intake for both WT mice and GLP-1R KO mice. There was a significant difference (P<0.01) for the mouse type in the 1h, 2h and 4h food intake by two way ANOVA. There were no significant effects on the interaction (P>0.05).

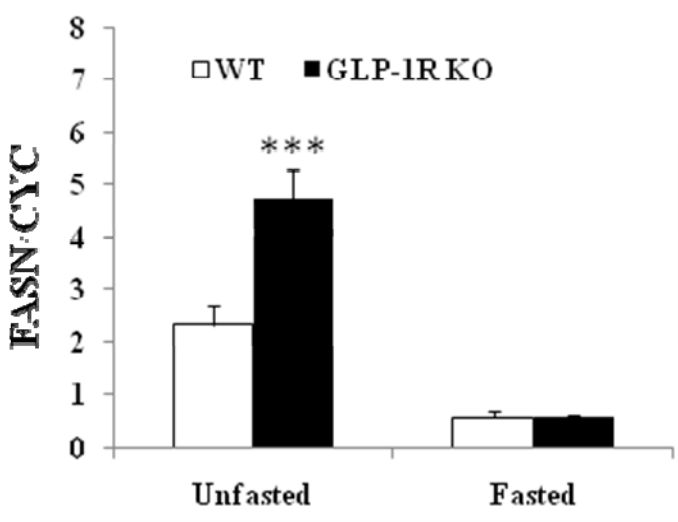

Figure 2. There was a significant difference (P<0.01) for the mouse type in FAS expression in unfasted condition by two way ANOVA. Data are means ± SEM for 8 mice each group. The fasting: P<0.0001, Interaction: P<0.01.

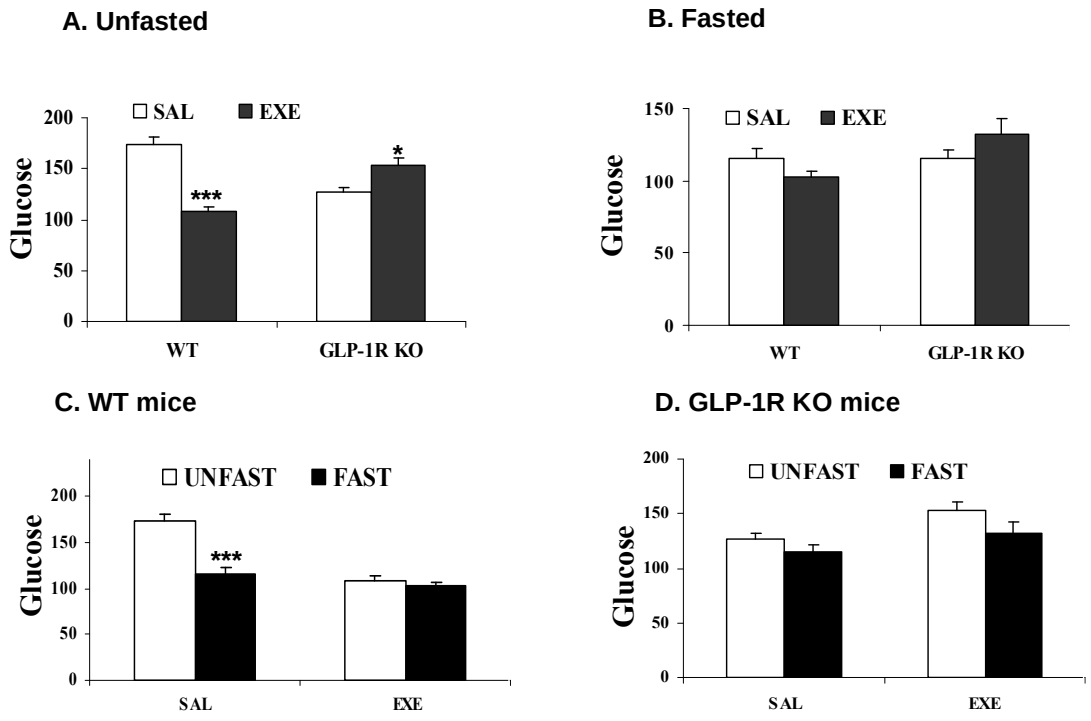

Figure 3. Exendin-4 decreases the blood glucose level in the unfasted condition in WT mice, but not in the fasted condition in GLP-1R KO mice. Data are means ± SEM for 8 mice each group. In the unfasted condition, mice: $P>0.05$, drug: $P<0.05$, Interaction: $P<0.0001$ by two way ANOVA. There were no significant effects on mouse type, drug, and interaction in the fasted condition. For WT mice, fast: $P<0.0001$, drug: $P<0.0001$, Interaction: $P=0.0001$. For GLP-1R KO mice, fast: $P<0.05$, drug: $P<0.01$, Interaction: $P>0.05$. *** $P<0.0001$ vs. controls for the WT mice. * $P<0.05$ vs. controls for the GLP-1R KO mice.

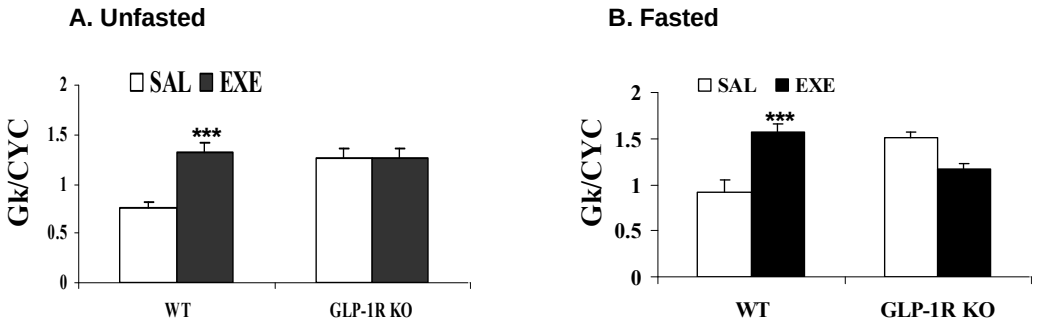

Figure 4. Exendin-4 increases the GK mRNA expressions of the liver samples in the unfasted condition and fasted condition for the WT mice, but not for the GLP-1R KO mice. Data are means ± SEM for 8 mice each group. In unfasted condition, mice: $P<0.05$, drug: $P<0.01$, Interaction: $P<0.01$ by two way ANOVA. In fasted condition, mice: $P>0.05$, drug: $P>0.05$, Interaction: $P<0.0001$. *** $P<0.001$ vs. controls for the WT mice.

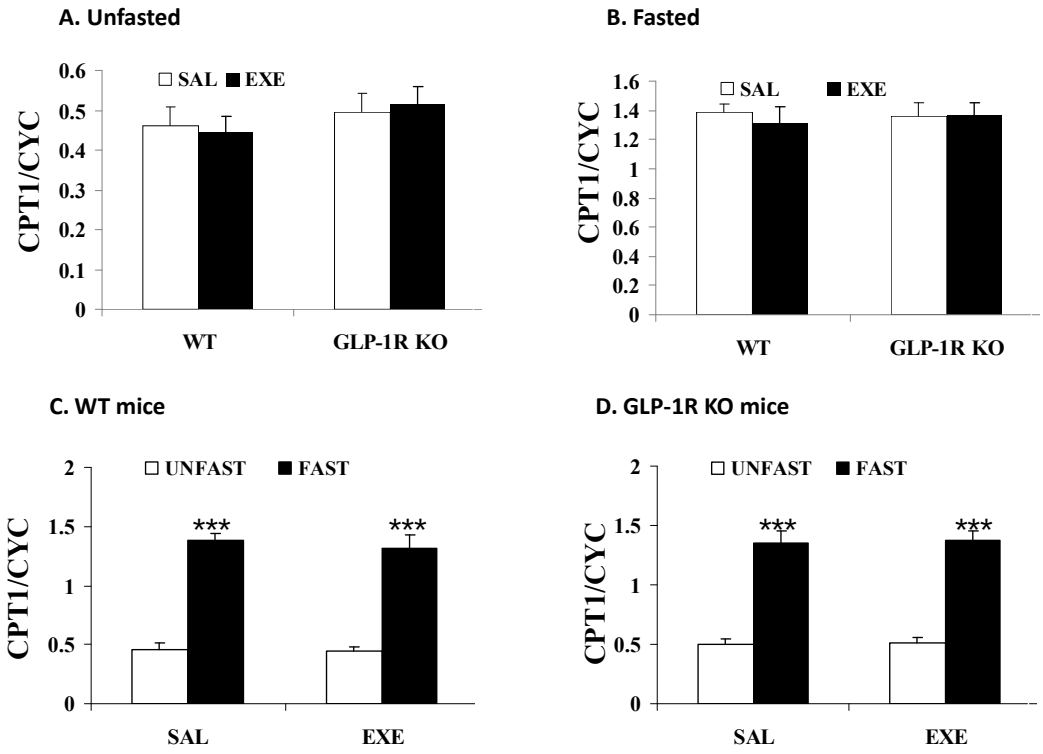

Figure 5. Exendin-4 has no effect in CPT1 mRNA expressions of the liver samples in the unfasted condition and the fasted condition for both WT mice and GLP-1R KO mice. There were significant effects of the fasting within the same treatment (vehicle or exendin-4), and for both WT mice and GLP-1R KO mice. Data are means ± SEM for 8 mice each group. There were no significant effects on mice, drug, and interaction in unfasted condition and fasted condition by two way ANOVA. For both WT mice and GLP-1R KO mice, there was a significant fasting effect ($P<0.0001$), but not an exendin-4 treatment effect ($P>0.05$) and no interaction effect ($P>0.05$). *** $P<0.0001$ vs. controls within the same treatment (vehicle or exendin-4), and for both WT mice and GLP-1R KO mice.

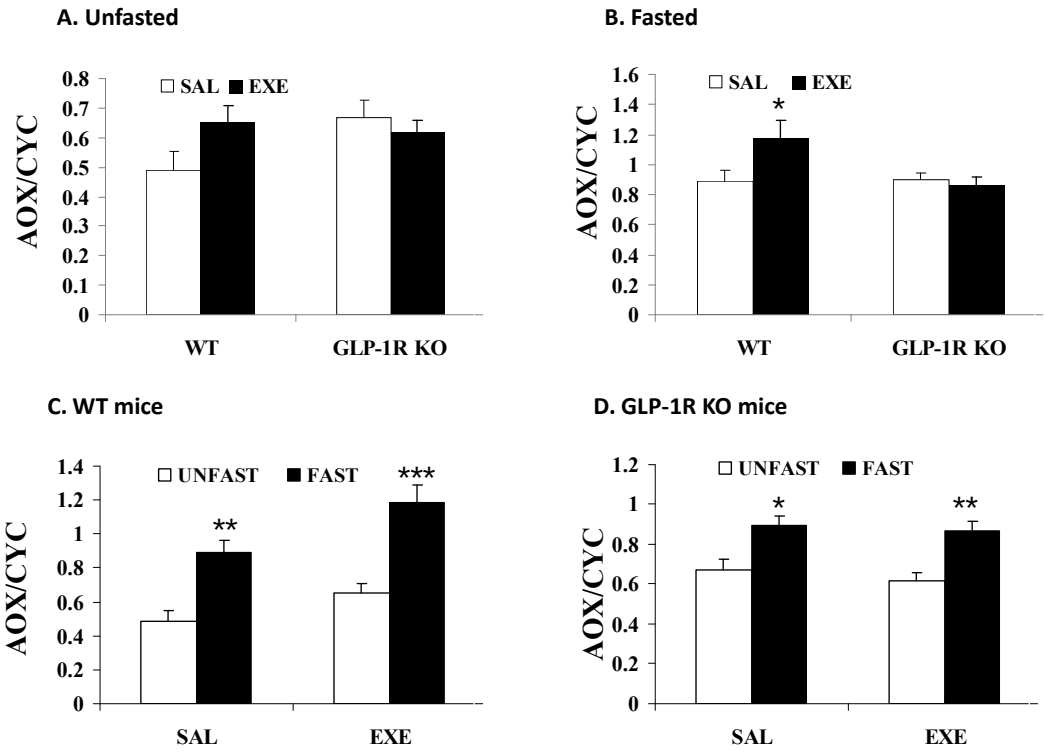

Figure 6. Exendin-4 increases the AOX mRNA expressions of the liver samples in the fasted condition in WT mice, but not for the unfasted condtion in GLP-1R KO mice. There were significant effects of the fasting within the same treatment (vehicle or exendin-4), and for both WT mice and GLP-1R KO mice. Data are means ± SEM for 8 mice each group. There were no significant effects on mice, drug, and interaction in unfasted condition by two way ANOVA. In fasted condition, mice: $P<0.05$, drug: $P>0.05$, Interaction: $P<0.05$. For WT mice, fast: $P<0.0001$, drug: $P<0.01$, Interaction: $P>0.05$. For GLP-1R KO mice, fast: $P<0.0001$, drug: $P>0.05$, Interaction: $P>0.05$. * $P<0.05$ vs. controls within the same treatment (vehicle or exendin-4), and for both WT mice and GLP-1R KO mice.

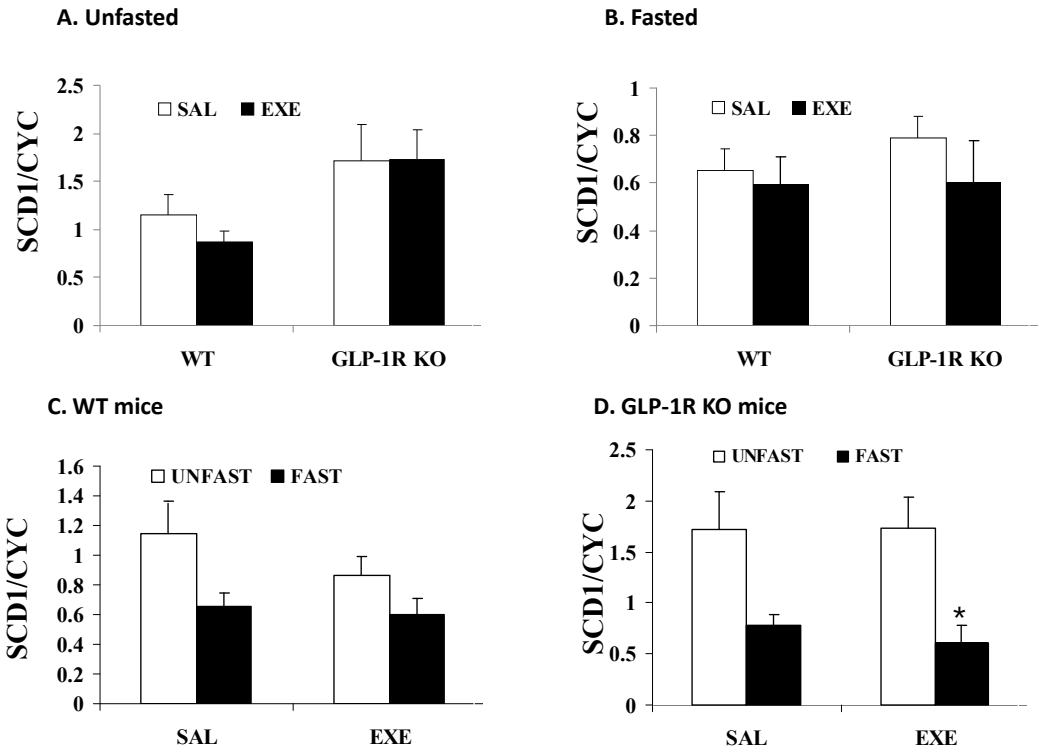

Figure 7. Exendin-4 has no effect in SCD1 mRNA expressions of the liver samples in the unfasted condition and the fasted condition for both WT mice and GLP-1R KO mice. There was only significant effects of fasting within the exendin-4 treatment for GLP-1R KO mice. Data are means ± SEM for 8 mice each group. There were no significant effects on mice, drug, and interaction in unfasted condition and fasted condition by two way ANOVA. For WT mice, fast: $P<0.05$, drug: $P>0.05$, Interaction: $P>0.05$. For GLP-1R KO mice, fast: $P<0.001$, drug: $P>0.05$, Interaction: $P>0.05$. * $P<0.05$ vs. controls within the exendin-4 treatment for GLP-1R KO mice.

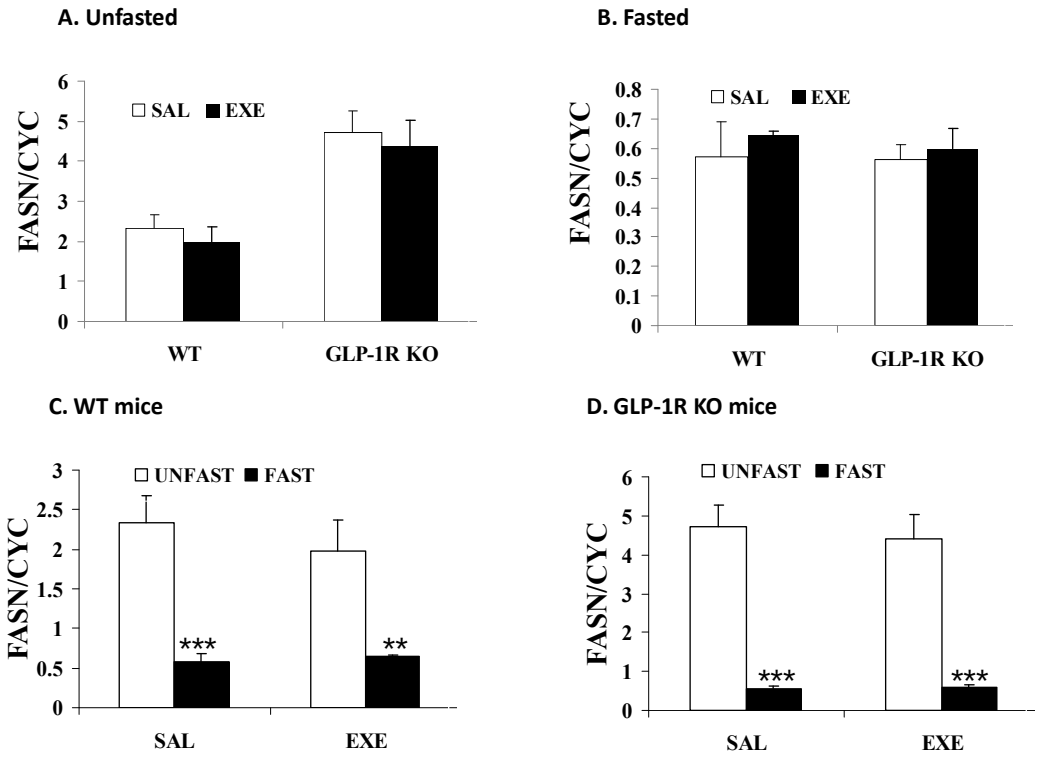

Figure 8. Exendin-4 has no effect in FAS mRNA expressions of the liver samples in the unfasted condition and the fasted condition for both WT mice and GLP-1R KO mice. There were significant effects of the fasting within the same treatment (vehicle or exendin-4), and for both WT mice and GLP-1R KO mice. Data are means ± SEM for 8 mice each group. In unfasted condition, mice: $P<0.0001$, drug: $P>0.05$, Interaction: $P>0.05$ by two way ANOVA. There were no significant effects on mice, drug, and interaction in fasted condition. For both WT mice and GLP-1R KO mice, there was a significant fasting effect ($P<0.0001$), but not an exendin-4 treatment effect ($P>0.05$) and no interaction effect ($P>0.05$). ** $P<0.01$ vs. controls within the same treatment (vehicle or exendin-4), and for both WT mice and GLP-1R KO mice.

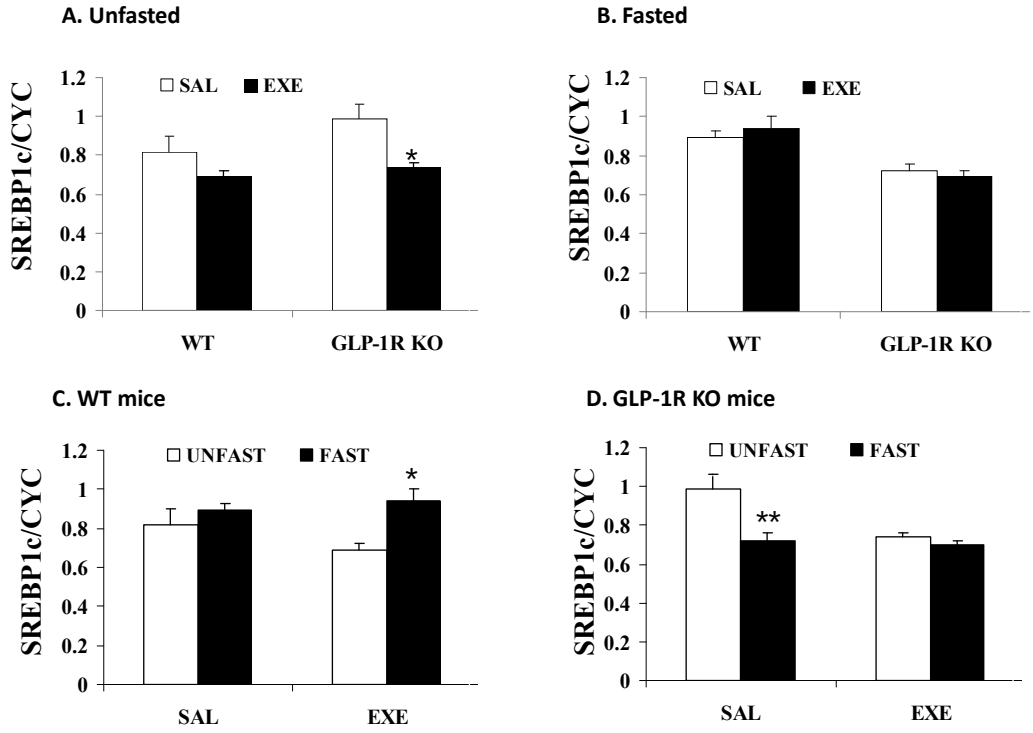

Figure 9. Exendin-4 decreases the SREBP1c mRNA expressions of the liver samples in the unfasted condition for GLP-1R KO mice, but not for the fasted condition and WT mice. Data are means ± SEM for 8 mice each group. In unfasted condition, mice: $P>0.05$, drug: $P<0.01$, Interaction: $P>0.05$ by two way ANOVA. In fasted condition, mice: $P<0.0001$, drug: $P>0.05$, Interaction: $P>0.05$. For WT mice, fast: $P<0.05$, drug: $P>0.05$, Interaction: $P>0.05$. For GLP-1R KO mice, fast: $P<0.01$, drug: $P<0.01$, Interaction: $P<0.05$. * $P<0.05$ vs. controls in unfasted condition for GLP-1R KO mice.

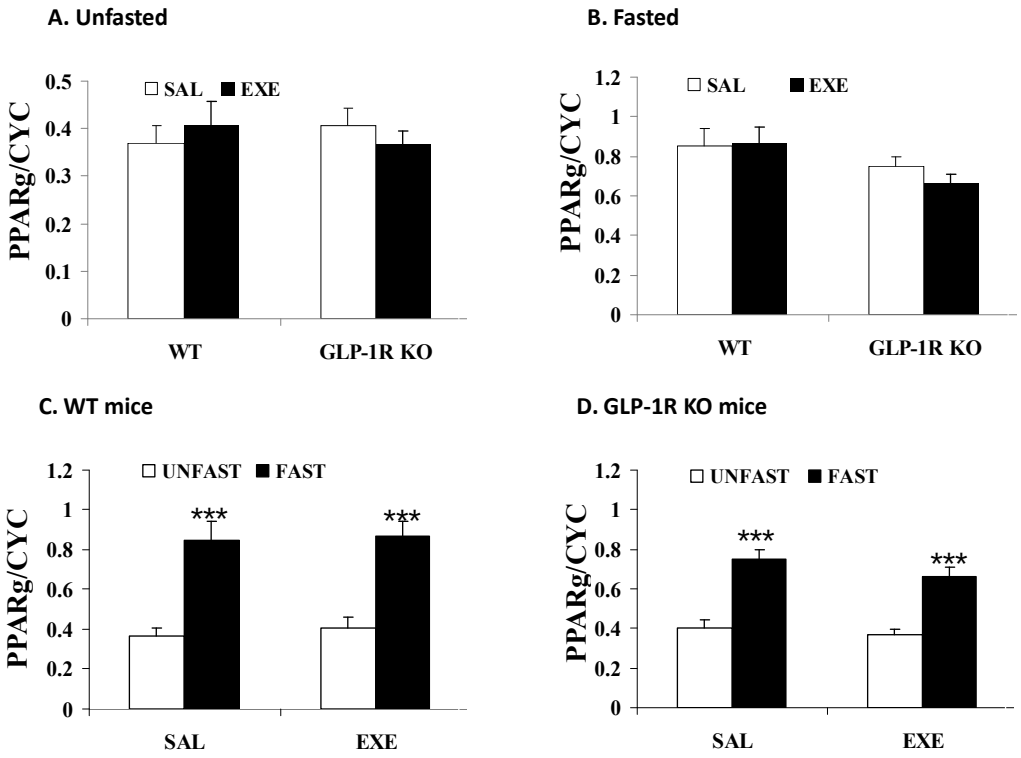

Figure 10. Exendin-4 has no effect in PPARγ mRNA expressions of the liver samples in the unfasted condition and the fasted condition for both WT mice and GLP-1R KO mice. There were significant effects of the fasting within the same treatment (vehicle or exendin-4), and for both WT mice and GLP-1R KO mice. Data are means ± SEM for 8 mice each group. There were no significant effects on mice, drug, and interaction in unfasted condition by two way ANOVA. In fasted condition, mice: $P<0.05$, drug: $P>0.05$, Interaction: $P>0.05$. For both WT mice and GLP-1R KO mice, there was a significant fasting effect ($P<0.0001$), but not an exendin-4 treatment effect ($P>0.05$) and no interaction effect ($P>0.05$). *** $P<0.0001$ vs. controls within the same treatment (vehicle or exendin-4), and for both WT mice and GLP-1R KO mice.

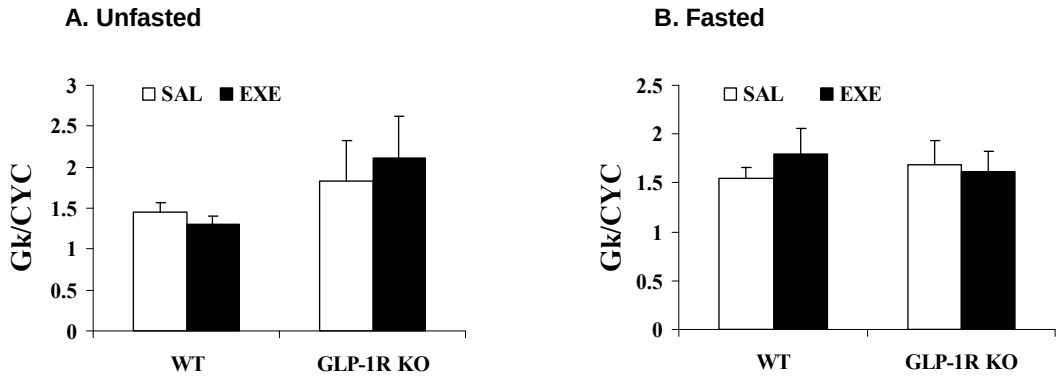

Figure 11. Exendin-4 has no effect in GK mRNA expressions of the brain samples in the unfasted condition and the fasted condition for both the WT mice and the GLP-1R KO mice. There were no significant effects on mice, drug, and interaction in unfasted condition and fasted condition by two way ANOVA.

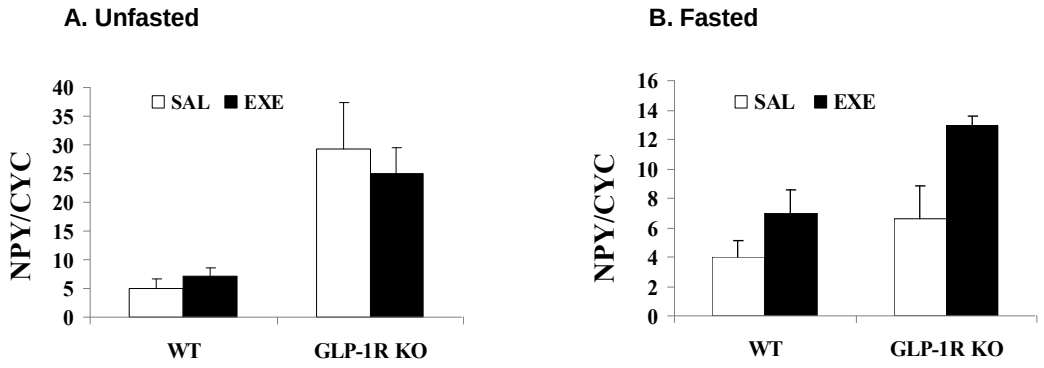

Figure 12. Exendin-4 has no effect in NPY mRNA expressions of the brain samples in the unfasted condition and the fasted condition for both the WT mice and the GLP-1R KO mice. In the unfasted condition, mice: $P<0.0001$, drug: $P>0.05$, Interaction: $P>0.05$ by two way ANOVA. In the fasted condition, mice: $P<0.05$, drug: $P<0.01$, Interaction: $P>0.05$.

Discussion

In this study, we investigated the effects of exendin-4 and GLP-1 receptor knock out on the nutrient sensing system and fatty acid metabolism. We demonstrated that the food intake of GLP-1R KO mice at 1h, 2h and 4h was significantly less than WT mice, and the FAS expression in liver was significantly higher in GLP-1R KO mice than WT mice in unfasted condition. Exendin-4 increases the GK expression in liver and increases the AOX expression in liver, which was associated with β-oxidation of free fatty acids.

To our knowledge, GLP-1 might have beneficial effects on improving the impairment of nutrient sensing system induced by aging. The up-regulated FAS gene expression might be one possible mechanism for higher body fat in GLP1R KO mice. The lowering body fat storage effect of exendin-4 may be in part due to increased fatty acid oxidation in the liver.

We measured the fasting-refeeding food intake at 1h, 2h and 4h for both WT mice and GLP-1R KO mice. The food intake of GLP-1R KO mice at each time was significantly less than WT mice. These data were consistent with our lab's preliminary data. Resistant starch was found to increase the fasting-refeeding food intake at 1h, 2h and 4h for 2 year old aged WT mice. Elderly persons have impaired ability to adjust food intake following over-eating or under-eating, resulting in impaired ability to defend their body weight (Roberts, Fuss et al. 1994; Hays and Roberts 2006). It may be because they have decreased NPY responsiveness to fasting in aged condition (Gruenewald, Marck et al. 1996; Kaneda, Makino et al. 2001). Resistant starch significantly increased the expression and release of glucagon-like peptide -1 (GLP-1) in the large intestine (Keenan, Zhou et al. 2006; Zhou, Hegsted et al. 2006). GLP-1R KO mice might have similar impairment of nutrient sensing system as aged mice, so they are less responsive to

fasting. All the data suggested that GLP-1 might have beneficial effects on improving the impairment of nutrient sensing system induced by aging.

We compared the genes related to fatty acid metabolism between WT mice and GLP-1R KO mice. We found that the FAS expression in liver was significantly higher in GLP-1R KO mice than WT mice in unfasted condition. There are no previous reports for abnormal body weight or feeding behavior of GLP1R KO mice, and even no evidence for abnormal body weight in 11- or 16-month-old aging GLP-1R KO mice, or high fat feeding in GLP-1R KO mice (Scrocchi, Brown et al. 1996; Scrocchi and Drucker 1998). But in our lab's preliminary data, GLP-1R KO male mice had significantly higher body weight and higher body fat compared with WT male mice after 12 weeks EC or RS diet feeding. And the body fat percentage of GLP1R KO mice is also significantly higher than WT mice. The up-regulated FAS gene expression might be one possible mechanism for higher body fat in GLP-1R KO mice.

We measured the GK expression both in liver and in hypothalamus. We observed an increase in the GK expression in liver by the exendin-4 treatment, but not in the hypothalamus. In our lab's preliminary data, resistant starch was found to increase gene expression of glucokinase in the ARC in the hypothalamus. But in this preliminary study, rats were fed resistant starch for 12 weeks and had consistently higher GLP-1 levels over a 24h period. In this study, the mice were treated with exendin-4 for just 7 days. It is possible that an increase of GK expression in the hypothalamus would require longer treatment with exendin 4.

In this study, we used a needle (Stoelting, Chicago, IL) which had an inner diameter of 0.52 mm to perform the micropunch of the arcuate nucleus of the slice of the brain under a microscope. Then, we transfered the tissue in the needle immediately to the test tube after the micropunch. The needle was so small that we did not get much tissue for the RNA extraction.

The RNA concentrations of some samples were too low to do RT-PCR, resulting in missing samples in each group. This may have affected the statistical results of the brain study.

Exendin-4 was demonstrated to be associated with fatty acid metabolism. Exendin-4 reduces body weight, decreases fat content of liver tissue, reduces hepatic oxidative stress, and reverses hepatic steatosis in ob/ob mice with 60 days treatment. The key regulators of *de novo* hepatic lipogenesis, SREBP-1c and SCD1, are down-regulated, and the key element in β-oxidation of free fatty acids, PPARα, is up-regulated in the liver tissue of the ob/ob mice with 60 days exendin-4 treatment. Besides these three genes, ACC, which is associated with *de novo* hepatic lipogenesis is down-regulated, and AOX, which is associated with β-oxidation of free fatty acids, is up-regulated in rat hepatocytes with exendin-4 treatment (Ding, Saxena et al. 2006). One group constructed a mouse model with elevated steady-state levels of exendin-4 by a single injection of a helper-dependent adenoviral (HDAd) vector which expresses exendin-4 chronically in vivo. When such mice are fed a high-fat-diet, the single injection of HDAd vector lowers body weight, improves glucose homeostasis, decreases hepatic fat, produces less liver total lipid and triglyceride levels and improves the adipokine profile. The ACC1, ACC2, SCD1, FAS, and PPAR-γ1, 2, which are associated with *de novo* hepatic lipogenesis are down-regulated in HDAd-Ex4 mice (Samson, Gonzalez et al. 2008).

In our study, we only observed an increase in AOX mRNA expression, which would be involved in fatty acid oxidation, in the fasted condition for WT mice. We did not see the effects of exendin-4 treatment on CPT1 mRNA expression, or on mRNA expressions of SCD1, FAS and SREBP1c, key regulators of *denovo* hepatic lipogenesis, and the PPARγ mRNA expression in the liver samples. One possible reason is that in the previous two studies, one mouse model is ob/ob, and the other is a high-fat-diet induced obese mouse model. In our study, we used a

normal mouse model fed a chow diet. These wild type mice are lean and the fatty acid metabolism may not be as active as the obese mice used in other studies. This could account for their non- responsive to the exendin-4 treatment. Another possible reason is that in the two previous studies, exendin-4 treatment was for 60 days in one, and in the other study the treatment lasted 15 weeks. However, our study was only a short-term study with treatment for 7 days. Many of the metabolic changes may not have had enough time to occur in our study. However, the increase of AOX mRNA expression in our study suggested that the exendin-4 treatment could increase fatty acid oxidation in liver.

In this study, we measured the NPY mRNA level in the arcuate nucleus in the arcuate nucleus of the hypothalamus in both unfasted and fasted mice. But we observed no increase of the NPY mRNA level in the fasting WT mice compared with the ad lib-fed WT mice (both vehicle treatment) This is not consistent with other literature reports. For 2-3 months old male mice, 24h food deprivation (fasting started at 7:00pm) increased hypothalamic NPY mRNA about 108 ± 6%, and the refeeding for 6 h significantly decreased the hypothalamic NPY mRNA level (Swart, Jahng et al. 2002). For 14-15 weekold male mice, 20h food deprivation (fasting started at 5:00pm) increased the hypothalamic NPY mRNA about 31% (Boswell, Nicholson et al. 1999). Compared with the ad lib-fed male control rats, the gene expression of the prepr-NPY mRNA in the arcuate nucleus was up-regulated by 43 ± 13% or 127 ± 29% after a 24 or 48 h food deprivation (Schwartz, Sipols et al. 1993).

An important reason for differences in our study versus previous studies, may be the length and timing of the fast. In this study, we fasted the mice at 9:00pm, 2 hours later after the lights were turned off. But in the literature, they all initiated the fasting before the lights were out. So the mice in our study had already eaten for 2 hours before fasting compared with other studies.

Such fasting can be called mild fasting because the mice may have had high intakes in the first two hours. Another reason may be that the mice in our study were about 3-5 months old, much older than the mice in the previous studies. Maybe they were less responsive to the removal of the food compared with the younger mice. Another possible reason may be the gender of the mice. The female mice used in our study may be less responsive to the removal of the food compared with the male mice used in other studies. So there ar plausible reasons why we did not observe the increase of the NPY mRNA level in the fasting WT mice compared with the ad lib-fed WT mice.

A study showed that the increase of NPY mRNA induced by 48h fasting could be significantly attenuated by the ICV injection of GLP-1 in rats (Seo, Ju et al. 2008). In our study, the GLP-1 agonist, exendin-4 did not reduce the NPY mRNA level in both the unfasted and fasted condition. The ICV injection of GLP-1 also dramatically inhibited the food intake in 48h fasted rats (Seo, Ju et al. 2008). In 7-10 week old male mice, exendin-4 could significantly reduced the 1h, 2h, 4h, 8h and 24h food intakes after 16-18h overnight fasting (Talsania, Anini et al. 2005). But in our study, we did not observe the food intake inhibitory effects of exendin-4. The food intake results are consistent with our NPY results. The reasons may be the same as why NPY did not change after fasting. The values of the cumulative food intake at each time are much lower in our study compared with the Talsania's study for both vehicle treatments (Talsania, Anini et al. 2005). The mice in our study were less hungry and so less responsive to fasting and the exendin-4 treatment. These values also demonstrated that the mild fasting designed in our study was the main reason why the food intake and NPY mRNA level were not affected by the exendin-4 treatment.

In conclusion, GLP-1 might have beneficial effects on improving the impairment of nutrient sensing system induced by aging. The up-regulated FAS gene expression might be one possible mechanism for higher body fat in GLP1R KO mice. The lowering body fat storage effect of exendin-4 may be in part due to the increase of the fatty acid oxidation in liver. Our findings provide a further understanding of the lowering of body fat storage effect of resistant starch, and also show the possible beneficial effect of resistant starch on the nutrient sensing system.

CHAPTER 4

CONCLUSIONS

The work in this thesis focuses on the effects of exendin-4 and GLP-1 receptor knock out on the nutrient sensing system and fatty acid synthesis and oxidation. We measured the food intake, GK, CPT1, AOX1, SCD1, FAS, SREBP1c and PPARγ mRNA expressions in liver, and the GK and NPY mRNA expressions in hypothalamus in WT mice and GLP-1R KO mice with vehicle or exendin-4 treatment. We showed that the food intake of GLP-1R KO mice at 1h, 2h and 4h of re-feeding after a mild fast was significantly less than for WT mice, and the FAS expression in liver was significantly higher in GLP-1R KO mice than WT mice in the unfasted condition. Exendin-4 increased the GK expression in liver, but not in the hypothalamus. Exendin-4 had no effects on the hypothalamic NPY expression and the 1h, 2h, 4h re-feeding food intake. We also found that extendin-4 only increased the AOX expression in liver, which was associated with β-oxidation of free fatty acids, but had no effect on the CPT1, SCD1, FAS, SREBP1c and PPARγ mRNA expressions in liver, which are all key regulators of fatty acid metabolism.

We provide evidence to indicate that GLP-1 might have beneficial effects on improving the impairment of the nutrient sensing system induced by aging. The up-regulated FAS gene expression might be one possible mechanism for higher body fat in GLP1R KO mice. The lowering of body fat storage effect of exendin-4 may be in part due to the increase of fatty acid oxidation in liver. These findings help us understand the lowering of body fat storage effect of resistant starch, and also introduce the possible beneficial effect of resistant starch on improving the impairment of nutrient sensing system induced by aging.

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VITA

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